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Impact of tomato wastes pyrolysis liquid against potato whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract:

Slow pyrolysis of tomato wastes, at temperature of 350 °C results form of Light Pyrolysis Liquid (LPL), which has further separation into aqueous fractions and organic layer. Fourier Transform Infrared spectroscopy (FTIR) was submitted to identify the component of each fraction. It demonstrated the presence of acetic acid, 1-amino-2-propanol, cresol, dimethoxyphenol, pyrogallic and anthracene. Toxicological experiment for every fraction was compared with the commercial formulations of thiamethoxam aganist the 2nd Instar nymphs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) pots trial. The result demonstrated that there was no significant differences between reduction percentages by using LPL and thiamethoxam. Consequently, biological evaluation test experiment was carried out by measuring reduction percentage of the adult stage of *B. tabaci* on potato plant spunta cv to study the effect of both Light Pyrolysis Liquid and thiamethoxam , the initial kill exceed after three days of application for LPL which reached 84.35% than after one day of application which was 73.9%. For instance, thiamethoxam initial kill reached 74% after three days of application. Subsequently, the residual effect was found to be 85.46 and 73.35 % for LPL and thiamethoxam, respectively. Our results concluded that, it can be used in integrated pest management as biopesticide as it results from plant by- product. Otherwise, there is a continues need for the development of new agrochemical products to provide growers with tools needed to address pest control problems specifically, reducing the risk on resistance development as it has a new mode of action due to presence of mixture compounds.

Introduction

Agricultural wastes have a high contents of lignin and cellulous in addition to some active ingredient (Abou Hussein and Sawan , 2010). Traditionally farmers get rid of these wastes by burning. Only few amounts go in forming composts. Buring procedure causes a serious impact on environment besides the adverse effect on public. Recently, a new trend was arising to utilize the value from these wastes. Slow pyrolysis away to achieve that. The thermal conversion

process (conventional pyrolysis) agricultural wastes are slowly heated in the absence of air. The thermal degradation of organic-based materials at slow heating rates (0.1 – 10 ^{0}C/S) and temperatures between 170 – 400 ^{0}C gives biochar and pyrolysis liquids (distillates) (Tiilikkala *et al.*, 2010). Pyrolysis liquid are found in different phases (Yanik *et al*., 2007). Pyrolysis has been used to generate many products since the times of ancient Egypt. Early products were used to caulk boats and embalm and mummify human remains (Murray *et al*., 2014). Furthermore, pyrolysis liquid can be used as biopesticides for pest management (Hossain *et al*., 2013).

Agricultural productivity remains a critical need to address due to the growing population in Egypt that is estimated to reach 120 million people by 2050. There is a demand for increasing food production. Potato (*Solanum tuberosum* L.) are grown and eaten in more countries than any other crop, and in the global economy it is the fourth most important crop after the three cereals maize, rice, and wheat and is one of the most important vegetable crops in Egypt for local consumption and exportation with an annual production of 325 million tons (Stat, 2012). In 2016, Egypt was ranked 14th in the world, with 5.0 million tons of potatoes produced (Faostat, 2016). On the contrary great annual losses have been caused by pests (Metspalu *et al.*, 2001).

The potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is key pest not only for vegetables but also for agronomic, horticultural, and ornamental crops throughout warm regions of the world (Brown, 1994) White flies, has strong flying capability more than 100 km (Byrne, 1999). In addition , it transmits many types of virues such as the begomoviruses, sweet potato leaf curl virus (SPLCV) and ipomoea leaf curl virus (ILCV), and the ipomovirus Sweet potato mild mottle virus (Valverde *et al*., 2004) .Moreover *M. persicae* also play a key role in the establishment and the dissemination of plant viruses (Boukhris-Bouhachem *et al*., 2017). These pests not only causing losses in crops through feeding on leaves, flowers, or fruit which resulting in reduction in yield but also the high densities can cause irregular ripening disorder in crops which is induced by phloem feeding and toxic saliva (Schuster *et al*., 1996). Furthermore, *B. tabaci* population has a problem of insecticide resistance which are widely diffused so that conventional chemical control doesn't give the prospect results (Castle *et al*., 2009).Thus, there is a continuing need for the development of new agrochemical products to provide growers with tools needed to address pest control problems. Globally, there is a great need to minimize the environmental risks resulting from pesticides leaching to ground water. The ability of the pyrolysis liquid to target certain agricultural pests could be an asset as a future simple and easy application. The pyrolysis liquid contains a mixture of chemical compounds which have a distinctive odor which can significantly repel pests (Booker *et al*., 2010). It is important to consider the type of biomass component in order to produce a pyrolysis liquid for potential pesticide development.

Therefore, the current work was conducted with the objective of study the possibility of convert farm wastes (tomato wastes) to useful product (pesticide).

Materials and methods

1. **Preparation of pyrolysis liquid from tomato wastes:**

Dried feedstock of tomato wastes leaves, roots and stems, approximately 100 kg were submitted to pyrolysis using RAUSSI mobile batch retort of the Egyptian-Finnish Agricultural Research Project in Sinai. Slow pyrolysis process was achieved at 350 ^{0}C. The retort was provided with cooling system. The gases and vapors were condensed. The condensables were collected as Pyrolysis Liquid that was left to stand for 24 hours.

2. **Separation and extraction of the light pyrolysis liquid:**

Analytical process was started with fractionated of Pyrolysis Liquids as two phases. Light Pyrolysis Liquid (LPL) complex chemical composition and precipitated tar at the bottom. To fractionate the LPL liquid–liquid extraction method was followed (Maggi and Delmon, 1994 and Vasalos *et al.*, 1994). The pyrolysis liquid components separation was based on polarity (figure 1) was performed in the laboratory of Plant Protection Institute at Ismailia Agriculture research station. The two fractions (LPL and Tar) were separated based on color differences. The organic solvent Dichloromethane (DCM) 100 ml was added to 100 ml LPL to separate organic compounds using separatory funnel. The obtained organic and the remaining compounds were calibrated. Both compounds subjected to partition procedures (Figure, 1).

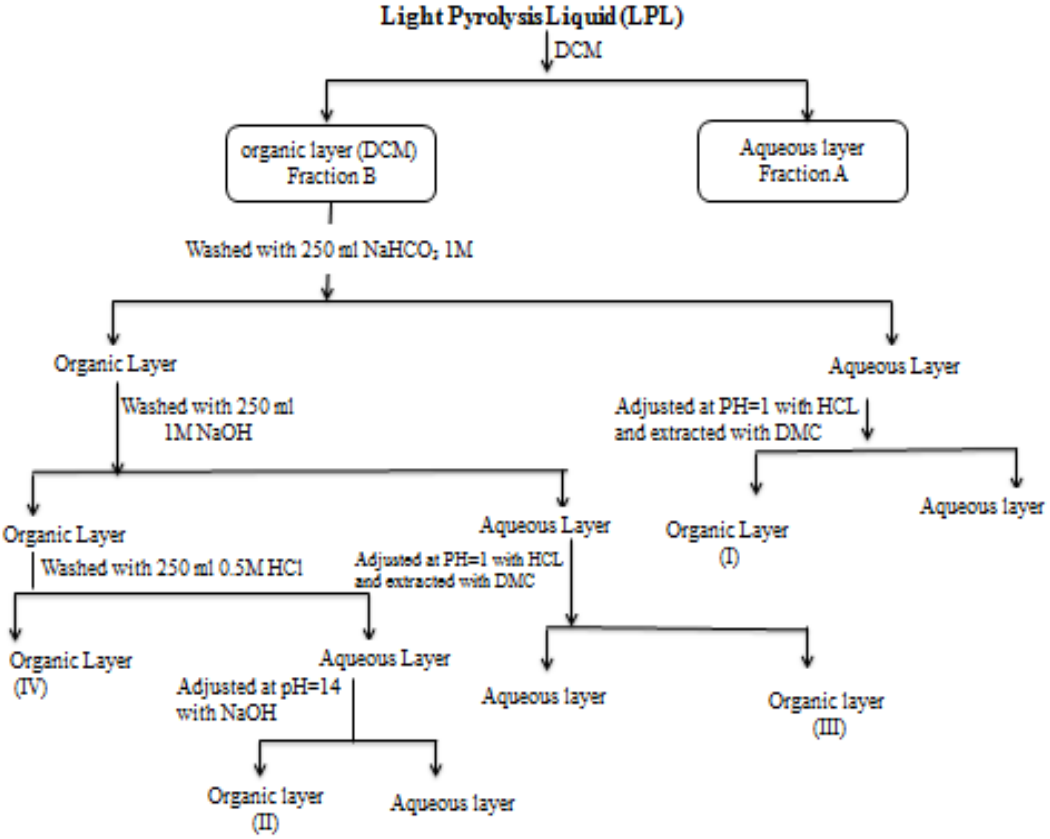


Figure (1) : Liquid-liquid fractionation scheme for Light Pyrolysis Liquid.

LPL were divided into aqueous fractions (Fraction A) and organic layer (Fraction B). Furthermore, the organic layer separated into four fractions .250 ml of $NaHCO_3$ was added to the organic layer which reacted with acids forming two layers (Organic and Aqueous layers). Separatory funnel was employed for

separation. 30 ml HCL was added to the aqueous layer to adjust the PH. Then 30 ml solvent DMC was added. The two layers were separated into organic (I) and aqueous layers. 250 ml NaOH was added to the organic layer to adjust the PH. Then 30 ml solvent DMC was added. The two layers were separated into organic and aqueous layers. 30 ml HCL was added to the aqueous layer to adjust the PH. Then 30 ml solvent DMC was added. The two layers were separated into Organic (III) and Aqueous layers. 250 ml HCL was added to the Organic layer to adjust the PH. The two layers were separated into organic (IV) and aqueous layers. 250 ml NaOH was added to the aqueous layer to adjust the PH. The two layers were separated into organic (II) and aqueous layers. Four glass columns chromatography (51 x 5.1 cm) were packed with silica gel to separate each compound from the fraction. Sample from every fraction was injected into the column using different mobile phase. The aqueous solution was dried with sodium disulphate (Na_2S0_4). Each solutions was transferred in a glass bottles to the laboratory of Plant Protection Institute at Ismailia Agriculture research station. Each fraction applied to bioassay to identify the active fraction against pests.

3. Analysis and characterization of the Light Pyrolysis Liquid:

Analysis and characterization of the final obtained compounds. The principal components of each was recorded by aid of IR spectroscopy. FT- IR spectroscopy of each sample relied on a Bio-Rad FTIS - 40 model, USA.

4. Toxicological experiments :

Potato plants *S. tuberosum* were grown in 15 cm diameter pots filled with a mixture of 1:1 sand and peat moss.

4.1. Maintenance of the strain:

Whitefly *B. tabaci* was collected from potato fields using a mouth aspirator and released on the grown potato plants pots. The strain was reared in muslin cages (0.60 x 0.60 x 1.00 m) .The cage contained four pots each planted with 2 potato plants. The cage was kept in control. Whitefly *B. tabaci* was reared for 2 months away from any contamination at 25 ± 2 °C , 50 ± 5 % RH and a 16-h photoperiod at the laboratory of plant protection institute at Ismailia Agricultural Research Station, Egypt (El-Zahi *et al*., 2017)

4.2. Tested compounds:-

Experiment was conducted to evaluate the efficacy of obtained compounds against the second instar nymphs *B. tabaci* on potato plant.

The following compounds were used throughout this study:

-Pyrolysis liquid fraction A .

-Pyrolysis Liquid fraction B (I ,II , III and VI).

-Light Pyrolysis Liquid (LPL).

-Commercial formulation of thiamethoxam (Thiamex 25% WG , MAC-GmbH, Germany) was tested in at their recommended dose.

The tested compound were sprayed by the aid of knapsack sprayer

4.3. Pots trial:

Whitefly *B. tabaci* was collected from potato strain. The adults could oviposit and put their eggs for 3h. To obtain *B. tabaci* immatures of uniform ages and the adult were removed. The infested leaves were labeled thereafter, *B. tabaci* eggs could develop for 9–10 days to second instar nymphs. Four series concentrations 10, 20, 40 and 80 % were used to determine the toxicity of the tested compounds on the second instar nymph. Mortalities were recorded on the second instar nymphs after 24 hours of application The mortality percentage

were corrected (Abbott, 1925). The criteria of mortality is flatted nymphs and easy to remove from the leaf surface with a fine brush (Horowitz *et al*., 1998) .

4.4.Cultivation of potato plants:

Invitro propagation of potato was performed on MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose (MS-3) and 0.8% agar to obtain virus-free material for infection assays. Explants were grown at 22C under a 16/8 h light/dark photo period.Virus-free potato minitubers cv. spunta were used after being harvested and kept at 4° C for periods of 1–6 months before cultivation under screen house to be protected it from insect virus carrier. While growing, the plants need to be protected from insect pests to avoid new disease infections. The soil was sandy loam type. All normal agricultural practices; i.e., irrigation, fertilization, and weed control for growing potato plants were performed. In this experiment, three screen houses with a dimension 30, 9 and 3 meter for length, width and height, respectively were utilized at the Agriculture Research Station, Ismailia. It was ploughed and prepared for planting. Each screen house area was divided into 4 rows with 30 meters long, separated by a gap of 0.5 meters. Spunta cv. was transplanted at distance of 10 cm in rows on 1st February on the two seasons of 2018 and 2019.

4.5.Screen house trail:

To evaluate the efficacy of LPL against the white fly *B. tabaci* (adult) (Mattos *et al*., 2019). The LPL was diluted with water with ratio of 1: 10 , respectively .Plastic containers (50ml) that have a semi open cover were full with the solution. The container were hanged , at a height around 1 - 1.5 meters with distance 3 meter far from each other. The reduction percentages were calculated. The numbers of the adults were recorded after 0*, 1 ,3, 7 and 10 days of application. Another screen house was served as control **(Henderson and Tilton, 1955).**

4.6. Statistical analysis:

Data obtained in both laboratory and screen house experiments were subjected to computerized statistical analysis. Duncan's multiple range tests was used to determine the significant differences between the mean values of the tested material using CoStat system for Windows, Version 6.311.

Results and discussion

1. **Preparation and separation of pyrolysis liquid from tomato wastes:**

The slow pyrolysis process on tomato straw reached 350 °C in 2 Hours and 45 minutes. The outputs of the cycle were 35.5 Kg biochar and 42 L pyrolysis liquid. With further separation of liquid, we found that about 10 L were precipitated tar and 32 L were Light Pyrolysis Liquid (LPL).The quantitative analysis obtained from liquid–liquid extraction which were used to fractionate the LPL (Figure, 2) was found to be about 20.4 % for aqueous fractions (Fraction A) and 74.4 % for the organic layer (Fraction B). Furthermore, 49.8, 0.9, 14.7 and 9 % for the acidic fraction (I), basic fraction (II), phenolic fraction (III) and hydrocarbon fraction (IV), respectively .

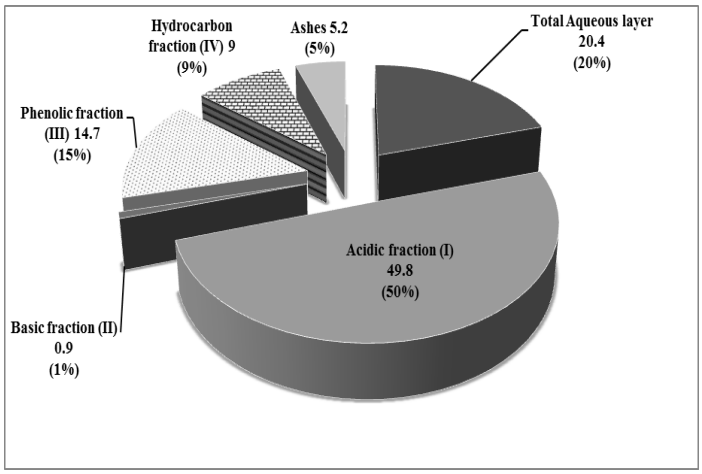
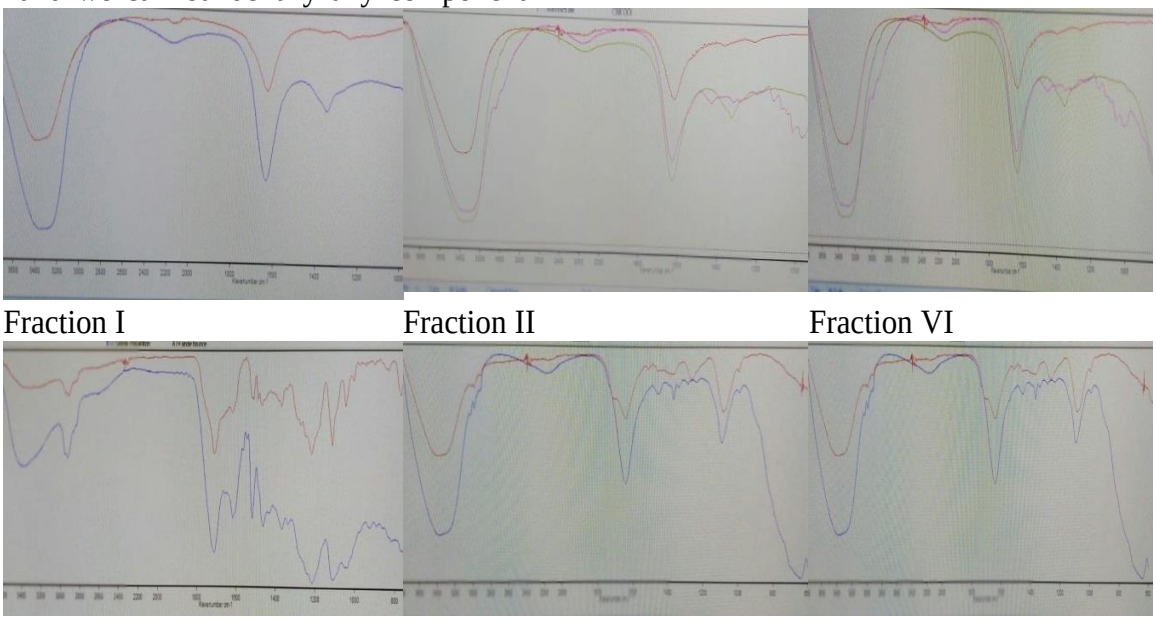


Figure (2): Liquid-liquid fractionation percentage for Light Pyrolysis Liquid.

2. Analysis and characterization of the Light Pyrolysis Liquid:

Fourier Transform Infrared spectroscopy (FTIR) was submitted to identify the component of each fraction automatically only the major compounds. As the result show in Figure (3) on one hand we cannot identify any component from the aqueous layer (fraction A) in which the chart showed to be mysterious, but on the other hand the organic layer (fraction B) we can clearly identify the acidic fraction (I) , basic fraction (II), phenolic fraction (III) and the hydrocarbon fraction (VI).



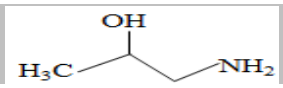
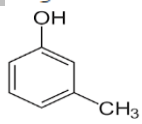
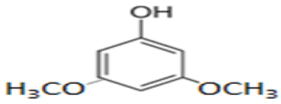
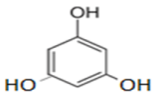
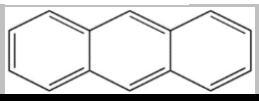
Fraction I Fraction II Fraction VI

Fraction III

Figure (3): The chart of major components in fraction B by FT- IR spectroscopy.

From the previous chart obtained from FT-IR spectroscopy shown in Figure (3) the peaks automatically identify the presence of acetic acid that found in the acidic fraction (I) besides the presence of 1-amino-2-propanol in basic fraction (II). Likewise, Cresol, Dimethoxyphenol and Pyrogallic that found in phenolic fraction (III). Finally the presence of Anthracene in the hydrocarbon fraction (VI).The six major chemical formula of each compound are shown in Table (1).

Table (1): Characterization of the major components in fraction by FT- IR spectroscopy.

Fractions		Chemical Formula	Chemical Name
Fraction A	**Aqueous Fractions**		**----**
Fraction B	**Acidic Fraction I**	CH_3-COOH	**Acetic acid**
	Basic Fraction II		**1-amino-2-propanol**
	Phenolic Fraction III		**Cresol**
			**Dimethoxyphenol**
			**Pyrogallic**
	Hydrocarbon fraction VI		**Anthracene**

3 .Toxicological experiments :

The efficacy of the used materials in terms of mortality against the 2nd instar nymph *B. tabaci* on potato plant after 24 hours of application pots trial is shown in Table (2). Data revealed that all used material reduced the mean numbers of the 2nd instar nymph compared with that on control. The increment in concentration and the used materials fluctuate from the mean number of the 2nd instar larva. We observed that the mortality percentage has a limit increase by increasing the concentration for every fraction. On the contrary, using the fractions and the pesticide had resulted in the mortality percentage. Data indicated that the highest percentage of reduction was by using Thiamex 25% WG followed by LPL, while there was no significant difference between them. The usage of fraction A showed 30% mortality, however Fraction B (I, II, III and IV) improved the mortality percentage, which were found to be 67.50, 51.25, 56.25 and 32.50 ,respectively.

Table (2) : Mortality percentage of the 2nd instar nymphs *Bemisia tabaci* pot trial .

Materials		Mortality % 2nd instar larva of whitefly *Bemisia* ***tabaci*** with different concentrations±SD			
Concentrations		**10%**	**20%***	**40%***	**80%***
Fraction A		**30.00±0.82**	**35.00±0.82**	**38.75±0.5**	**47.50±1.3**
Fraction B	**I**	**67.50±1.3**	**63.75±1.7**	**62.50±1.7**	**60.00±1.4**
	II	**51.25±0.5**	**56.25±0.9**	**58.75±1.5**	**62.50±1.5**
	III	**56.25±0.9**	**60.00±0.8**	**61.25±0.9**	**63.75±0.9**
	IV	**32.50±1**	**31.25±0.5**	**38.75±0.5**	**42.50±1**
LPL		**87.25±0.9**	**92.50±0.6**	**93.75±0.5**	**93.75±0.5**
Thiamex 25% WG					**90±1.5**
Control					**2.5±0.5**
LSD at 0.05 probability level					**0.5**
***Concentration show phytotoxicity on potato plant.**					

Screen house Trail

The experiment was conducted to evaluate the efficacy of 10% LPL and Thiamethoxam against whitefly, *B. tabaci* on potato during 2018 and 2019. Data in Table (3) summarized the assessment of reduction percentage with time (Day). It shows that after ten days of application, the lowest population (1.9/leaf) with reduction percentage of about 85.97 % was observed by using the LPL. On the contrary with the using of Thiamethoxam, the population reached 3.75/leaf with reduction percentage of about 71.69 %. Data also showed that the initial kill increase after three days of application of LPL which reached 84.35% than after one day of application which was 73.9%. For instance, Thiamethoxam initial kill reached 74% after three days of application. Subsequently, the residual effect was found to be 85.46 and 73.35 % for LPL and Thiamethoxam, respectively.

Table (3): Assessment of reduction percentage on the adult stage of whitefly *Bemisia tabaci* by time.

Material	**Mean population of the adult stage whitefly *Bemisia tabaci* / potato leaf (Assessment of Reduction % by Time(Day) after application)**					**Reduction Percentage**	
Time	0*	1	3	7	10	Initial kill	Residual Effect
LPL	11.50	03.00 (73.9)	02.00 (84.35)	02.00 (84.95)	01.9 (85.97)	84.35	85.46
Thiamethoxam	11.25	03.00 (73.33)	03.25 (74.00)	03.25 (75.00)	03.75 (71.69)	73.33	73.35
Control	11.25	11.25	12.50	13.00	13.25	--	--
LSD at 0.05 probability level							**2.24**

In fact , the Pyrolysis Liquid obtains from agricultural wastes has a unique chemical and physical properties. It can be used in IPM as biopesticide as it results from plant wastes . Otherwise, there is a continus need for the development of new agrochemical products to provide growers with tools needed to address pest control problems Specifically, reducing the risk on resistance development as it has a new mode of action due to presence of mixture compounds. For instance, the pyrolysis liquid has a broader period of application so more life stages can control. In the meantime, there is a demand for increasing food production

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Effectiveness of Different Fungicides Formulations and Certain Ground Spraying Equipment in Controlling Wheat Stripe Rust in Egypt

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Wheat stripe rust, chemical control, low volume ground spraying techniques and equipment.

Abstract:

Wheat (*Triticum aestivum* L.) is of the most important cereal crop for many world's populations. It is the most important staple food of about two billion people. However, the production and productivity of wheat is affected by various biotic and abiotic stresses. Among the biotic stresses, stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is one of the most widespread and damaging diseases of wheat. Field experiments were carried out in an area of about three kirats planted with the susceptible variety (Giza160), during seasons 2017/2018 and 2018/2019 in 15th and 30th March in wheat field located at Sakha, Kafrelshiekh Governorate. The selected area was split into 30 plots including 3 control plot. Propiconazole, azoxystrobin + cyproconazole and cyproconazole + propiconazole were sprayed with the rate of recommended dose rate and three treatments sprayed with water as control by using Hydrulic Matabi evolution sprayer (56 L./fed.), Knapsack motor sprayer (Arimitsu) (43 L./Fed.) and Rotary Matabi sprayer (18 L./fed.). Data indicated that, all tested compounds revealed significant influenced on wheat stripe rust pathogen (Pst). Data of two successive sprays gave the same results since propiconazole occupied the first rank in reducing rust severity. The best fungicide in this respect were propiconazole followed by cyproconazole + propiconazole, however azoxystrobint + cyproconazole was the least in this regard. It could be recommended to use these compounds with LV spraying equipment with not less than (18L/Fed.). Also, the best equipment in this respect were Rotary (Matabi) followed by Hydraulic (Matabi) and Knapsack motor sprayer (Arimitsu) the tested equipment under study. Hence, the yield production was high in the best fungicide and equipment. The rate of performance of Arimitsu sprayer was 15.25 Fed./day, but the lowest rate of performance was Rotary Matabi sprayer since it could spray only 2.3 Fed./day.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop for the majority of world's populations. It is the most important staple food of about two billion people (36% of the world population). Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). However, the production and productivity of wheat is affected by various biotic and abiotic stresses. Among the biotic stresses, stripe (yellow) rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most widespread and damaging diseases of wheat, causing great losses in yield and grain quality (Line, 2002 and Chen, 2005). Grain losses caused by this devastating pathogen have been reported to be 10-70 % besides affecting the quality of grain and forage (Chen, 2005). The frequency of epidemics and damage caused by stripe rust is different in each country. In Egypt, stripe rust is the most common and important wheat disease. It caused severe losses in grain yield (Abu El-Naga *et al*., 2001). A lot of methods were available to control wheat rusts. Growing resistant cultivars and applying synthetic fungicides were commonly used as the two main strategies to successfully control yellow rust in many countries, worldwide. Yield and quality losses were related to reductions in green leaf area resulting from pustule formation on infected leaves. Different management options, such as use of resistant varieties, However, the disease became one of the worst diseases, affecting almost every released and registered variety in the country. Under such condition, when the inoculum level was very high, the use of fungicide is mandatory to obtain optimum yield.

Variety resistance has been ultimately the best option for managing stripe rust in the long term. However, in the short to medium term growers planting moderately susceptible varieties were reliant on the use of fungicides either at sowing (in-furrow on fertilizer or seed treatments) or in-crop (application of foliar fungicides), or a combination of both options. The development of new pathotypes of the stripe rust fungus, which reduced the resistance of selected commercial varieties, could made make fungicide intervention necessary in other situations. Chemical control of cereal rusts was extensively studied by many investigators using different applications in many locations foliar spray fungicides against wheat rusts have been known for many years and most of them were used as protects. Majority of interest was directed to the type, dosage of fungicides used in Egypt, while a lesser attention was given to the application methods. Hindy (1992) recorded significant variation in the spray deposit due to spray technique, arrangement of the nozzles and rate of application. The world attention was directed to minimization of spraying volumes and costs of control pests which might been achieved by using a cheap and effective fungicide or using recent ground spraying technique with low cost of application per feddan and more homogenous spray coverage (Magdoline *et al*., 1992 and Matthews, 1992). Concluded that the optimum droplet size for spraying insecticide and fungicide application should be ranged between 50-150 μm, which gave best control results of the target disease with minimum fungicide and minimum ecosystem contamination. According to Bouse *et al*., (1986), Gohich (1983),

Reichard *et al*. (1977) and Yates and Cowden (1985), the droplet size was a combined function of spraying technique chemical formulation and ambient conditions. Thus, this study was carried out to investigate the effect of fungicides and equipment controlling wheat stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* on wheat plants under field conditions in Egypt.

Materials and methods

1. Fungicides:

Trade and common name of fungicides and dosage applied :

- Propiconazole (Telet®), 25% E.C., with concentration 25 cm^3/ 100L water.
- Azoxystrobin + Cyproconazole (Amestar extra®), 28% S.C., rate dose 300 cm^3/ fed.
- Cyproconazole + Propiconazole (Minara®), 41% E.C., rate dose 200 cm^3/fed.

2. Spraying equipment tested on wheat plants:

Three ground application machines were selected to perform the scope of this work as follows:

- Hydrulic Matabi sprayer (56L/fed.).
- Knapsack motor sprayer (Arimitsu) (43 L/Fed.).
- Rotary Matabi sprayer (18 L/fed.).

The tested equipment could be represented according to the technical categorization mentioned in Table (1). Calculations of productivity caliberation and rate of performance were recorded as described by Hindy (1992).

3.Execution of field experiments:

3.1.Description of the Study Area:

The experiment was carried out at Sakha Agriculture Research Center during 2018 and 2019 growing seasons. Wheat planted at 15 th November and two successive sprays takes place on 15th and 30th Mars. The studied area was located in North Delta region, in wheat field located at Sakha Research Station, Kafrelshiekh Governorate. (31° 08 North and 30° 56 East). Climatic condition of the studied area was typically arid Mediterranean climate. the experiment was done under local meteorological conditions of 23°C average temperature, 55% average RH and 2 m/sec. average of wind velocity. The area of 3 kirats was divided into 30 equal plots each plot was, 300 cm^2 between treatments were not cultivated as barrier zones to avoid drift spray between treatments, spraying operations have not been done with any fungicides before execution the field experiment. The experimental field was sprayed with recommended dose of Propiconazole, Azoxystrobin + Cyproconazole and Cyproconazole + Propiconazole by using Hydraulic Matabi evolution sprayer (56 L./fed.) Knapsack motor sprayer (Arimitsu) (43 L./Fed.) and Rotary Matabi sprayer (18 L./fed.) , respectively and three treatments sprayed with water as a control treatment.

3.2.Treatments and experimental design:

Two common wheat cultivars, namely Giza 160 , which had been susceptible to stripe rust, was used for the experiment. A factorial randomized complete block design (RCBD) used with three replications. The selected area of three kirats was split into 30 equal plots including 3 control plots. Fertilizer rate and crop husbandry practices, such as cultivation and weeding were carried out according to the recommended practices. In order to evaluate the tested compounds and equipment on them, before spraying, and post-treatment recorded after days from treatment to determine the effect of the tested chemicals. the 2nd spray takes place after 15days from the 1st spray.

Evaluation of three certain systemic fungicides; propiconazole, azoxystrobint

+ cyproconazole, and cyproconazole + propiconazole and three equipment; Hydraulic Matabi sprayer (56 L./fed.) Knapsack motor sprayer (Arimitsu) (43 L./Fed.) and Rotary Matabi sprayer (18 L./fed.). as well as control un-treatment (water). The experimental unit was a plot included 18 rows with 9 m long and 30 cm. apart, each row received 30g. of seeds using broadcasting method of sowing. The experiment was surrounded with a spreader of highly susceptible varieties; irrigation, fertilization, weed control *etc*. were applied according to the technical recommendation of the crop as normal. Artificial inoculation was performed using the methods of Tervet and Cassel (1951) as mentioned before. The inoculation was concentrated on the spreader plants, on the other hand each fungicides was applied soon after inoculation and repeated 7 days later. The application was carried out at the proper time and correct doses were applied.

4. Calibration and performance adjustment of the tested equipment:

4.1.Collection of spray deposit:

Before spraying each wheat treatment, a sampling line constructed of five wire holder fixed in diagonal line inside each treatment to collect the lost spray between plants; each wire holder top had a fixed water sensitive paper (Novartis Cards®) on it, also, the water sensitive paper cards put on five plants ; to collect the droplets deposit on wheat leaves at both upper and lower levels of plant, were designed according to Hindy (1989). Cards were collected and transferred carefully inside paper. Involved data to the laboratory for measuring and calculating the number of droplets/cm² and its volume mean diameter (VMD) µm in all treatments was done.

4.2. Determination of spray deposit:

Number and size of blue spots (deposited droplets) on the water sensitive papers (Novartis cards®) measured with scaled monocular lens (Strüben) ® (15X) Japanes lens. Volume mean diameter (VMD) µm and number of droplets in one square centimeter (N/cm²) were estimated according to Hindy (1992).

Table (1): Techno-Operational data, calibration and rate of performance of certain ground sprayers applied on Wheat field during season (2017/2018).

Items	Hydraulic (Matabi) evolution sprayer	Rotary (Matabi) sprayer	Knapsack motor sprayer (Arimitsu)
Type of atomization	Hydraulic	Spinning disc	Pneumatic Mechanical
Nozzle type	Hollow cone 800	One restirector	Air shear nozzle
Pump type	Hydraulicair pump	-	Centrifugal fan
Number of nozzles	1	1	1
Pressure (bar)	5	-	-
Spray tank (L.)	20	2	20
Rate of application (L/fed.)	56	18	34
Working speed (Km/h.)	2.4	2.4	2.4
Swath width (m.)	1.5	1.0	5.0
Flow rate (L/min.)	0.8	0.172	1.630
Spray height (m.)	0.5	0.5	0.5
Type of Spraying	Target in all treatments		
Productivity * (fed./h.)	0.86	0.570	2.860
Rate of performance*(fed./day)	3.4	2.3	15.25

* Number of spraying hours = 8 hours daily. *Number of workers =2.

* Calculations of productivity and rate of performance after Hindy (1992).

4.3. Calculation and data analysis:

4.3.1. Rust severity was recorded soon after disease onset and 3 times thereafter with 10 days intervals following the methods adopted by **Petroson *et al*. (1948)**. Evaluation of efficacy of each fungicide were computed according to the following formula adopted by **Rewal and Jhooty (1985).**

$$\% \text{ Efficacy} = \frac{\% \text{ infection in the control} - \% \text{ inf. in treatment}}{\% \text{ Infection in the control}}$$

Yield components expressed as 1000 kernels weight and test weight were estimated at harvest stage.

4.3.2. Statistical analysis of each experiment was performed each season individually using Dunkan's New Multiple Range Test according to **SAS (1996)** .

4.4.Phytotoxic effect:

It was determined by recording any color change, leaf curling or flaming up to 15 days after each spraying, according to **Badr *et al*. (1995).**

Results and discussion

1.Chemical control of stripe rust of wheat:

Data presented in Tables (2) and(3) and Figures (1) and (2) revealed that the evaluation of three certain systemic fungicides and three certain equipment as affected with wheat stripe rust infection in terms of disease severity, on cv. Giza 160 during 2017/18 and 2018/2019 growing seasons. These data indicated that a significant difference was observed between one and two spray application of Propiconazole, however, the rest of fungicides showed significance in this regard, in particular using Rotary Matabi . Under the stress of one spray application a significance was observed between each of three certain systemic fungicides and control treatment. The efficacy of the tested fungicides under the stress of one spray application ranged between (28.57% and 71.43%) and between (75.00% and 93.75%) in case of two spray application.

Data of two successive sprays gave the same result since Propiconazole occupied the first rank in reducing rust severity. The best fungicide in this respect were propiconazole followed by cyproconazole + propiconazole, however azoxystrobint + cyproconazole was the least in this regard. In the second season 2018/2019, data in Table (5) run in a parallel line with those previously mentioned in the first season, since the fungicide Propiconazole was in the first rank (one and two successive sprays). Also, the best equipment in this respect were Rotary (Matabi) followed by Hydraulic (Matabi) the tested equipment under study.

Severe stripe rust epidemic was recorded in the tested wheat cultivar during the second growing season; 2018/2019, than that in the first season; 2017/2018 (Table, 4). The obtained data of disease severity, and infection type (IT), were combined to calculate average coefficient of infection (ACI) was assessed in the cultivar during an epidemic in the two growing seasons. Where, the final stripe rust severity for the tested wheat cultivar varied from one year to another, as affected by the slightly changes in environmental conditions between the two years under study. In addition to, changes occurring in these Pst races population (Figures, 1 and 2).

Table (2): Evaluation of three equipment with three fungicides against stripe rust infection on wheat cultivar; Giza160 in terms of ACI, during 2017/2018.

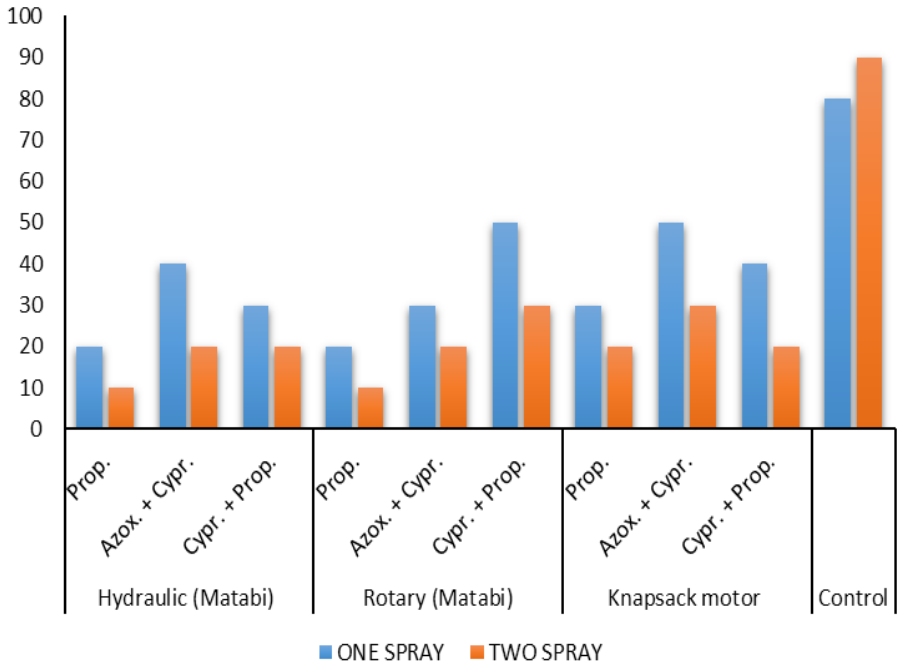
Equipment	Fungicides	A	B	Diff.	Efficacy %	
					A	B
Hydraulic (Matabi)	Propiconazole	20[d]	5d	15	71.43	93.75
	Azoxystrobint + Cyproconazole	30[c]	10c	20	57.14	87.50
	Cyproconazole + Propiconazole	20[d]	10c	10	71.43	87.50
Rotary (Matabi)	Propiconazole	10[e]	5d	5	85.71	93.75
	Azoxystrobint + Cyproconazole	20[d]	5d	15	71.43	93.75
	Cyproconazole + Propiconazole	20[d]	5d	15	71.43	93.75
Arimitsu	Propiconazole	20[d]	5d	15	71.43	93.75
	Azoxystrobint + Cyproconazole	50[b]	20b	30	28.57	75.00
	Cyproconazole + Propiconazole	30[c]	10c	20	57.14	87.5
Control	Water	70[a]	80a	10	0	0
L.S.D. (1%)	-	0.112	0.884	-	-	-
(5%)	-	0.086	0.649	-	-	-

A = first spray ,B = second spray. Numbers followed by the same letter at the same column are not significantly different.

Table (3): Evaluation of three equipment with three fungicides against stripe rust disease severity on wheat cultivar; Giza160 in terms of ACI conducted at Sakha during 2018/2019.

Equipment	Fungicides	A	B	Diff.	Efficacy %	
					A	B
Hydraulic (Matabi)	Propiconazole	20[e]	10[d]	10	75	88.89
	Azoxystrobint + cyproconazole	40[c]	20[c]	20	50	77.78
	Cyproconazole + Propiconazole	30[d]	20[c]	10	62.5	77.78
Rotary (Matabi)	Propiconazole	20[e]	10[d]	10	75	88.89
	Azoxystrobint + cyproconazole	30[d]	20[c]	10	62.5	77.78
	Cyproconazole + Propiconazole	50[b]	30[b]	20	37.5	66.67
Arimitsu	Propiconazole	30[d]	20[c]	10	62.5	77.78
	Azoxystrobint + cyproconazole	50[b]	30[b]	20	37.5	66.67
	Cyproconazole + Propiconazole	40[c]	20[c]	20	50	77.78
Control	Water	80[a]	90[a]	10	0	0
L.S.D. (1%)	-	0.128	0.132	-	-	-
(5%)	-	0.091	0.094	-	-	-

A = first spray , B = second spray. Numbers followed by the same letter at the same column are not significantly different.



Figure(1): Effect of wheat stripe rust severity on wheat cultivar; Giza160 after treatments with different fungicides using three equipment, during 2017/18.

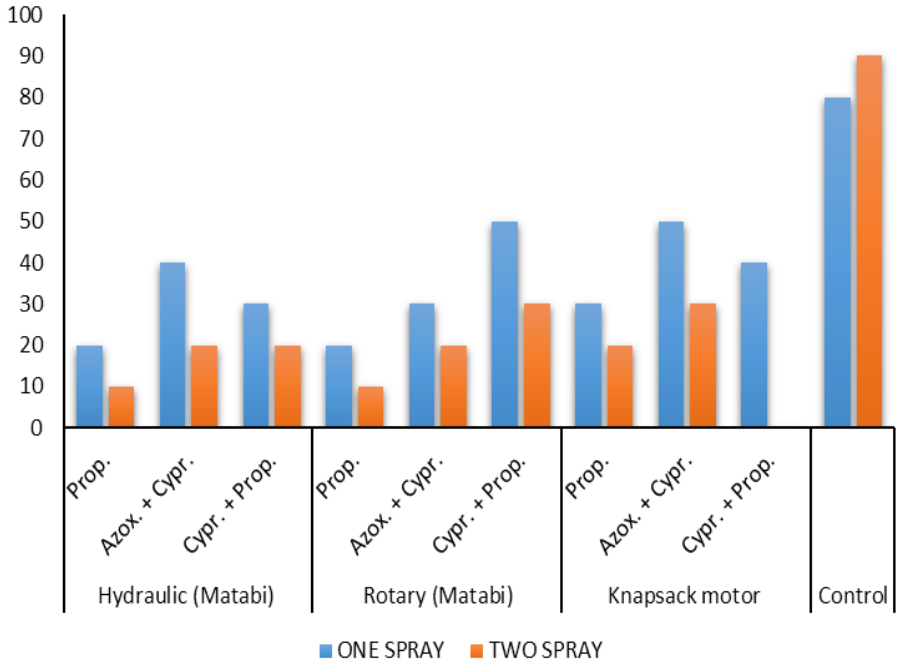


Figure (2): Effect of wheat stripe rust severity on wheat cultivar; Giza160 after treatments with different fungicides using three equipment, during 2018/2019.

As regard to the effect of the application on 1000 k.w. and test weight the presented data indicated the presence of significance between one or two sprays. In Tables (4) and (5) and Figures, (3) and (4) revealed that one spray resulted in the presence of significance between each of the tested three certain

systemic fungicides under study. Data analysis of the study revealed that, the best fungicide in this respect were propiconazole followed by cyproconazole + propiconazole, however azoxystrobint + cyproconazole was the least in this regard. The increase over the control treatment was achieved with the application of propiconazole (one spray and two sprays). The increase over control in case of one spray ranged between (18.12%, 23.85% and 32.43%), and between (29.24%, 35.10% and 36.44%) with Knapsack motor sprayer, Hydraulic (Matabi) and Rotary (Matabi), respectively, in case of two sprays application.

Table (4): Evaluation of three fungicides and three equipment against stripe rust infection on wheat cultivar; Giza160 in terms of 1000 k.w. and test weight conducted at Sakha during 2017/2018.

Equipment	Fung.	1000 kernel weight (g)					Test weight -1000 ml (g.)				
		A	B	Diff.	Increase over control		A	B	Diff.	Increase over control	
					A	B				A	B
Hydraulic (Matabi)	F1	45.7^{d}	61.6^{b}	8.10	23.85	35.10	668^{d}	789^{b}	121	16.17	30.93
	F2	44.4^{g}	55.2^{i}	10.8	21.62	27.57	645^{f}	735^{f}	90	13.18	25.85
	F3	47.7^{b}	55.8^{g}	15.9	27.04	28.35	650^{e}	738^{f}	88	13.85	26.15
Rotary (Matabi)	F1	51.5^{a}	62.9^{a}	12.8	32.43	36.44	710^{b}	779^{c}	69	21.13	30.04
	F2	45.1^{e}	58.2^{d}	11.4	22.84	31.31	678^{c}	758^{e}	80	17.40	28.10
	F3	47.1^{c}	59.9^{c}	13.1	26.11	33.26	675^{c}	772^{d}	97	17.04	29.40
Arimitsu	F1	42.5^{i}	56.5^{e}	14	18.12	29.24	785^{a}	799^{a}	14	28.66	31.79
	F2	44.8^{f}	55.9^{f}	11.1	22.32	28.48	602^{g}	780^{c}	178	6.977	30.13
	F3	44.0^{h}	55.4^{h}	11.4	20.91	27.83	785^{a}	798^{a}	13	28.66	31.70
Control		34.8^{j}	39.9^{j}	5.18	0.00	0.00	560^{h}	545^{g}	15	0	0
L.S.D. (1%)		0.096	0.079				4.262	6.761			
	(5%)	0.077	0.054				3.116	4.937			

A = First spray , B = Second spray. Numbers followed by the same letter at the same column are not significantly different.

As for, the effect of fungicides on the test weight, data in the same Tables (4) and (5) and Figures (3) and (4) run in a parallel line with those previously mentioned, since the fungicide propiconazole was in the first rank (one and two sprays). On the other hand, increase over control in case of one spray ranged between (6.98% and 28.66%) and between (25.85% and 31.79%) with the application of Cyproconazole + Propiconazole, Propiconazole, respectively in the first season 2018/2019.

Table (5): Evaluation of three fungicides and three equipment against stripe rust infection on wheat cultivar; Giza 160 in terms of 1000 k.w. and test weight conducted at Sakha during 2018/2019.

Equipment	Fung.	1000 kernel weight (g)					Test weight -1000 ml (g.)				
		A	B	Diff.	Increase over control		A	B	Diff.	Increase over control	
					A	B				A	B
Hydraulic (Matabi)	F1	49.99^{e}	51.41^{e}	1.42	16.96	22.19	599.00^{e}	600.66^{i}	16.0	8.12	12.49
	F2	50.37^{d}	52.93^{b}	2.56	17.59	24.43	625.00^{b}	602.60^{g}	22.4	11.90	12.77
	F3	50.52^{c}	52.36^{d}	1.84	17.83	23.61	595.00^{g}	605.00^{e}	7.51	10.60	13.11
Rotary (Matabi)	F1	47.83^{h}	50.48^{f}	2.65	13.21	20.76	592.33^{i}	611.66^{d}	19.3	7.09	14.06
	F2	48.92^{g}	50.05^{g}	1.13	15.15	20.08	594.66^{h}	601.00^{h}	6.34	7.45	12.54
	F3	50.65^{b}	52.64^{c}	1.99	18.05	24.01	609.00^{e}	619.66^{b}	10.7	9.63	15.17
Arimitsu	F1	47.44^{i}	49.79^{h}	2.35	12.50	19.66	597.33^{f}	614.66^{c}	17.3	7.86	14.48
	F2	49.23^{f}	50.49^{f}	1.26	15.68	20.78	602.33^{d}	603.66^{f}	25.7	8.63	12.92
	F3	51.84^{a}	53.52^{a}	1.68	19.93	25.26	628.00^{a}	623.66^{a}	20.0	12.30	15.71
Control		41.51^{j}	40.00^{i}	1.51	0.00	0.00	550.33^{j}	525.66^{j}	24.7	0.00	0.00
L.S.D. (1%)		0.022	0.072				0.090	0.134			
	(5%)	0.012	0.058				0.063	0.098			

A = First spray, B = Second spray. Numbers followed by the same letter at the same column are not significantly different.

As regard to the fungicides evaluation during 2018/2019, data presented in Table (5) revealed that, the application of either one or two successive sprays reduced rust severity comparing with the untreatment control. Concerning the effect of the tested fungicides on 1000 k.w. and test weight run in a parallel line with those previously mentioned in the first season 2017/2018.

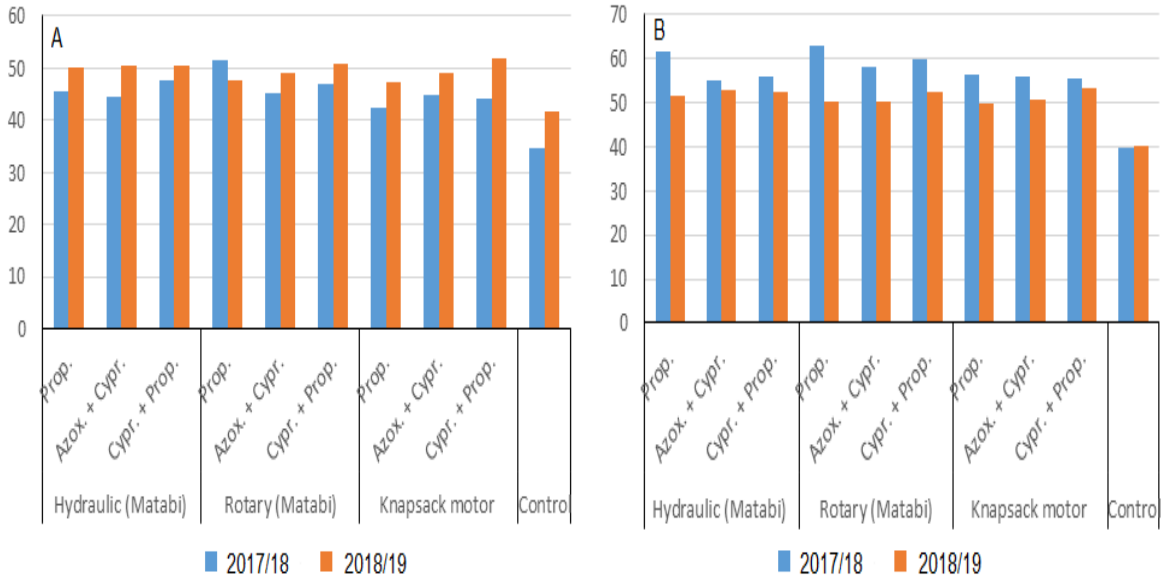


Figure (3): Evaluation of three fungicides using three equipment against stripe rust infection on the Giza160 in terms of 1000 k.w. conducted at Sakha during seasons 2017/2018 and 2018/2019, A = First spray and B = Second spray..

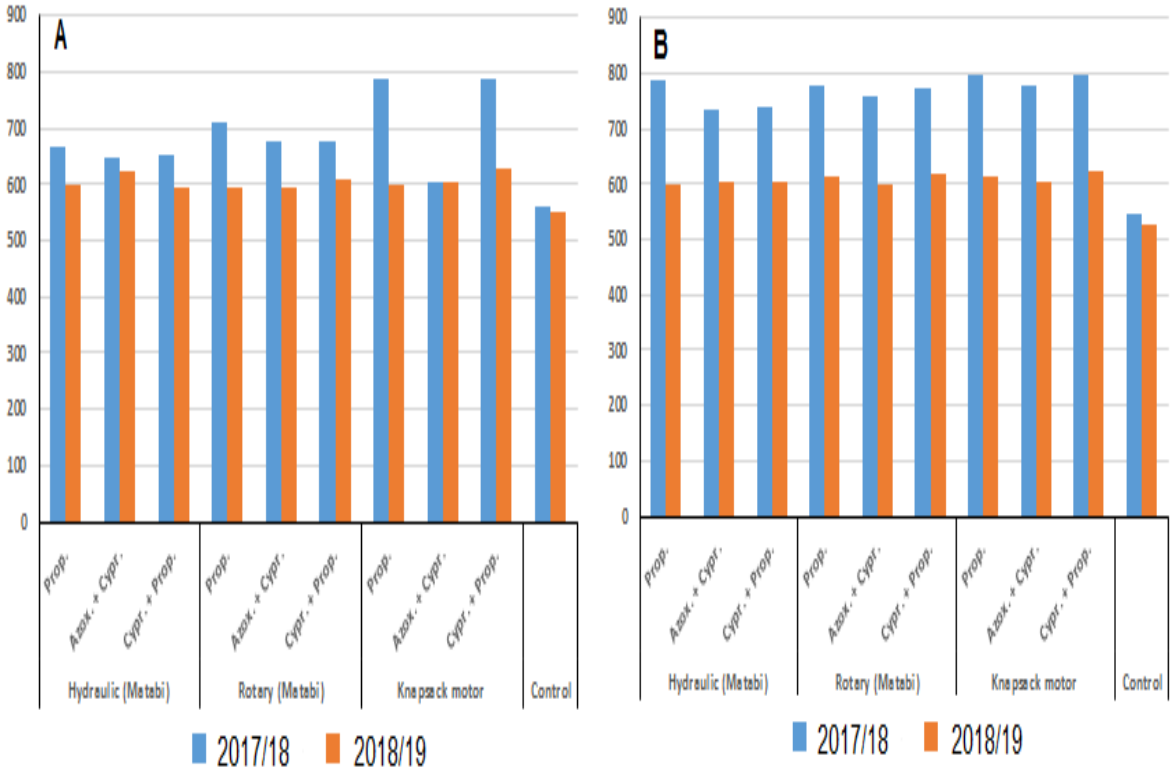


Figure (4): Evaluation of three fungicides using three equipment against stripe rust infection on the Giza160 in terms of test weight conducted at Sakha during seasons 2017/18 and 2018/19, A = first spray and B = second spray.

2. Spray coverage on wheat leaves of fungicide used:

Data in Table (6) showed that ,in the case of propiconazole using Hydrulic Matabi evolution sprayer (56L/Fed.), Knapsack motor sprayer (Arimitsu) (43 L/Fed.) and Rotary Matabi sprayer (18 L/Fed.), respectively the droplet sizes were 152, 143 and 130 µm and N/cm^2 were 157, 183 and 121 for the same equipment. In the case of azoxystrobint + cyproconazole using Hydraulic Matabi sprayer (56L/Fed.), Knapsack motor sprayer (Arimitsu) (43 L/Fed.) and Rotary Matabi sprayer (18 L/Fed.), respectively the droplet sizes were 153, 132 and 147 µm and N/cm^2 were 158, 184 and 119 for the same equipment. In the case of cyproconazole + propiconazole using Hydraulic Matabi sprayer (56L/Fed.), Knapsack motor sprayer (Arimitsu) (43 L/Fed.) and Rotary Matabi sprayer (18 L/Fed.), respectively the droplet sizes were 164, 153 and 134 µm and N/cm^2 were 136, 180 and 117 for the same equipment.

Table (6): Spraying coverage on wheat plants and ground holders produced by certain ground spraying equipment, at season 2017-2018 using total recommended dose rate tested fungicides against wheat stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* at Kafrelshiekh Governorate.

Equipment	Hydraulic (Matabi) sprayer						Knapsack motor sprayer (Arimitsu)						Rotary (Matabi) sprayer					
Application rate L./fed.	56						34						18					
Insecticide	Propiconazole		Azoxystrobin+ Cyproconazole		Cyproconazole +Propiconazole		Propiconazole		Azoxystrobin+ Cyproconazole		Cyproconazole +Propiconazole		Propiconazole		Azoxystrobin+ Cyproconazole		Cyproconazole +Propiconazole	
	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD	N/cm^2	VMD
Upper level	188	139	189	159	184	139	199	139	200	138	193	158	133	134	136	145	129	122
Lower level	126	164	164	127	132	146	167	146	168	125	166	148	109	126	102	148	105	146
Mean	157	152	158	153	136	164	183	143	184	132	180	153	121	130	119	147	117	134
Ground	50	143	52	142	52	134	50	131	48	148	48	146	20	101	22	145	18	136
Sapry lost % on ground	13.7	_	14	_	14.1	_	12	_	11.5	_	11.8	_	7.8	_	8.5	_	7.1	_

3.Lost spray on ground of fungicides produced by equipment :

3.1.Hydraulic Matabi sprayer (56L/Fed.):

Data in Table (6) showed that the lost spray percentages which were 13.7,14 and 14.1% from the total spray volume in the case of recommended dose of propiconazole, azoxystrobin + cyproconazole and cyproconazole + propiconazole, respectively.

3.2.Knapsack motor sprayer (Arimitsu) (43 L/Fed.):

Data in Table (6) showed that the lost spray percentages which were 12,11.5 and 11.8 % from the total spray volume in the case of recommended dose of propiconazole, azoxystrobin + cyproconazole and cyproconazole + propiconazole, respectively.

3.3.Rotary Matabi sprayer (18 L/Fed.):

Data in Table (6) showed that the lost spray percentages which were 7.8,8.5 & 7.1% from the total spray volume in the case of recommended dose of propiconazole, azoxystrobin + cyproconazole and cyproconazole + propiconazole , respectively.

The rate of performance of Arimitsu sprayer was 15.25 Fed./day. It was the best equipment, but the lowest rate of performance was Rotary Matabi sprayer since it could spray only 2.3 Fed./day.

Table (7): Relationship between field spray quality of Fungicides by Knapsack motor sprayer (Arimitsu) (34L.\Fed.) , Rotary (Matabi) sprayer (18L.\Fed.) and Hydraulic Matabi sprayer (56L.\Fed.) at 2017\2018 and 2018\2019 seasons against stripe rust of wheat at Kafrelshiekh Governorate.

Equipment	Hydraulic (Matabi) sprayer			Knapsack motor sprayer (Arimitsu)			Rotary (Matabi) sprayer		
Application rate L./fed.	56			34			18		
Insecticide	Propiconazole	Azoxystrobin+ Cyproconazole	Cyproconazole +Propiconazole	Propiconazole	Azoxystrobin+ Cyproconazole	Cyproconazole +Propiconazole	Propiconazole	Azoxystrobin+ Cyproconazole	Cyproconazole +Propiconazole
	Spray Quality	Spray Quality	Spray Quality	Spray Quality	Spray Quality	Spray Quality	Spray Quality	Spray Quality	Spray Quality
Upper level	0.74	0.84	0.76	0.7	0.69	0.82	1	1.07	0.95
Lower level	1.3	0.77	1.1	0.87	0.74	0.89	1.16	1.45	0.95

S.Q = Spray quality. = VMD/N/cm^2= Spray quality (degree of homogeneity).
The spray height is constant ~ 0.5 meter in all treatments.
VMD= Volume mean diameter ,N/cm^2= Number of droplets/cm^2.

Data in Table (7) showed that homogeneity of spray coverage was high and in case of Rotary Matabi followed by Arimitsu and Hydraulic Matabi. Also The following remarks and results were obtained: There was no Phytotoxic effect on wheat leaves after application treatments with pesticides in all treatments there was no change in the leaves color, and no leaf curling or flaming up phenomena was happened in case of all treatments and there was a highly significant differences between both the distribution percentages of droplet sizes(LSD= 5.85 for equipment,5.9 for levels and 5.8 for compounds) and for the droplets number/cm^2 (LSD= 5.3 for equipment,5.31 for levels and 5.3 for compounds) at 5% .

Wheat stripe (yellow) rust, caused by *P. striiformis* is highly destructive disease of wheat. Under favorable conditions, stripe rust can cause yield losses of up to 100% in susceptible varieties (Roelfs, 1985). The main strategy for the controlling of wheat stripe rust in Egypt would remain focused on the development of resistant cultivars and chemical options are the two principal methods of wheat rust management strategies implemented in most wheat producing areas of the world. To come up with this, several new fungicides have been evaluated against wheat rusts and are being used in wheat as rusts management options and to sustain wheat production and productivity. Concerning the evaluation of three systemic fungicides in controlling stripe rust of wheat through the application of two successive sprays , using three equipment the obtained results indicated that the fungicide Tilt (Propiconazole) exhibited the lowest rust reaction ca 5 in terms of disease severity. Either of the tested fungicides showed high significant difference between one and two sprays applications. It must be noticed here is that Amistar extra (Azoxystrobint + cyproconazole) exhibited the highest disease severity ca 50. Severe stripe rust epidemic was recorded in the tested wheat cultivar during the second growing season; 2018/19, than that in the first season; 2017/2018 (Table, 5). The obtained data of disease severity, and infection type (IT), were combined to calculate average coefficient of infection (ACI) was assessed in the cultivar during an epidemic in the two growing seasons. Where, the final stripe rust severity for the tested wheat cultivar varied from one year to another, as affected by the slightly changes in environmental conditions

between the two years under study. In addition to, changes occurring in these Pst races population (Figures, 1 and 2). Thus the obtained results confirmed the elapsed ones with few exceptions. The distinction of Tilt (Propiconazole) as effective in the increase over control with second treatment i.e. two sprays. Similar results were recorded by Boshoff *et al.*, (2003); Jorgensen *et al.*, (2003) and Covarelli and Orfei (2005) and Shahin (2008), who indicated that the dynamic nature of stripe rust required the induction of new wheat cultivars with new genetic constitutions and/or the quick intervention with new fungicides having recent active ingredient, other than those available in the market, especially at the critical times of epiphytotics .

The fungicide treatments were effectiveness in reducing wheat stripe rust disease severity and improving crop yield. Field experiment was carried out on infected area with wheat stripe rust pathogen *P. striiformis* f. sp. *tritici* at early season on wheat plants. For evaluation the field performance of Low-Volume spraying machines; Hydrulic Matabi evolution sprayer (56L/Fed.), Knapsack motor sprayer (Arimitsu) (43 L/Fed.) and Rotary Matabi sprayer (18 L/Fed.), respectively ; to spray propiconazole, azoxystrobin + cyproconazole and cyproconazole + propiconazole with total recommended dose. A satisfactory coverage was obtained on bean plants, the droplet spectrum was obtained in field experiment was agreed with the optimum droplet sizes which mentioned by (Matthews, 1992), in case of low volume equipment. Data indicated that, all tested compounds revealed significant negative influenced on Wheat stripe rust pathogen Puccinia *striiformis* f. sp. *tritici*. Data of two sprays gave the same result since Propiconazole occupied the first rank in reducing rust severity. The best fungicide in this respect were Propiconazole followed by cyproconazole + propiconazole, however Azoxystrobint + Cyproconazole was the least in this regard. It could be recommended to use this compound with LV spraying equipment with not less than (18L/Fed.). Also, the best equipment in this respect were Rotary (Matabi) followed by Hydraulic (Matabi) and Knapsack motor sprayer (Arimitsu) the tested equipment under study. The rate of performance of Arimitsu sprayer was 15.25 Fed./day, but the lowest rate of performance was Rotary Matabi sprayer since it could spray only 2.3 Fed./day. The best obtained result was Rotary Matabi sprayer (18 L/Fed.) spray volume, 140 μm and 115 droplets/cm^2 and the lost spray on ground was 7.1 %., and these results agreed with Hindy *et al.* (2004), Genidy *et al.* (2005) which recommended KZ oil and Pyriproxyfen followed by Agerin using low volume spraying because of reducing the time lost in process filling the machines, improve the homogeneity of the spray solution on the plant leaves and saving the lost spray on the ground, these results also in agreement with Bakr *et al.* (2014) they recommended by using Profenofos followed by Pyriproxyfen and Spinosad with Agromondo sprayer (20L/fed.). Dar *et al.* (2019) showed that Motorized Knapsack sprayer (Agromondo) (20 L.Fed.) was the best equipment to control seadling pests at early seson of Cotton. The rate of performance of Knapsack motor sprayer (Arimitsu) was 15.25 Fed./day. It was the best equipment, but the lowest rate of performance was Hydraulic sprayer (Matabi) since it could spraying only 3.4

Fed./day. Also, the lowest spray volume , the lowest percentage 7.1% of lost spraying between plants occurred by Rotary Matabi sprayer (18 L/fed.), this results was agreed with Hindy *et al.* (1997), who mentioned that, there was a positive relationship between rate of application and lost spray on ground.

Also, the best equipment in this respect were Rotary (Matabi) because the horizontal long ,stripe and narrow leaves which pick the small size droplets with high surface tension more than gravity and still on wheat leaves more than large size droplets followed by Hydraulic (Matabi)and Knapsack motor sprayer (Arimitsu) the tested equipment under study. The rate of performance of Arimitsu sprayer was 15.25 Fed./day, but the lowest rate of performance was Rotary Matabi sprayer since it could spray only 2.3 Fed./day. Spray Quality were near to 1 in case of Rotary Matabi sprayer which indicated high spray coverage homogeneity and best stripe rust of wheat controlling , this results was agreed with (Matthews,1992) .

It could be recommended to use propiconazole with total recommended dose followed by cyproconazole + propiconazole, azoxystrobint + cyproconazole rate with low volume (LV) spraying equipment with not less than (18L./Fed.) which revealed successful results. There was a negative complete correlation between (VMD) and the disease severity of Wheat while there was a positive complete correlate between N/cm² and the disease severity of wheat in all treatments.

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Effect of some biocontrol agents and biopesticides against tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) on tomato crop at Alexandria, Egypt

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Abstract:

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetables around the world. In Egypt, tomato is widely grown vegetable crop annually in 2-3 plantations. Many pests attacking tomato crop causing very serious damage, one of the recent devastating exotic pests is the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *T. absoluta* insect is very difficult to control. Therefore, this study was conducted to find an effective and suitable management approach against tomato leaf miner. The experiment was carried out in the tomato plantation during two consecutive seasons for autumn (15th of August 2018) and spring season (15th of March 2019) at the Experimental Station Farm, Abies, Alexandria Governorate, Egypt. According to the infestation reduction rate of *T. absoluta*, the data detected that the treatment (Bio, *T.a*), release of the egg parasitoid, *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) with rate 8 cards/¼ feddan showed successful results (42.71%) to management *T. absoluta*, followed by the foliar spray in both of (Bio, N.) treatment (application of Fytomax N based on Azadirachtin 1% extracted from the Neem tree seeds with rate 1ml./L. of water) and (Bio, *B.t*) treatment (application of *Bacillus thuringiensis* (Diple D.F.) with rate 100 gm./¼ feddan) 35.90% and 33.75%, respectively, lastly use of (Ch,Co.) treatment (application of chemical pesticide (Coragen) based on chlorantraniprole 20% SC with rate 15 cm./¼ feddan) (8.41%). The yield production and cost benefit were recorded. Results revealed that release of the egg parasitoid, *T. achaeae* as a biocontrol agent followed by application of Fytomax N (azadirachtin) as a biopesticide, bacteria of *B. thuringiensis* formulate (Dipel D.F.) and fungi of *Metarhizium anisopliae* formulate (Lycomax) as biocontrol agents performed best in reducing *T. absoluta* infestation, increase of yield production and cost benefit, comparing with apply of traditional agriculture practices (i.e. chemical pesticide, hand picking and destruction of infested leaves and fruits).

Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera) is native pest of neotropical region. In 2006, it was identified in Spain and after that it has spread to most of Europe, Africa, West, Central and South Asia (Sridhar *et al*., 2014 and Venkatramanan *et al*., 2017). It is originated come from South America, rapidly invaded different European countries and spread very fast along the Mediterranean Basin including Egypt (Desneux *et al*., 2010). In Egypt, *T. absoluta* was first detected in 2009 on tomatoes at Marsa Matrouh (northwestern Egypt), then the pest rapidly spread to the upper and lower regions of Egypt (Moussa *et al.*, 2013 and Salama *et al.*, 2015). It is oligophagous moth feeding on solanaceous crops and one of the key pests of tomato production (Garcia and Espul, 1982 and Germain *et al*., 2009). Tomato (*Solanum lycopersicum* L.) is considered one of the most economically important vegetables around the world. *T. absoluta* is one of the major devastating exotic pests attacking tomato crops in many regions of the tomato-producing worldwide. Severe infestation with *T. absoluta* can potentially result in significant damage by feeding on all aerial parts of tomato plant and affects both yield and fruit quality. If *T. absoluta* is not properly managed, it is causing 80–100% crop loss in the field and in protected cultivation (Desneux *et al*., 2010; Khanjani, 2013 and Ramzi Mansour *et al*., 2018).

In order to reduce the excessive use of insecticides in tomato fields, environmentally control strategies have been developed, including cultural control measures (e.g. crop rotation, selective removal and destruction of infested plant material) (Korycinska and Moran, 2009) and the use of entomopathogens (Urbaneja *et al*., 2012). Also, lepidoptera pheromones have been used for insect monitoring and mating disruption, which is a great biotechnological tool to successfully reduce *T. absoluta* infestations (Cherif and Kaouther, 2014). This involves only the follow-up of male flight activity during the growing season, which aims at deciding the most appropriate timing for applying either pestcticide treatments or biological control (Caparros Megido *et al*., 2013).

Biological control using natural enemies would be the concerted use as a major component of any integrated pest management (IPM) program for controlling *T. absoluta*. Egg parasitoid species of family Trichogrammatidae are considered efficient biological control agents and are widely used commercially for the suppression and control of lepidopterous pests on many crops (Agamy, 2003 and Ballal *et al*., 2016). They are easy to rear and release either in open fields or protected crops (Chailleux *et al.*, 2012). Selection of the appropriate *Trichogramma* species for controlling a given insect pest is a crucial factor to the success of biological control program (Desneux *et al.*, 2010; Mills, 2010 and Chailleux *et al.*, 2012). The parasitic wasp *Trichogramma achaeae* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) has been suggested as a possible biological control agent against *T. absoluta* (Pasquale *et al.*, 2015). On the other hand, botanicals have been the oldest tool used for the control of insect pests. Several plants exhibit antifeedent properties against an array of insects. Among them Neem is one of the

important plants still find a place in modern pest management programmes (Kona *et al.*, 2014).

Tomato is known as the main host of *T. absoluta* and feeds, develops and reproduces also on other solanaceous plants such as potato, tobacco, eggplant, pepper, aubergines, black nightshade and several weeds such as jimson weed (Pereyra and Sanchez, 2006). However, the main damage is usually observed on the leaves and fruits, but inflorescences and stems can also be affected. Eggs of *T. absoluta* are deposited chiefly on the leaves, singly or in small groups, and the larvae attack leaves, stems and fruits. Larvae of *T. absoluta* feed on the mesophyll of the leaf leaving only the epidermis intact with its feces, which subsequently widens and then the damaged tissue dries. Under intense attack, the damaged leaves turn yellow, wither, and senescence; the fruits are destroyed; and the plant is ultimately die (Maluf *et al*., 1997).

Current management of *T. absoluta* in Egypt as a part of Mediterranean Basin, is mainly based on treatment with chemical insecticides (González-Cabrera *et al.*, 2011). At present, depending on the cropping system and infestation intensity, the main control tools used against tomato leaf minor, *T. absoluta* rely too heavily on conventional insecticides that have led to the development of insecticide resistance (Haddi, 2012). In addition, the problems of using chemical control are further exacerbated by awareness of environmental pollution, toxicity to natural enemies and increasing risks to human and mammals (Tillman, 2000). Therefore, the use of insecticides has become subordinated to other control methods, such as biological control singly and/or in integrated with other methods as use of aggregation pheromones and biopesticides that have gained more credibility in the last decades (Senior *et al.*, 2001 and Agamy, 2003).

This experiment aimed to identify a non-chemical pesticide approach to control *T. absoluta* at Alexandria Governorate, Egypt. So, the present study was conducted to manage of *T. absoluta* population using some biocontrol agents and biopesticides comparing with use of conventional practices (i.e. chemical pesticide, hand picking and destruction of infested leaves and fruits) in tomato field at Alexandria Governorate, Egypt, by estimating natural rate of infestation.

Materials and methods

1. Description of the study area:

The field experiment was carried out at the Experimental Station Farm, Abies, Faculty of Agriculture, Alexandria University, Alexandria Governorate, Egypt. An experimental area of one hectare (equal 2.5 feddan) (feddan = 4200 m^2) was planted with tomato (*Lycopersicon esculentum* Mill) variety Gold stone. Experimental area cultivated during two consecutive tomato plantations seasons for autumn (15^{th} of August 2018) and spring season (15^{th} of March 2019). Minimum, maximum temperature and relative humidity (RH) throughout the experimental period in the region was recorded from the metrological station: 623180 (HEAX), Ministry of Agriculture, Alexandria.

2. Experimental design:

The experimental was conducted a Randomized Complete Block design with 8 treatments and 3 replicates during two consecutive tomato plantations seasons. Each replicate per plot was

contained 3 rows with 15 plants / row at 60 x 45cm spacing, for a total of 45 plants.

3. Experimental materials:

Delta traps supplied with *T. absoluta* pheromone were placed (4 traps/ feddan) at all the experimental plots and hung at a height of 1 m. The pheromone capsule was changed once a month and the sticky plate once a week. All the treatments started when more than 3 adults were caught in the traps. The number of 3 caught adults has been selected to prove the field pest presence without economic threshold correlations. After that, the pheromone traps were removed from all the field experiment. In Table (1) is given the treatments, trade name, scientific name, abbreviations of experimental treatments and application rate of each treatment. The experimental materials were diluted with tap water and applied at field rates based on the dose recommended by the companies. The treatments were (Bio, *T.a*) = release of the egg parasitoid, *T. achaeae* with rate 8 cards/¼ feddan as a biocontrol agent, were kindly hanged directly in the field on the tomato plants; (Bio, *M.a*) = application fungi of *Metarhizium anisopliae* (Lycomax) ½Kg./¼ feddan in soil as a biocontrol agent; (Bio, *B.t*) = application bacteria of *Bacillus thuringiensis* (Diple D.F. 9.4% WG) 100 gm./¼ feddan by foliar spray as a biocontrol agent; (Bio, N) = application of Fytomax N as a biopesticide, based on Azadirachtin 1% extracted from the Neem tree (*Azadirachta indica*) seeds with rate 1ml./L. of water by foliar spray; (Bio, Ph.T) = application of Pheromone lures through installation of delta sex pheromone trap with rate one trap/¼ feddan; (Ch, Co.) = application chemical pesticide of Coragen 20%SC, based on chlorantraniprole with rate 15 cm./¼ feddan; (H) = Hand picking and destruction of infested leaves and fruits; (C) = control (untreated), which left without any practices carried out by the farmers. Foliar sprays were applied by using a knapsack sprayer **(Molla *et al*., 2011)**.

4. Estimation the percent infestation by *Tuta absoluta:*

The plants in all treatments were weekly visually checked. Ten plants of medium row were used for data collection from each replicate/ plot before treatment as well as 3rd, 7th and 14th days after applied the experimental treatments. Data on number of healthy and infested plants; leaves and fruits infestation by leaf miner of *T. absoluta* were counted and recorded from all the experimental treatments per plot. Infested leaves per each treatment were placed in a plastic bag and taken to the laboratory. Leaves were examined under binocular microscope (no. of mines in the leaves/plant) and larvae of *T. absoluta* live or dead as well as mines were counted before treatment and in intervals 3rd, 7th and 14th days after applied the experimental treatments.

On the other hand, per ten plants percent infestation, leaves infestation per plant and fruits damage (visual estimation) by leaf miner per plant were calculated. To assess the effect of treatments on reduction in fruits damage, numbers of infested and uninfested tomato fruits from 10 plants were counted from each replicate in different control methods. After that, percent reduction in fruits infestation over control after two consecutive tomato plantations seasons was measured. Subsequently in the end of tomato plantations spring season 2019, fruits were weekly visually checked and

harvested, then weighted. The data on yield production of tomato was recorded.

5. Cost-benefit of yield:

At the end of harvesting, cost-benefit of using all treatments and control were estimated according to the following formula: -

Cost benefit = cost of yield production – control costs.

The control costs included the costs of purchasing the experimental materials and labor crops cost at each treatment.

6. Data analysis:

The data recorded on different parameters were analyzed statistically by using Co-Stat computer software program (2004) for analysis of variance. ANOVA was made by F-variance test and the differences between treatment means were compared by Fisher's LSD test (Al-Rawi and Khalf-Allah, 1980).

Table (1): The different treatments applied on tomato crop infestation by *Tuta absoluta* during two consecutive tomato plantations seasons 2018-2019 at Abies, Alexandria Governorate, Egypt

Treatments	Trade name	Scientific name	Application rate
a) Bio-agents			
1. Egg parasitoid	1.1.*Trichogramma achaeae* **(Bio, *T.a*)**	*Trichogramma achaeae*	8 cards/¼ feddan
2. Entomopathogens	2.1. Lycomax **(Bio, *M.a*)**	*Metarhizium anisopliae*	½ Kg./¼ feddan
	2.2. Diple D.F. 9.4% WG **(Bio, *B.t*)**	*Bacillus thuringiensis*	100 gm./¼ feddan
b) Recommended pesticides			
3. Biopesticide	3.1. Fytomax N **(Bio, N.)**	*Azadirachta indica*	1ml./L.
	3.2. (Pheromone lures) **(Bio, Ph.T)**	----------	one trap/¼ feddan
4. Chemical pesticide	4.1. Coragen 20% SC **(Ch.Co.)**	Chlorantraniliprole	15 cm./¼ feddan
5. Hand picking and destruction of infested leaves and fruits (H)			
6. Control (untreated) (C)			

- Parasitoid cards of *Trichogramma achaeae* were provided by Agriculture Research Centre, Giza, Egypt.
- Lycomax, Diple D.F and Fytomax N were obtained from Russell IPM Company, UK.
- Pheromone lures were obtained from pheromone production unit, Plant Protection Research Institute, Agriculture research center, Alexandria, Egypt.
- Coragen 20% SC was obtained from Shoura Chemicals Company.

Results and discussion

1. Effect of different treatments on tomato plant, leaf and fruit infestation:

Effect of different treatments on percent plant, leaf and fruit infestation by *T. absoluta* during two consecutive tomato plantations seasons in experimental area, Abies, Alexandria Governorate, Egypt is presented in Table (2). In autumn season (15th of August, 2018), the lowest plant (46.63%) and leaf (15.07%) infestation was recorded in treatment (Bio, *T.a*), release of the egg parasitoid, *T. achaeae* with rate 8 cards/¼ feddan as a biocontrol agent followed by foliar spray in both of (Bio, N.) treatment (application of Fytomax N based on Azadirachtin 1% extracted from the Neem tree seeds with rate 1ml./L. of water) as a biopesticide and (Bio, *B.t*) treatment (application bacteria of *Bacillus thuringiensis* (Diple D.F. 9.4% WG) with rate 100 gm./¼ feddan) as a biocontrol agent, respectively. On the contrary maximum infestation was recorded in untreated treatment (control). The similar trend was also found in spring season (15th of March 2019).

While, other results revealed that spring season was highest population density of *T. absoluta* followed by summer season (Tabikha and Abdel Nasser, 2015). In several Egyptian tomato producing areas, the degree of damage by this insect even reached 100% (Moussa *et al*., 2013). Under plastic greenhouse conditions in Nasr city (Cairo area, northern Egypt), infestation began in the third week of March and both the highest numbers of *T. absoluta* larvae and percentage of tomato infestation occurred in July (Ata and Megahed, 2014).

Effect of different treatments on fruit infestation by *T. absoluta* per plant during two consecutive tomato plantations seasons 2018-2019 at Abies, Alexandria Governorate, Egypt is showed in Table (2). In autumn season, the treatment (Bio, *T.a*) had the lowest fruit infestation (11.44%) followed by (Bio, N.) treatment (12.80%) and (Bio, *B.t*) treatment (13.23%). Maximum fruit infestation (19.97%) was recorded in control (untreated treatment). The same trend was also found in spring season. Accordingly, maximum reduction of fruit infestation over control after two consecutive tomato plantations seasons was also found in treatment (Bio, *T.a*) (42.71%) followed by (Bio, N.) treatment (35.90%) and (Bio, *B.t*) treatment (33.75%) comparing with use of conventional agriculture practices, chemical pesticide (Ch, Co.); hand picking and destruction of infested leaves and fruits (H) in tomato field at Alexandria Governorate, Egypt. While, no significant differences were observed between treatments (Bio, N.), (Bio,*B.t*), (Bio, *M.a*) and (Bio, *T.a*).

All treatments reduced population density of tomato leaf miner significantly. This study also revealed that after 7 -10 days of treatment application *B. thuringiensis* and *M. anisopliae* indicated effect on the larvae of *T. absoluta*, can be supposed that, the establishment of bacteria and fungi on the larvae of insect pests take some days. While, the application of Neem seed extract against larvae of *T. absoluta* resulted after 3 - 4 days. The results of the present experiment are quite like that of (Trindade *et al*., 2000; Hamdy and Walaa, 2013; Shalaby *et al*., 2013 and Shiberu and Getu, 2018).

2. Effect of different treatments on tomato yield:

The results indicated in the end of tomato plantations spring season, 2019 that the treatment (Bio, *T.a*), release of the egg parasitoid, *T. achaeae* with rate 8 cards/¼ feddan as a biocontrol agent provided the highest yield (4.08 ton / ¼ feddan) followed by (Bio, N.) treatment, foliar spray of Fytomax N based on Azadirachtin 1% extracted from the Neem tree seeds with rate 1ml./L. of water as a biopesticid and (Bio, *B.t*) treatment, application bacteria of *B. thuringiensis* (Diple D.F. 9.4% WG) as a biocontrol agent with rate 100 gm./¼ feddan (3.95 and 3.88 ton/¼ feddan, respectively) (Table, 3). Accordingly, Minimum tomato yield (2.66 ton/¼ feddan) was recorded in control (untreated treatment).

3. Cost benefit of yield:

Cost benefit of different treatments for managing *T. absoluta* after two consecutive tomato plantations seasons 2018-2019 at Abies, Alexandria Governorate, Egypt is presented in Table (3). The cost benefit was the highest (5841.85 L.E) in treatment (Bio, *T.a*) followed by both of treatment (Bio, N), and treatment (Bio, *B.t*) (5699.04 and 5607.45 L.E, respectively). Data shown that the treatment (Bio, *T.a*), release of the egg parasitoid, *T. achaeae* with rate 8

cards/¼ feddan as a biocontrol agent proved to be effective considering reduction of *T. absoluta* infestation, increase of yield production and cost benefit. On other hand, using chemical pesticide (Ch, Co), application of Coragen 20%SC with rate 0.5ml./L. of water gave the low yield production and cost benefit. So, considering that the result after two consecutive tomato plantations seasons 2018-2019, the treatment (Bio, *T.a*) may be recommended for managing tomato leaf miner, *T. absoluta* at Abies, Alexandria Governorate, Egypt. Besides, the other advantages of using the safe biocontrol agents directly on the yield production and indirectly on the environment and living agents.

Generally, seasonal rate of infestation in biocontrol agents and biopesticides treatments was less than the chemical pesticide treatment, especially treatment with release of the egg parasitoid, *T. achaeae* showed least percentages of fruits infestation (11.44% and 3.38% in autumn and spring seasons, respectively) compared with (16.42% and 4.05% in autumn and spring seasons, respectively) in the chemical pesticide treatment Coragen 20%SC (chlorantraniprole). In the same trend, Laham *et al.* (2009) reported that chlorantraniliprole is a relatively new insecticide for the control of lepidoptera and selected other species, Because of the new mode of action on both larvicidal and ovicidal activity. Also, Chlorantraniliprole has negligible impact on key parasitoids, predators, and pollinators by use field rates. However, the efficiency of chemical control of tomato leaf miner infestations has been poor because of the endophytic habit of its larvae, which are protected in the leaf mesophyll or inside fruits (Cocco *et al.*, 2013).

In the present study, obtained results agree with Agamy (2003) and Mills (2010) who stated that egg parasitoid species of family Trichogrammatidae are considered efficient biological control agents and are widely used commercially for the suppression and control of lepidopterous pests on many crops. They are easy to rear and release either in open fields or protected crops (Chailleux *et al.*, 2012). Also, Abd El-Hady (2014) stated that increasing the number of released parasitoids caused significant increase of parasitization and the seasonal rate of infestation was obviously less than the pesticides' treatments and relatively than the bio-rational solutions. Goda *et al.* (2015) revealed that oophagous parasitoid would play crucial role to the success of biological control program for management of tomato leaf miner, *T. absoluta*. Obtained results revealed that possible to reduce the tomato leaf miner impact by applying Fytomax N (Azadirachtin), which showed promising results in controlling *T. absoluta*. Researchers have focused on the use of botanical extracts, oils and plant powders, which are cheap, of short persistence and of low mammalian toxicity were indicated that many of these plant materials show a broad spectrum of activity against insect pests, such as lethal, antifeedant, repellent and growth regulatory effects (Shiberu and Getu, 2018). Fytomax N had great efficacy towards *T. absoluta* and rates of infestation were always less, because Fytomax N prevents or interferes with an insect's development. It has an ovicidal effect and controls target pests by contact as well as by ingestion. It acts as repellent, antifeedant, and interference

with the molting process of insect pest. Treated insects stop feeding and growing. Nevertheless, few biopesticides are effective against *T. absoluta* and selective to beneficial insects at the same time (Goda *et al*., 2015). Also, found that Azadirachtin caused high mortality in *T. absoluta* larvae allowing only 2.5–3.5% survival (Tomé *et al.*, 2013). While, Serviciode Sanidad (2008) recommended that use of Azadirachtin as a preventive spray cause light infestations (< 30 adult catches per week) of *T. absoluta*.

The studies that focused on the effect of *B. thuringiensis* on *T. absoluta* have been performed that the commercial formulates based on *B. thuringiensis* may be a good control alternative for *T. absoluta* as other insect pests. It is a Lepidoptera-specific microbial, which ingested and disrupts the mid gut membranes (Giustolin *et al*., 2001; Niedmann and Meza-Basso, 2006 and Mallia, 2009). The *B. thuringiensis* is highly efficient in controlling *T. absoluta*, because the first instar larvae were the most susceptible than the second and third instar larvae. This result has shown that the impact of *T. absoluta* can be greatly reduced by spraying only *B. thuringiensis* with no need for chemical insecticides (González-Cabrera *et al.*, 2011).

Hence, it is important to regularly survey solanaceous plants for the occurrence of *T. absoluta* and document natural enemies attacking different stages of the pest. It is crucial to educate farmers on pest stages and symptoms of damage caused by *T. absoluta*. So that, farmers can determine initiate action on time and prevent spread of the pest. It would also be useful to help the farmers in identifying potential biocontrol agents of the pest, *T. absoluta*. In conclusion, applying biocontrol agents or biopesticids like in this study achieved best rates of reduction of *T. absoluta* infestation at Abies, Alexandria Governorate, Egypt in two consecutive tomato plantations seasons 2018-2019. Further studies are needed for other tomato plantations as different rates of the *Tuta absoluta* population are expected. It is concluded that the identification of biocontrol agents against *T. absoluta* with high efficacy and fruits quality, may serve as an important in management programmes of *T. absoluta* as ecofriendly manner.

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Table (2): Effect of different treatments on percent plant, leaf and fruit infestation by *Tuta absoluta* during two consecutive tomato plantations seasons 2018-2019 at Abies, Alexandria Governorate, Egypt.

Treatments	% Plant infestation		% Leaf infestation		% Fruits infestation		% reduction over control after two consecutive tomato plantations seasons
	Autumn season (15th of August, 2018)	**Spring season** (15th of March, 2019)	**Autumn season** (15th of August, 2018)	**Spring season** (15th of March, 2019)	**Autumn season** (15th of August, 2018)	**Spring season** (15th of March, 2019)	
(Bio, *T.a*)	46.63**c**	43.04**c**	15.07**f**	5.93**c**	11.44**c**	3.38**c**	42.71
(Bio, *M.a*)	60.20**b**	50.17**b**	19.22**c**	7.58**c**	13.37**bc**	3.66**bc**	33.05
(Bio, *B.t*)	58.64**b**	49.96**b**	16.97**de**	7.26**c**	13.23**bc**	3.64**bc**	33.75
(Bio, N.)	49.14**c**	44.49**c**	16.06**ef**	7.26**c**	12.80**bc**	3.58**bc**	35.90
(Bio, Ph.T.)	80.43**a**	63.74**a**	23.35**b**	10.09**b**	18.29**ab**	4.28**ab**	17.78
(Ch, Co.)	58.99**b**	50.90**b**	17.69**d**	7.53**c**	16.42**abc**	4.05**abc**	8.41
(H)	81.28**a**	64.40**a**	23.43**b**	14.52**b**	16.98**abc**	4.05**abc**	14.97
(C)	84.64**a**	66.94**a**	25.40**a**	15.54**a**	19.97**a**	4.47**a**	-------
Level of significance	**	**	**	**	**	**	
CV%	5.33	8.51	3.00	5.17	7.00	9.06	

Abbreviations of Experimental Treatments

(Bio, *T.a*) = release of the egg parasitoid, *Trichogramma achaeae* with rate 8 cards / ¼ feddan.
(Bio, *M.a*) = application fungi of *Metarhizium anisopliae* (Lycomax) ½ Kg. / ¼ feddan in soil.
(Bio, *B.t*) = application bacteria of *Bacillus thuringiensis* (Diple D.F. 9.4% WG) 100 gm. / ¼ feddan by foliar spray.
(Bio, N.) = application of Fytomax N (based on Azadirachtin 1% extracted from the Neem tree seeds) 1ml / L of water by foliar spray.
(Bio, Ph.T)= application of Pheromone lures through installation of delta sex pheromone trap with rate one trap / ¼ feddan.
(Ch, Co) = application chemical pesticide of Coragen 20%SC (chlorantraniprole) 15 cm. / ¼ feddan.
(H) = Hand picking and destruction of infested leaves and fruits.
(C) = control (untreated).

Table (3): Estimated yield production of tomato, control costs and cost benefit in the experimental area of different control methods against *Tuta absoluta* in the end of tomato plantations spring season, 2019 at Abies, Alexandria Governorate, Egypt.

Treatments	Yield production Ton / ¼feddan	Cost of yield production L.E. / ¼feddan	Control costs L.E. / ¼feddan	Cost benefit (L.E.)
(Bio, *T.a*)	4.08	6120	278.15	5841.85
(Bio, *M.a*)	3.84	5760	210.64	5549.36
(Bio, *B.t*)	3.88	5820	212.55	5607.45
(Bio, N.)	3.95	5925	225.96	5699.04
(Bio, Ph.T.)	3.12	4680	134.40	4545.60
(Ch, Co)	3.00	4500	294.59	4205.41
(H)	3.03	4575	198.77	4376.23
(C)	2.66	3990	---------	3990

[Treatments: Same as indicated under Table 2]

- Cost of yield production (L.E. / ¼feddan) = Yield production (Ton / ¼feddan) X Price of Ton yield production (~ 1500 L.E. / Ton)
- Control costs (L.E. / ¼feddan) = Costs of purchasing the experimental materials and labor crops cost at each treatment.
- Cost benefit (L.E.) = Cost of yield production – control costs.

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Effect of plant extracted oils on biological aspects and silk production of mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

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Abstract:

The effects of feeding silkworm larvae *Bombyx mori* L. (Lepidoptera: : Bombycidae) (Egypt hybrid, Giza) on mulberry leaves supplemented with three plant extracted oils; sesame oil (*Seassamum indicum* L.), olive oil (*Olea europaea*.) and nigella sativa oil (*Nigella sativa* L.) at three concentrations (0.5, 1.0 and 2.0 %) for one time (At the beginning of the fourth instar) and two times (At the beginning of fourth and fifth instars) on some biological and technological characters were studied. Sesame oil (0.5%) decreased the grown larval mortality and increased the cocooning percentage, the moth emergency rate and cocoon shell weight, but 1.0% concentration increased fecundity, cocoon weight and silk filament size. Olive oil (2%) increased the content silk ratio while nigella sativa oil (0.5%) caused more length and weight of reelable filament silk.

Introduction

The mulberry silkworm, *Bombyx mori* L. (Bombycidae, Lepidoptera) is one of the most economically important insects not only on the national level but also internationally. The production of high quality and quantity of natural silk depends mainly on larval feeding (Parra, 1991). The mulberry silkworm, *B. mori* . is reared successfully as the main source of natural silk. Recently, considerable attention has been given to improve rearing techniques of silkworms to increase the production of raw silk in Egypt to meet with the higher demands for industrial purpose. Furthermore, developing and improving the practical and applicable techniques for increasing the productivity of silkworm i.e. silk and eggs production is necessary. So, the nutritional studies on the silkworm, *B. mori* L. are of much importance pertaining to its productivity and the nutritional value of local indigenous plants, wild herbs and edible seeds rich in protein are of great significance. Therefore, it has been reported recently that better production of cocoon crops and eggs is possible when mulberry leaves are supplemented with certain nutritional materials (Singh *et al*., 1993; Zannoon, 1994; Ashour, 1997; El-Sayed *et al.*, 1998 and Mesbah *et al.*, 2000).

The present investigation studied the effects of some plant extracted oils as

nutritional additives on biological aspects and the effects on quantitative characteristics of cocoons and silk filament of mulberry silkworm *B.mori* .

Materials and methods

Biological and technological studies were done to determine the effects of addition some plant extracted oils.

1.Materials:

1.1. Mulberry leaves, *Morus alba* variety balady.

1.2. The mulberry silkworm, *B. mori* (Egypt hybrid, Giza) were obtained from the Sericulture Research Department, Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation in Giza, Egypt.

2.Plant extracted oils:

2.1. Sesame oil (*Seassamum indicum*, L.)

2.2. Olive oil (*Olea europaea*.)

2.3. Nigella sativa oil (*Nigella sativa* L.)

The procedures of plant extraction were produced at the Food Technology Research Institute, Agriculture Research Center, Giza. Three concentrations were used for each oil (0.5%, 1% and 2%) prepared by (Harvey and John, 1898).

2.Methodes:

2.1. Rearing technique:

Rearing mulberry silkworm was carried out under controlled laboratory conditions of 26 ± 2 °c and 70 ± 5 % RH. Rearing procedures were achieved according to Krishnaswami (1978) rearing technique. Mulberry leaves were dipped in each concentration of each used material for one minute and left to dry and then fed to mulberry silkworm larvae. The control leaves were dipped in distilled water. Each concentration was offered to two groups of silkworm larvae, the first group was fed with treated leaves only one time at the beginning of the 4th larval instar, while the second group was fed two times at the beginning of the 4th and 5th larval instars. Using three replicates (50 larvae) for each concentration.

2.2. Biological studies:

The following biological aspects under this investigation were studied :

2.2.1. Larval mortality (%)

2.2.2. Larval duration (day)

2.2.3. Cocooning percentage (%)

2.2.4. Adult emergence (%)

2.2.5. Fecundity of female (number of deposited egg/ female).

2.3.Technological studies:

2.3.1. Cocoon indices:

Ten resulted fresh cocoons from each replicate were collected, cleaned, weighed and carefully cut. The pupae and exuviate were removed, and cocoon shells were weighed. Silk content ratio was calculated according to formula by Tanaka (1964).

$$\text{Silk content ratio (\%)} = \frac{\text{Weight of fresh cocoon shell (mg)}}{\text{Weight of fresh cocoon (mg)}} \times 100$$

2.3.2. Reelable silk filament parameters:

Another ten cocoons from each replicate of the resulted fresh cocoons were collected; oven dried and reeled by individual reeling machine. The length (m) and weight (mg.) of the dried reelable filament were determined. The sizes of reelable filaments were calculated according to Tanaka (1964) formula:

$$\text{Silk filament size (dn)} = \frac{\text{Weight of silk filament (mg)}}{\text{Length of silk filament (m)}} \times 9000$$

2.4. Statistical analysis:

The obtained results were subjected to statistical analysis of variance (LSD) and the data were presented as means according to Snedecor and Cochran (1982) method using software COSTAT program.

Results and discussion

Effect of fnriching mulberry leaves with some plant extracted oils on biology and silk production of silkworm *Bombyx mori* :

1. Biological aspects:

1.1. Larval mortality:

The 1st concentration (0.5%) in all treatments exhibited the least larval mortality percentage such as, Sesame oil recorded (6.5 and 5.0 %), followed by *Nigella sativa* oil recorded (7 and 6%) if using them one time and two times, respectively.

1.2. Larval duration:

Sesame oil at 1.0% concentration, Olive and *Nigella sativa* oils at 0.5% concentration recorded the shortest larval duration (9 days) when used one time or two times comparing the control which recorded (11 days) (Table,1). These results may be attributed to the richness of sesame oil with many essential nutrients as phosphorous, iron, magnesium, manganese, zinc, vitamin B1 and fatty acids which consider an essential indicator of the nutritional value of the oil, these nutrients have beneficial and very positive effect on larval healthy growth, as documented by (Anilakumar *et al*., 2010; Hassan, 2012 and Rahman *et al*., 2007). Similarly, More than 200 different chemical compounds have been detected in olive oil including sterols, triterpenic alcohols, fatty acids (oleic acid), triacylglycerols hydrocarbons (squalene and carotenoids), chlorophylls, tocopherols, aliphatic alcohols, and volatile compounds. Furtheremore, it is a source of at least 30 phenolic compounds. These compounds are strong antioxidants and radical scavengers. And have antimicrobial activity against many bacterial strains, which enhanced larval immune system as reported by (PanelKellie and Peter, 2002 and Servili and Montedoro, 2002). These results in accordance with those of Al-Jabre *et al*. (2005) demonstrated that black seed (*Nigella sativa*), its oil and extracts showed a wide spectrum of favorable biological activities act as antimicrobial, immune stimulant, anti-inflammatory and anti-oxidant (Al-Ghamdi, 2001). Also, Morssy (2009) reported that the significantly decreased of larval mortality and the duration of silkworm *Bombyx mori*, as a result to treating larvae with three plant extract oils (lime, clover and jojoba oils) with 3 concentrations (0.5, 1.0 and 2.0%). Moreover, Prasad *et al*. (2001) recorded that the silkworms fed with mulberry leaves supplemented with potato leaf extract recorded the lower larval mortality and larval duration.

Table (1): Effect of some plant extracted oils as nutritional additives on mortality (%) and grown larval duration (day) of *Bombyx mori* .

Treatments	Conc. (%)	Larval mortality (%)			Larval duration (day)		
		4th instar	5th instar		4th instar	5th instar	
			Treated in 4th only	Treated in 4th and 5th		Treated in 4th only	Treated in 4th and 5th
Sesame oil	0.5%	5.00	6.50	5.00	5.00	10.00	9.00
	1%	5.00	7.50	6.00	6.00	9.00	9.00
	2%	6.50	8.50	9.50	7.00	11.00	11.00
	Mean	5.50	7.50	6.83	6.00	10.00	9.67
Olive oil	0.5%	5.00	7.50	6.11	6.00	9.00	9.00
	1%	6.50	8.00	7.83	5.00	9.00	9.00
	2%	7.50	8.50	8.00	7.00	11.00	10.00
	Mean	6.33	8.00	7.31	6.00	9.67	9.33
Nigella sativa oil	0.5%	6.00	7.00	6.00	5.00	9.00	9.00
	1%	7.50	8.50	9.00	6.00	11.00	10.00
	2%	8.50	10.50	12.00	7.00	12.00	12.00
	Mean	7.33	8.66	9.00	6.00	11.00	10.33
Control		10.00	12.00		7.00	11.00	
LSD 5% for concentration		2.137**	2.215**	1.489***	0.851***	1.424***	1.263***
LSD 5% for compound		2.087**	0.0274*	0.0478*	ns	ns	Ns

1.3. Percentage of cocooning:

Adding of sesame oil (0.5%) caused the highest percent of cocooning being 88.42 and 92.10% when mulberry leaves were treated one time and two times, respectively in comparison 61.11% for the control (Table,2).

1.4. Emergence percentage (%):

Sesame and *Nigella sativa* oils at 0.5% concentration of each and olive oil at 1.0% concentration caused significant increasing of adult emergence percent (85 and 90%) if used one time and two times, respectively, comparing 70% for control group (Table,2).

1.5. Female fecundity (No. of eggs/female):

The tested oils cleared that sesame and olive oil at 1.0% concentration exhibited the highest number of deposited eggs per female recording 374 eggs/female followed by *Nigella sativa* oil at 0.5% concentration recording 360 eggs/female when used two times, while control group recorded 303 eggs/female (Table,2).

The improvement of these biological characters under study might be owing to the presence of high levels of flavonoids, tannins and alkaloids in sesame oil characterized by a high level of antioxidant activity and attributed to the antibacterial, antifungal, and antiviral properties of *S. indicum* (Rice-Evans *et al*., 1995 and Bankole *et al*., 2007). Likewise, natural Phytochemicals (phenols and triterpenes) present in olive oil as important bioactive molecules against diseases, exert different biological activity, including antioxidant, anti-inflammatory and antiviral effects and used as defense against microbial and fungal invasion (Hollman and Katan, 1999 and Eastwood, 1999). In harmony with the fore mentioned results, El-Sayed (1999) reported that the mixture of honey and Black Cumin (*Nigella sativa*) seeds increased silk production and number of deposited eggs/female. Also, Mahmoud

et al. (2012) evaluated three types of honey; Carob [*Ceratonia siliqua*], Seder [*Ziziphus* sp.] and Black Cumin honey [*Nigella sativa*] which contains all the good qualities and benefits of black cumin seeds, compared to the untreated control. The evaluated types of honey to more or less extent increased the egg productivity. In the same manner, Xu *et al.* (1992) found that from 3rd- to 5th instar larvae *Bombyx mori* fed on mulberry leaves soaked in extracts of *Brassica campestris* pollen and royal jelly increased the cocoon formation and oviposition. Shoukry *et al.* (1998) recorded that mulberry leaves supplemented with two volatile oils Ploughman's oil and Jasmine oil on silkworm *B. mori*, increased percentage of cocoon production compared with the control.

Table (2): Effect of some plant extracted oils as nutritional additives on cocooning %, emergence % and fecundity of mulberry silkworm *Bombyx mori* .

Treatments	Conc.	Cocooning %		Emergence %		Fecundity (No.egg/female)	
		Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars
Sesame oil	0.5%	88.42	92.10	85.00	90.00	341	352.3
	1%	84.73	87.36	85.00	90.00	332	374
	2%	71.66	77.54	80.00	80.00	321	328.3
	Mean	81.60	85.66	83.33	86.67	331.3	342.5
Olive oil	0.5%	85.79	89.47	80.00	85.00	338.6	345.6
	1%	89.83	91.44	85.00	90.00	361.3	374.3
	2%	74.05	75.13	80.00	80.00	325	337
	Mean	83.22	85.34	81.67	85.00	341.7	352.3
Nigella sativa oil	0.5%	86.70	90.42	85.00	90.00	348.3	360.6
	1%	77.29	80.54	80.00	75.00	334	342.3
	2%	69.39	74.86	75.00	70.00	312	310
	Mean	77.79	81.94	80.00	78.33	331.4	337.6
Control		61.11		70.00		303	
LSD 5% for concentrations		1.703***	4.101***	6.140***	4.877***	1.616***	1.525***
LSD 5% for compounds		13.990*	13.226**	6.149**	12.190*	26.157*	32.582*

2. Technological studies:

2.1. Cocoon indices:

2.1.1. Fresh cocoon weight (g):

The weight of fresh cocoon was significantly higher with Sesame oil at 1.0% (1.400 and 1.416 g) when used one and two times compared to control cocoon 1.105 g. (Table, 3).

2.1.2. Shell cocoon weight (g):

All the tested treatments induced significant increase over the control especially; Olive oil (1.0%) recorded the best means recording 0.222 and 0.236 g when used one and two times. while Sesame oil (0.5%) recorded 0.240 g when used two times, comparing with control 0.150 g. (Table, 3).

2.1.3. Cocoon shell ratio (%):

The olive oil (2.0%) recorded (18.695 and 18.898%) ratio of silk content if using it one and two times, respectively. Meanwhile, Sesame oil at o.5% recorded 17.868 and 18.320 g, when used one time and two times, respectively (Table, 3).

Table (3): Effect of some plant extracted oils as nutritional additives on cocoon indices of *Bombyx mori* .

Treatment	Conc. (%)	Fresh cocoon weight (g)		Shell cocoon weight (g)		Silk ratio (%)	
		Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars
Sesame oil	0.5%	1.220	1.310	0.218	0.240	17.868	18.320
	1%	1.400	1.416	0.210	0.216	15.000	15.254
	2%	1.200	1.140	0.174	0.172	14.500	15.087
	mean	1.273	1.288	0.200	0.209	15.789	16.220
Olive oil	0.5%	1.285	1.302	0.200	0.215	15.564	16.513
	1%	1.265	1.322	0.222	0.236	17.549	17.851
	2%	1.150	1.180	0.215	0.223	18.695	18.898
	mean	1.233	1.268	0.212	0.224	17.269	17.754
Nigella sativa oil	0.5%	1.250	1.305	0.208	0.230	16.640	17.624
	1%	1.186	1.196	0.185	0.191	15.598	15.969
	2%	1.167	1.173	0.177	0.179	15.167	15.260
	mean	1.201	1.224	0.190	0.200	15.801	16.284
Control		1.105		0.150		13.537	
LSD 5% for concentration		0.152*	0.144**	0.0262***	0.0222***	1.524***	1.324***
LSD 5% for compound		ns	Ns	0.0287**	0.0422*	2.379*	2.346*

2.2. Reeled silk filament parameters:

2.2.1. Silk filament length (m):

Nigella sativa oil at 0.5% exhibited the highest length of reeled silk filament of cocoon recording (1145.5 and 1180.6 m) when used one and two times respectively. Followed by sesame oil at 1% concentration recorded (1100 and 1125.2 m) respectively, when used one and two times. While the control recorded 845.8 m (Table,4).

2.2.2. Silk filament weight (g):

The heaviest weight of reeled silk filament (0.223 and 0.224 g) was recorded for *Nigella sativa* oil at 0.5% for adding one and two times, respectively. While, the least weight of reeled silk filament (0.130 g) for control (Table,4).

2.2.3. Silk filament size (dn):

All the plant oils means induced highly significant increase over the control especially with sesame oil 1.781 dn. with using two times (Table,4).

The enhancement in cocoon and silk filament characters may be referred to haemolymph protein improvement, as a result to rearing larvae with the nutritional oil extracts under investigation, as documented by Mbaebie *et al.* (2010) reported that *S. indicum* is a good source of protein, carbohydrate, minerals and crude fibre. Similarly, Crews *et al*. (2006) stated that, Sesame oil contains significant amounts of the lignans sesamin and sesamolin compounds, which have beneficial effects on serum lipid levels and give sesame oil a marked antioxidant activity, because of the responsibility of the lignans for the great stability of sesame oil to oxidation. In the same way, *N. sativa* seeds contain fixed oil, proteins, alkaloids, saponins, and essential oil. The biological effects of *N. sativa* are attributed to its various characterized constituents, as documented by (Ali and Blunden, 2003).

Murugappan *et al.* (1996) mentioned that feeding larvae with mulberry leaves soaked in 1% jaggery solution which improved the cocoon

characters (cocoon weight by 45% and shell weight by 30%). Also, Shoukry *et al.* (1998) recorded that the cocoon production, the weight of shell cocoon, silk content ratio and cocoon weight were increased when mulberry leaves were supplemented with two volatile oils Ploughman's oil and Jasmine oil. And Prasad *et al*. (2001) recorded that the silkworms fed with mulberry leaves supplemented with potato leaf extract recorded the highest cocoon weight, shell ratio and shell weight.

Zannoon (1994) found that, different solutions of bee honey (i.e. citrus honey, clover honey and cotton honey), mixture of equal volumes of the three types of honey plus pollen grains offered to silkworm larvae *Bombyx mori* as nutritional additives gave longer and heavier filament of reeled cocoons with no change of its size., Kuntamalla and Rao (2004) found that the leaf extract of neem (1.0%) added to mulberry leaves offered to silkworm caused highest filament length and silk ratio. In connection, Khalil *et al.* (2006) stated that addition of various concentrations of anise extract showed that the higher concentration gave the best values in terms length of filament and weight of the cocoons. But, Kumar *et al.* (2009) cited that blue green algae spirulina (100 ppm, 200 ppm and 300 ppm foliar spray) caused that Silk filament length is significantly higher at 300 ppm concentration compared to control.

Feeding silkworm larvae two times at the beginning of fourth and fifth instars on mulberry leaves dipped in Sesame oil (0.5 % or 1.0 %) decreased the grown larval mortality and increased the cocooning percentage, the moth emergency rate, cocoon shell weight and filament size.

Table (4): Effect of some plant extracted oils as nutritional additives on reeled filament characters of the *Bombyx mori*.

Treatment	Conc. (%)	Silk filament length (m)		Silk filament weight (g)		Silk filament size (dn)	
		Treated in 4^{th} instar only	Treated in 4^{th} & 5^{th} instars	Treated in 4^{th} instar only	Treated in 4^{th} &5^{th} instars	Treated in 4^{th} instar only	Treated in 4^{th} &5^{th} instars
Sesame oil	0.5%	996.6	1021.3	0.188	0.212	1.697	1.867
	1%	1100.0	1125.2	0.179	0.190	1.464	1.519
	2%	1008.4	1015.6	0.217	0.221	1.935	1.958
	mean	1035	1054.0	0.194	0.207	1.698	1.781
Olive oil	0.5%	921.5	956.5	0.161	0.177	1.572	1.663
	1%	1045.5	1100.1	0.205	0.218	1.764	1.782
	2%	912.0	922.0	0.183	0.188	1.805	1.835
	mean	959.6	992.8	0.183	0.194	1.713	1.760
Nigella sativa oil	0.5%	1145.5	1180.6	0.223	0.224	1.751	1.707
	1%	1016.3	1043.5	0.195	0.204	1.726	1.758
	2%	1007.8	1020.5	0.185	0.191	1.651	1.683
	mean	1056.5	1081.5	0.201	0.206	1.709	1.716
Control		845.8		0.130		1.383	
LSD 5% for concentration		145.174*	146.141**	.0211***	.0227***	0.212**	0.247**
LSD 5% for compound		114.164*	133.871*	0.033**	.0295***	0.255*	0.235*

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Resistance induction in lima bean plants by silicon and ascorbic acid

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Abstract:

Abiotic elicitors of plant defense can induce plant resistance effective against various insect pests. Vegetable crops are currently infested with several lepidoptera caterpillars causing economically devastating crop losses. To date, there is no one treatment or technique has been found to be effective in all cases to control insect pest infestations. In this study the possible effects of silicon and ascorbic acid to induce resistance in lima bean against feeding activity of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae were evaluated under greenhouse and laboratory conditions. Results indicated that the foliar spray of silicon (1%) and ascorbic acid (0.2%) as individual application on lima bean 30 days after seedlings emergence reduced the feeding activity of the third instar larvae at 5 days after treatment by 29.6 and 19.2%, respectively. The best efficacy was obtained using a combined treatment by soil drench of silicon 1% and foliar spray by a mixture of (silicon 1% and ascorbic acid 0.2%) (1:1 v/v) showed feeding inhibitory activity of 35.7% and larval mortality of 32.7%. This combined treatment exhibited also significant increase in the activities of two enzymes (polyphenol oxidase and peroxidase) involved in plant defense mechanism. Consequently, the use of silicon and ascorbic acid for induced resistance of plant to insects could be contributed in reducing the use of conventional insecticides.

Introduction

Insect herbivores are responsible for about 15- 35 % of crop annual losses in vegetable production in Egypt and worldwide. Because of less agrochemicals being used and less new insecticides coming on the market due to environmental concern, research efforts are now being directed to find acceptable and safer alternatives which are required for economically viable and

environmentally safe crop protection measures. One such possible alternative to synthetic pesticides is host plant resistance by using more resistant varieties and induction of plant resistance by biotic or abiotic elicitors including silicon (Basagli *et al*., 2003; Cherif *et al*., 1994; Eldoksch and El-Sebae, 2009) and ascorbic acid (Felton and Summers, 1993).

Silicon is not considered an essential element for most plants but research findings indicated that absorption of soluble silicon by plants is beneficial to crops via inducing resistance and protection against pest attack (Epstein, 1994 and Massey *et al*., 2006).

The protection in plants by silicon could be due to its accumulation and polymerization in the plant cells, to form a mechanical barrier as silica – cuticle double layers that difficult to be attacked by the insect pests (Massey *et al*., 2006; Ma and Yamaji, 2006 and Teixeira *et al*., 2017). Furthermore, mechanical barriers are not the only defense mechanism against external agents. Investigations with cucumber plants have shown induced resistance of silicon – treated plants to the fungus *Pythium* spp., resulting from the accumulation of phenolic compounds, lignin and phytoalexins (Cherif *et al*., 1994 and Fawe *et al.*,1998). Materials that induce such defense response in plants called elicitors that can trigger the induced resistance process. Freitas *et al*. (2012) evaluated the use of silicon in integrated management of diamondback moth, as a physical barrier, reducing the use of pesticides for cabbage insect control and found that mortality was high in treatment with 12kg/ha of silicon , they concluded that silicon damaged larval jaw, limiting ingestion and causing high mortality.

Defense related proteins and ascorbic acid in higher plants have received also considerable attention as sources of resistance against insect pests. Felton and Summers (1993) indicated that ascorbic acid is essential for both nutritive and antioxidant functions in phytophagous insects. They indicated that the plant enzyme ascorbate oxidase retains activity in the digestive system of the herbivore *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and high levels of the enzyme are present in several host plants. The enzyme oxidizes L- ascorbic acid to dehydro-L-ascorbic acid, a potentially toxic product. The oxidation of ascorbic acid also produces active oxygen species such as the highly reactive hydroxyl radical, and the nutritional quality of protein for larval feeding was significantly reduced by treatment with ascorbic acid and ascorbate oxidase.

In the present study, using lima bean plants the effect of silicon and ascorbic acid applied alone and in combination as soil or foliar treatment on feeding activity, and mortality of Spodoptera caterpillars were tested. The effect of silicon and ascorbic acid treatment on plant defensive enzymes, peroxidase (POX) and polyphenol oxidase (PPO) was also investigated.

Materials and methods

1. Test plants:

Lima bean seeds (*Phaseolus lanatus* L.) were sown in sandy loam soil in 10 cm diameter plastic pots and grown in greenhouse conditions. Plants could grow for 30 days before exposure to chemical treatment and 35 days before exposure to Spodoptera caterpillars. Plants were placed in rows according to each treatment and six replicate plants were made for each treatment. Plants were watered as needed through the

entire experiment, soil treatment was carried out using 100 ml solution of formulated Mg silicate applied alone with the rate of (1.0%) or in combination with ascorbic acid (0.2%) as 1:1 ratio using drench method.

2. Experimental design:

Chemical treatment in pot experiment consisted of : T1- control (spray with water containing 0.1 % Tween 80), T2- soil treatment with magnesium silicate by drench method (1.0 %), T3- foliar treatment with magnesium silicate, Mg Si (1.0 %) ,T4- foliar treatment with ascorbic acid (0.2%), T5- combined treatment (Soil and foliar treatment) including soil drench with magnesium silicate, 1.0% and (foliar treatment with magnesium silicate, 1.0% + ascorbic acid, (0.2 %) , (1:1, v/v).Tween-80, 0.1% (detergent) was added in each of the prepared solution to allow equal distribution of solution on the lima bean plant leaf surface and to improve the ability of the plant to absorb the solution more readily. Water + tween-80 was used as a control solution. The plants were sprayed by a 500 ml hand spray bottle until run off 5 days prior to the application of the Spodoptera caterpillars bioassays.

3. Bioassays:

3.1. Shorth- term 24 h caterpillars feeding:

A laboratory strain of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was continuously reared on castor bean leaves was used in the bioassays. Five third instar larvae of the caterpillars were placed together with a treated and weighted lima bean leaf from exogenously sprayed lima bean plants in a petri dish (9 cm diameter) or from plants grown in treated soil and allowed to consume the diet for 24 hrs. Six replicates were used for each treatment including the control. The leaves were removed after 24 hrs exposure and weighed to determine the percentage eaten by the larvae. The average of feeding activity of 30 larvae per each treatment was calculated. The feeding inhibitory activity of leafy diet was determine using the equation of Wada and Manukata (1968) for feeding ratio = B/A x 100, Where A = amount of diet consumed in control and B = amount of diet consumed in the treated diet.

3.2. Long – term (12 days) caterpillars feeding:

The second instar larvae of *S. littoralis* were selected for assaying the effect of silicon and ascorbic acid applied alone or in combination and other treatments on lima bean seedlings and their potential resistance to feeding activity of Spodoptera caterpillars. The larvae could consume foliage for 12 days on the different treatments and control leaves present in plastic vials, each vial contains five 2nd instar larvae were placed together on treated lima bean leaf which was changed every two days by fresh one. The vials were covered and maintained at about 26 °C. After 12 days of exposure, the larvae were removed and then they were transferred back to the normal leafy diet. The vials were checked daily for pupation.

3.3. Enzymatic activity determination:

Lima bean leaves were macerated with a mortar and pestle and 10 ml of potassium phosphate buffer (0.1mol/L; pH 6.0) were added to 0.2 g of macerated leaves. The resulting solution was resting for one hour at 4 ºC and the agitation for three times and then the solution was centrifuged at 13000 g for 15 minutes, at 4 ºC for obtaining the supernatants (enzyme extract) which used for enzyme activity determination. The activity of

peroxidase (POX) and polyphenol oxidase (PPO) were measured by spectrophotometry via the increase of optical density (OD), using OD 470 min^{-1} g^{-1} and OD 420 min^{-1} g^{-1}, respectively, following methodology described by Mohammadi and Kazemi (2002) with minor modification . The substrate utilization by peroxidase and polyphenol oxidase were guaiacol / H_2O_2, and catechol, respectively. The enzymatic activity was expressed as units per gram of fresh weight (ug^{-1}). One activity unit was defined as the increment of 0.1 absorbance unit per minute. The result was reported as mean ± SD.

3.4. Statistical analysis:

Data of lima bean leaf weights consumed by *Spodoptera* larvae were statistically analyzed using analysis of variance (ANOVA) with multiple comparison tested with the Duncan's multiple range test method. The *P*-value (0.05) was used for deciding the degree of significance of the different treatments.

Results and discussion

1. Short - term bioassay:

The effects different treatments of soil magnesium silicate (MgSi, 1%), foliar MgSi 1% and foliar ascorbic acid (AA, 0.2%) as well as the combined treatment of soil MgSi and foliar (MgSi + AA) (1:1, v/v) on caterpillar anti-feeding activity during 24 hrs exposure period are presented in Table (1). The data indicated that the combined treatment of soil drench with Mg silicate and foliar application with the mixture of ascorbic acid and Mg Si (1:1) gave the highest feeding inhibitory activity against 3rd instar larvae of spodoptera caterpillars for 24 h feeding period with 35.7 % feeding inhibition followed by foliar Mg silicate, foliar ascorbic acid and then soil drench using Mg silicate with 29.6, 19.2 and 17.4 % reduction in feeding activity respectively compared with the control.

Table (1) : Anti-feeding activity of soil and foliar - applied magnesium silicate (MgSi) and ascorbic acid (AA) against 3rd instar larvae of *Spodoptera* caterpillars.

Treatment	Concentr-ations %	Avg. wt of died consumed per 5 larvae during 24h (mg ±SD)	Feeding ratio (and of control)	Feeding inhibition %
T1: control	-	115±12d	100	0.0
T2: soil MgSi	1.0	95±15c	82.6	17.4
T3: foliar MgSi	1.0	81±8b	70.4	29.6
T4: foliar AA	0.2	93±11c	80.8	19.2
T5: Soil MgSi and Foliar MgSi + AA (1:1)	1.0 + (1.0 + 0.2)	74±7a	64.3	35.7

Mean values followed by different letters differ significantly at 5% level using Duncan's multiple range test

2. Long- term bioassay:

Results after 12 days feeding period of 2nd instar larvae of spodoptera caterpillar are presented in Table (2). The data showed that the effect of the combined treatment of soil drench by Mg silicate (1%) and foliar spray by (Mg silicate (1%) + ascorbic acid (0.2%) (1:1, v/v) exhibited the highest larval mortality (32.7%) followed in a descending order by foliar spray with Mg silicate (24.4%) then, foliar spray treatment with ascorbic acid and then soil drench with Mg silicate with larval mortality of 19.4 and 17.7 %

respectively. Larval mortality was based on % of larvae that did not pupate.

The data of long-term bioassay of caterpillars feeding activity indicated that foliar spray by silicon or ascorbic acid alone made lima bean plants more resistance to spodoptera larvae damage compared with the control. Also, treatment of soil with silicon alone or in combination with ascorbic acid made the plant leaves more resistance to the consumption by *Spodoptera* larvae.

Treatments of soil with silicon alone, foliar spray with silicon alone as well as foliar spray with ascorbic acid alone caused reduction in pupation compared with the control with percent pupation 82.3, 75.6 and 80 6% respectively. Ma and Yamaji (2006) indicated that following silicon uptake by the roots, silicic acid is rapidly translocated to the shoot and leaves, and with increasing Si concentration in the plant sap silicon is polymerized to form amorphous silica and silicon increases the resistance of plants to the green aphid *schizaphis graminum* (Rond.). The conducted results agree with conclusion of many investigators. Shalata *et al.* (2001) reported that the antioxidant and pro-oxidant properties of ascorbic acid are becoming increasingly appreciated for induction of plant resistance against insect pests. Rice cultivar with low tissue silicon is associated with increased susceptibility to insect pests as well as increased problems with crop lodging. Basagli *et al.* (2003) and Keeping and Kvedaras (2008) reported that silicon proved to be as a plant defense against insect herbivory and the application of sodium silicate is deposited on cell wall material forming silica – cuticle double layers and silica cellulose double layer in the leaves and stems of the treated plants that affect the performance of insect pests.

Table (2) : Effect of magnesium silicate (MgSi) and ascorbic acid (AA) applied alone and in combination on some biological aspects of *Spodoptera* carerpillars.

Treatment %	Method of application	Larval mortaility	Avg. days to pupation	Pupation %
T1: control	-	5.9	19.8±0.4c	94.1
T2: MgSi (1.0)	Soil dernsh	17.7	16.9±0.7b	82.3
T3: MgSi (1.0)	Foliar spray	24.4	14.8±0.8a	75.6
T4: AA (0.2)	Foliar spray	19.4	17.3±0.4b	80.6
T5: MgSi & MgSi / AA (1:1)	Soil dernsh + Foliar spray	32.7	14.1±0.1a	67.3

-Different letters indicate significantly difference results at 5% level using Duncan's multiple range test.

3. Enzymatic activity determination:

Results of enzymatic activity determination are presented in Table (3). The data indicated that the greatest PPO activity occurred in the combined treatment of soil Mg silicate and foliar Mg silicate mixed with ascorbic acid (1:1) with (420 ug^{-1} fresh weight), followed by the treatment of foliar Mg silicate alone and foliar ascorbic acid alone (374 and 306 ug^{-1} fresh weight respectively) and then the treatment of soil Mg silicate which showed the least PPO activity (261 ug^{-1} fresh weight). All treatments showed higher activity than the control (144 ug^{-1} fresh weight) (Table, 3).

Table (3) : Polyphenol oxidase (PPO) and peroxidase (POX) activities (mean±SD) in lima bean plants.

Treatment	Enzyme activity of Polyphenol oxidase (PPO) ug^{-1} fresh weight	Enzyme activity of peroxidase (POX) ug^{-1} fresh weight
T1 control	144±11d	152±18d
T2 soil MgSi	261±21c	270±31c
T3 foliar MgSi	374±16b	390±17b
T4 foliar AA	306±28b	345±15b
T5 soil MgSi + Foliar MgSi + AA (1:1)	420±18a	511±15a

-Different letters indicate significantly different results at 5% level using Duncan's multiple range test.

The combined treatment of soil Mg silicate and foliar Mg silicate + ascorbic acid (1:1) (511 ug^{-1} fresh weight) showed the higher POX activity than the treatments with foliar Mg silicate and foliar ascorbic acid which had an intermediate activity (390 and 345 ug^{-1} fresh weight, respectively), treatment of soil Mg silicate (270 ug^{-1} fresh weight) showed the least POX activity but all of these treatments showed higher activity than that of the control (152 ug^{-1} fresh weight) (Table, 3). Khattab (2007) indicated that polyphenol oxidase (PPO) and peroxidase (POX) play an important role in the defense mechanism of cabbage plants (*Brassica olereacea* var. capitata) against phloem sucking aphid (*Brevicoryne brassicae* L.) by increasing enzymatic activity. Peroxidases play an important role in defense against other insects (Dowd and lagrimini, 1997) and their activity has reported to increase as a response to herbivory or wounding. In addition, POX can contribute to insect resistance by quinone oxidation which can bind to protein to reduce digestibility in insects. Polyphenol oxidase is the major anti-nutritive enzyme induced also in response to wounding and insect herbivory. This enzyme oxidizes phenolic compounds to quinone reactive molecule which can interact with other biological molecules (Dowd and Lagrimini, 1997).

The application of silicon compounds and ascorbic acid in crop management may provide a viable component of integrated management of insect pests because they leaves no pesticide residues in food or the environment and can be easily integrated with other pest management practices (Liang *et al.*, 2003 and Massey *et al.*, 2006). Consequently, the use of silicon and ascorbic acid for induced resistance of plant to insects would be contributed in reducing the use of conventional insecticides.

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Egg production and life cycle of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) reared on three cereals

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Abstract:

The effect of three cereal hosts, wheat, sorghum and corn, on the egg production by *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae) under mass rearing condition was examined. The rearing of moths on sorghum yielded significantly larger amount of eggs (21.45 ± 0.004 g) than the eggs produced by wheat and corn reared moths (12.17 ± 0.004 g), which was also significantly larger than the amount of eggs produced by the moths and (3.79 ± 0.008 g), respectively. These amounts of eggs were produced by the moths over a period of 60 day. The chemical analysis of different cereals revealed that no one cereal was richer than another in all nutrients. The three cereals did not substantially differ in their water content. The fat content of corn was like that of sorghum, but both were significantly higher than the fat content of wheat. While corn had the highest carbohydrate content, it had the lowest protein content among the three cereals. Wheat had significantly higher content of protein than did sorghum or corn, but had intermediate carbohydrate content between corn and sorghum. Therefore, none of the obtained results regarding larval development, fecundity or body weight could be attributed to the concentration of a single category of nutrients in the three cereals used for the rearing of insects.

Introduction

Angoumois grain moth *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae) is a small moth with a slender 5-7 mm long body when wings are folded and 10-16 mm wingspan. The moth attacks many host plants, both in field and store room. It infests kernels of corn, sorghum, wheat, rice and other crops. With an adult lifespan of about two weeks, a female lay eggs either singly or in batches of variable sizes; one female could lay up to 100 eggs, but the average is much lower (Dobie *et al.*, 1984). Newly-laid eggs are white but they

quickly change to a reddish color. They are oval with the anterior (micropylar end) truncate and bearing longitudinal ridges and weaker transverse ridges (Carter, 1984). Following hatching, the larva may walk outside the host kernel for 24 h, before making a hole in the kernel and staying inside until pupation (Mahmoud, 2011). However, in stored grains as well as during the mass rearing process, the larva is rarely seen, because it mostly completes its development within a single grain. After entering the kernels primarily in the germ end or its periphery, the larvae rarely move from one grain to the other. Two or three larvae may develop in single grains of maize, but only one adult is produced from a single grain in case of other hosts such as wheat or sorghum (Cox and Bell, 1981). Full-grown larvae spin silken cocoons around themselves within the hollow grains and become inactive for 2 d before pupation, and within 7 to 12 d, depending on laboratory conditions, the adult moths emerge (Akter *et al.*, 2013).

The most important biotic factor that affects rearing of stored grain moths is species or strain of cereal host. The cereal species or strain used as food affect many parameters of the life cycle of the moth (Saljoqi *et al.*, 2015). Even the physical forms in which the cereals are supplied to the insect, i.e., whole grain, crushed grain, broken grain or flour, can be an important determinant of the rate of food ingestion and growth (Uberoi, 1961). *S. cerealella* has variable preferences for certain cereals, depending on the size, texture, coat and structure of the grain, as well as grain's moisture content and rate of weight loss during storage (Hamed *et al.*, 1992 and Nadeem *et al.*, 2011). This variable preference indicates that the nutritional quality of cereals and their physicochemical properties shape the biology of the moth. For example, in the mass rearing of *S. cerealella*, egg production, which is evidently the outcome of the fecundity of females in the culture, is affected by the nutritional quality of host grain (Cônsoli and Filho, 1995 and Borzoui *et al.*, 2017). In addition, the development rate of *S. cerealella* is largely determined by the nutritive and physical characteristics of cereals (Gomez *et al.*, 1983 and Hamed and Nadeem, 2012). Although several studies have been conducted to test the effects of different stored products on the development and fecundity of *S. cerealella* (Shazali and Smith, 1985; Cônsoli and Filho, 1995; Hansen *et al.*, 2004 and Khan *et al.*, 2010), little is known about the effects of cereal species on the moth in a mass rearing system. The aim of the present study is to examine the effect of different cereal species on the life cycle of *S. cerealella* and amount of eggs produced in a mass rearing program of the insect.

Materials and methods

1.Mass rearing of *Sitotroga cerealella* :

To test the effect of cereal on the egg production by *S. cerealella*, the moth was mass-reared on three types of grains: wheat, *Triticum aestivum* L. (Var. Sedes1); sorghum, *Sorghum vulgare* L. (Var. Giza 15); and corn, *Zea mays* L. (Single Hybrid 10). The rearing was carried out in the mass rearing unit at the Plant Protection Research Institute, Agricultural Research Center in Assiut. The three types of cereals are recorded as hosts for *S. cerealella* (Ayertey and Ibitoye, 1987 and Trematerra and Gentile, 2002). The grains were obtained from the Department of Seeds, Directorate of Agriculture, Assiut.

2.The rearing device:

The setup used for mass rearing of the moth was basically the same as

described by Hamed and Nadeem (2010). The rearing chambers consisted of two main elements, grain-holding frames (trays) and emerging boxes. Each grain frame measured 34.5 × 24.3 × 2 cm and could hold about 1 kg of grain. The length and width of the frame could be changed to alter its size in order to fit inside emerging cages of any form but the depth of the crib did not exceed 2 cm to prevent excessive heating. The frame consisted of two screen walls of steel meshes (17 × 30) held 2 cm apart by 3 alometal spacers, and had their top open. The mesh walls held the grain within the frame and allowed the adult moths to pass through. Three emerging boxes were used in the experiment. Each emerging cage measured 54 × 40 × 30 cm and consisted of two distinct parts. The upper part was a bottomless cage with racks to hold 6 grain frames. The racks were arranged such that the frames stand beside each other, 7 cm apart, and held in a slanted position (ca. 25° to vertical) at a uniform manner. This slanted arrangement increases the exposed areas of the frames, thus reducing probable heating of grains inside the frame. The frames were held in position by upper and lower racks. The three walls, the door and the roof of this part of the emerging box were covered with muslin to prevent the escaping of the adult moths, while allowing air exchange. The second and lower part of the emerging cage consisted of a plastic funnel 54 cm in length, 40 cm in width, and 38 cm in height. Each funnel functioned to lead eggs and adults down to a plastic bottle. The emerging boxes were all mounted on a metal stand about 65 cm high. The setup is shown in Fig. 1.

3.Running the setup:

An amount of 6 kg of wheat, corn, or maize was used to fill the frames in each of the three rearing chambers. Each amount of cereal was mixed with enough water and boiled at 100ºC for about 10 minutes to get rid of contamination, kill mites and other unwanted organisms, soften the grains, and create cracks on the surface to enhance infestation and development of the *S. cerealella* larvae. The grains were then left to cool down and on the next day they were loaded in the holding frames and placed in the chambers. In the beginning of rearing, eggs of *S. cerealella* were obtained from the laboratory and used to infest grains in the frames. Two days before infestation, fresh *S. cerealella* eggs of uniform age (0-24 h) were thoroughly cleaned of mites. Equal amounts of eggs, 1 g each, were placed in small containers and left uncovered for two days at 27ºC. This period is the time expected for neonate larvae to approach hatching. The eggs from each container were then evenly scattered on one grain-holding frame, and the frames were fixed in place inside the emerging cage. Accordingly, 1 g of eggs was used to infest 1 kg of cereals.

Figure (1): Two of the cages used for the mass rearing of *Sitotroga cerealella*. 1. Steel mesh, 2. Upper part of the cage, 3. Funnel, 4. Metal stand and 5. Collecting plastic jar.

The eggs produced by moths reared on each type of cereal were daily collected and weighed. Starting from the first appearance of eggs on each host cereal, this was done for 60 successive days, a period roughly assumed to cover two generations of the moth. Adults that happened to escape the rearing chambers and come down with eggs into the collecting bottles were carefully taken and kept in labelled jars until death. In these jars the moths had the chance to mate and the females laid eggs that were sieved through a fine mesh fixed near the bottom of the jar. The eggs produced by these moths were also collected on a daily basis and combined with the eggs obtained from their original rearing chamber on the same day. The whole setup was kept in a rearing room under conditions of 26 ±2ºC., >60 % RH, 14:10 L: D cycle. The experiment was repeated 7 times and the amount of eggs produced using the three different cereals were compared.

4.Host cereal and the weight and size of eggs:

To test whether any variation in the amount of eggs produced from the three mass reared cultures was due to difference in egg weight or due to difference in the number of produced eggs, samples of equal numbers (500) of eggs from the three cultures were weighed. The determination of egg weight by quantifying the mass of several hundreds of eggs has been adopted by other researchers (Hamed and Nadeem, 2012). Before weighing, the eggs were cleaned from scales, cereal or insect remnants. In addition, samples of more than 200 eggs from each culture were examined under light microscope, where the length and the width of eggs were determined with the aid of an ocular micrometer.

5.Cereal type on the life cycle of *Sitotroga cerealella*:

5.1. Larvae:

Since life cycle characteristics such as larval period, pupal period, survival and adult longevity cannot be drawn from the mass rearing process, *S. cerealella* was reared on wheat, sorghum or corn in a separate experiment. Eggs taken from the cultures maintained on wheat, sorghum and corn were separately kept until hatching. Several 300 neonate larvae were taken and reared on the corresponding host cereal. Rearing the larvae on the same host on which their parents have developed was done to avoid possible negative effects that would arise in case they were reared on a different host **(Barron, 2001)**. For each host cereal, the 300 larvae were divided into 6 equal groups and placed in 250 ml plastic tubes supplied with cleaned cereals ad libitum. The conditions of temperature, relative humidity and light regime were the same as mentioned for the mass rearing experiment. The tubes were covered at the top with a piece of muslin and were inspected daily until the appearance of dark circular spots on the exterior surface of the grains. These spots were carefully opened to observe the end of larval stage. The duration of larval stage and survival of larvae were then calculated. However, since the larval developmental takes place within the chamber inside the grain, determining durations of various larval instars was not possible.

5.2. Pupae:

As the pupae appeared, grains containing them were transferred into petri dishes 2 cm high and 14 cm in diameter and kept under the same conditions as larvae. They were checked daily until the emerging of adults and the

pupal period and pupal survival were calculated.

5.3. Adults:

Newly emerged moths were taken daily from the petri dish, weighed singly using a 4-decimal place balance. To investigate fecundity on each type of the grains, paired (1 female and 1 male) newly emerged moths were transferred to glass vials 10 cm long and 1.5 cm in diameter, each provided with grains pasted on stripes of paper to serve as oviposition sites. The vials were daily visited where any paper stripes with eggs stuck to them were replaced with new ones. This was done until the death of moths, where the adult longevity was determined. The deposited eggs were counted with the aid of a binocular light microscope. Lengths of the pre-oviposition period (the period between emergence and the first oviposition incidence), oviposition period (the period from the first oviposition to the last) and post-oviposition period (the period between the end of oviposition and the death of female) were recorded. Samples of eggs were also observed daily to determine the time of hatching. The incubation period and the total development time (egg-to-adult) were then calculated.

6.Chemical analysis of the grains:

To examine how the effects of cereal type on the moth could be attributed the chemical structure of the cereal, samples from the three cereals were analyzed. The grains were milled using a laboratory grinder prior to analysis. Ten gm samples were placed in previously weighed glass tubes and transferred into an electrically heated oven at 100°C and left for two hours. The tubes were removed from the oven and cooled to room temperature in a desiccator. After weighing, the procedure was repeated until the difference between two consecutive weights was smaller than 2% of the original weight (i.e., less than 2 mg). The weight loss was then considered the moisture content. Fat content was determined using a 16-h Soxhlet extraction with petroleum ether according to the Association of Official Analytical Chemists methods (AOAC, 1999). Samples of the dried powdered cereals weighing 10 g each were submitted to extraction on Soxhlet extractor during 16 h, after which the extracts were filtered through small, hardened paper into weighed vessels. Vessels containing residue were dried for 1 h in an oven at 100°C, weighed, and the total crude fats were calculated. The anthrone sulfuric acid method was used to estimate the carbohydrate content according to Laurentin and Edwards (2003). Two hundred mg anthrone reagent was mixed with 30 ml distilled water, 8 ml absolute ethyl alcohol, and 100 ml concentrated sulfuric acid in a conical flask under continuous cooling in an ice bath. Ten mg samples of the dry powdered grains were mixed with 10 ml hydrochloric acid (8N) in test tubes and heated in a boiling water bath for 1 hour. The solutions were cooled, filtered and the supernatant was completed to 10 ml by adding distilled water. A volume of 0.1 ml of the extract was mixed with 4.5 ml of the prepared anthrone reagent mixture. The mixture was heated in a boiling water bath for 7 min, after which it was cooled under tap water. The absorbance of the developed blue green color was measured at 620 nm against a blank containing only water and anthrone reagent. The results were then calibrated against previously known data for glucose concentration in distilled water. The nitrogen content was determined using the method of Micro-Kjeldahl distillation following digestion

with sulphuric acid, basically as described in Khalid and Shadeed (2015). Samples of 1g of grain powder were taken in Pyrex digestion tubes and 30 ml of conc. H_2SO_4 were carefully added, followed by the addition of 10 g potassium sulphate and 14 g copper sulphate. The solution was heated until it became colorless and then allowed to cool, diluted with distilled water and transferred into 800 ml Kjeldahl flask. Three or four pieces of granulated zinc and 100 ml of 40 % NaOH were added and the flask related to the splash heads of the distillation apparatus. Next, 25 ml of H_2SO_4 (0.1 N) was taken in the receiving flask and distilled; it was tested for completion of reaction. The flask was removed and titrated against NaOH (0.1 N) solution using Methyl Red indicator for determination of nitrogen, which in turn gave the protein content.

7.Calculations and statistical analysis:

Data regarding the egg production in the mass rearing of *S. cerealella*, the parameters in the experiment where the moths were reared on the three cereal types, the nutrient contents of cereals, the results were analyzed using one-way ANOVA, followed by Tukey-test for multiple comparisons when significant differences were observed. All the tests were conducted according to Fowler *et al*. (1998), aided by Microsoft Excel software.

Results and discussion

The collected eggs of *S. cerealella* were substantial enough to be weighed during a period of about 60 days in each trial. In addition, although the three types of cereals were infested with the eggs of *S. cerealella* at the same time in the beginning of each trial, the first appearance of eggs produced by the three cultures was not simultaneous. The first yield of eggs was obtained from moths reared on sorghum, followed by those reared on wheat, whereas the corn reared culture was the latest to produce eggs. The time taken by the three cultures to begin producing eggs was 37.71±2.07, 35.71±1.87 and 39.71±2.58 days for wheat, sorghum and corn, respectively. The differences between the three periods of time are not significant (ANOVA; F = 0.429, P = 0.659). The weights of eggs obtained every 5 days were summed and the values from the 7 trials were averaged and shown in Figure (2). Two obvious weight peaks were observed for the sorghum reared culture, whereas the wheat reared and corn reared cultures each had one peak of egg weight. The culture kept on sorghum yielded the largest amount of eggs, followed by the wheat reared culture, then the corn reared culture. The cumulative weight of eggs collected over 60 days, the period during which the cultures were yielding eggs almost daily, is shown in Figure (3). The difference between the weight of eggs obtained from the sorghum reared culture and eggs of the other two cultures began to become significant starting from day 5 in the collecting period. Starting from day 25, the difference between cumulative weights of eggs produced by the three cultures became significant (ANOVA, F = 10.03; $P < 0.001$; *Tukey*-test applied at $P < 0.05$). Whether the observed difference in the amount of eggs produced from the three mass reared cultures is due to difference in egg weight or due to difference in the number of produced eggs, equal numbers of eggs (500) from the three cultures were weighed and the results are shown in Fig. 4. Eggs from the corn reared culture were significantly heavier than those from the other two cultures (ANOVA, F = 14.32; $P < 0.001$).

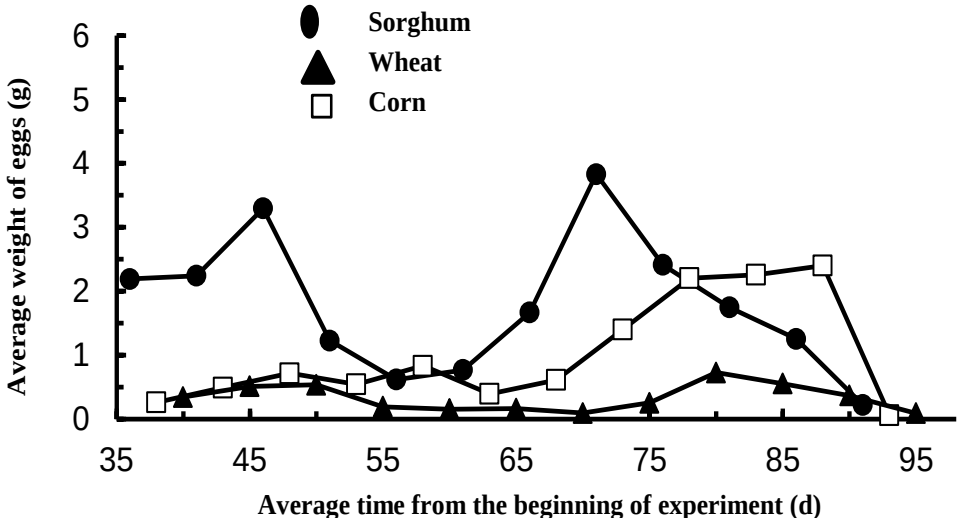

Figure (2): Weight of eggs obtained from mass-reared cultures of *Sitotroga cerealella* kept on 6 kg of wheat, sorghum or corn.

Each point represents the eggs collected over 5 days and is plotted as the mean of the 7 replicates.

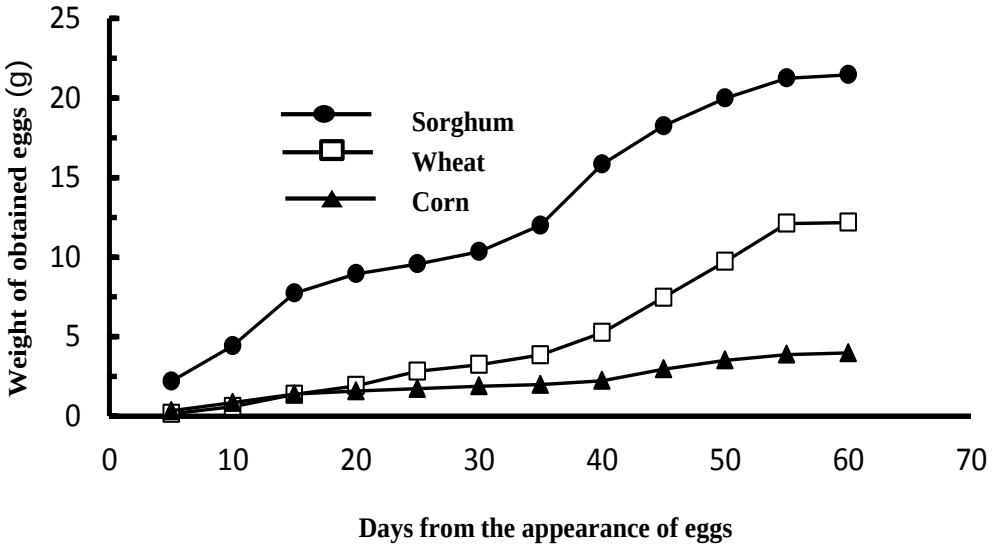

Figure (3): Cumulative weight of eggs obtained from the mass rearing of *Sitotroga cerealella* on 6 kg of wheat, sorghum or corn.

Each point represents the mean of 7 replicates. The difference between the weight of eggs from the sorghum reared culture and the other two cultures began to become significant starting from day 5. The three weights differed significantly from each other starting from day 25 (ANOVA; $F = 10.03$; $P < 0.001$; *Tukey*-test at $P < 0.05$).

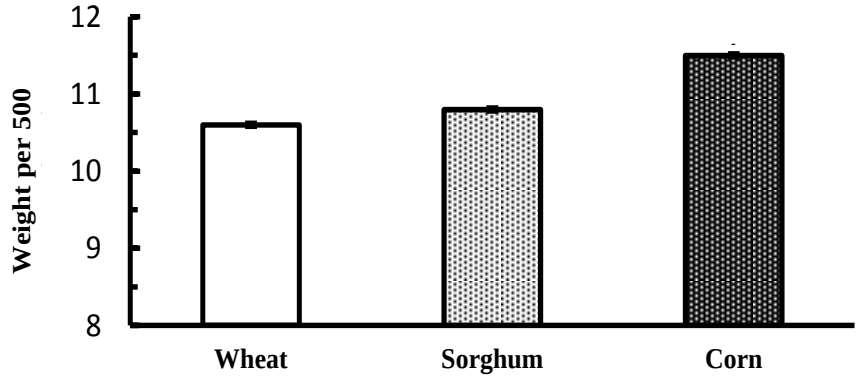

Figure (4): Weight of 500 egg of *S. cerealella* reared on wheat, sorghum or corn grains. N = 10 (500 eggs, each).
Means denoted with different letters are significantly different (ANOVA, $F = 14.32$; $P < 0.001$; *Tukey*-test at $P < 0.01$).

The eggs of *S. cerealella* exhibited remarkable variation in size in relation to host cereal. In general the eggs were elongate and oval in shape that ranged in color from pale yellow to white in the three cultures. Eggs from the corn reared culture were significantly larger, in terms of both width and length, than the eggs obtained from the wheat reared culture. The latter were also significantly larger than those obtained from the sorghum reared culture (ANOVA, $F = 8.93$; $P < 0.001$). The size of eggs taken from the three cultures is shown in Figure (5).

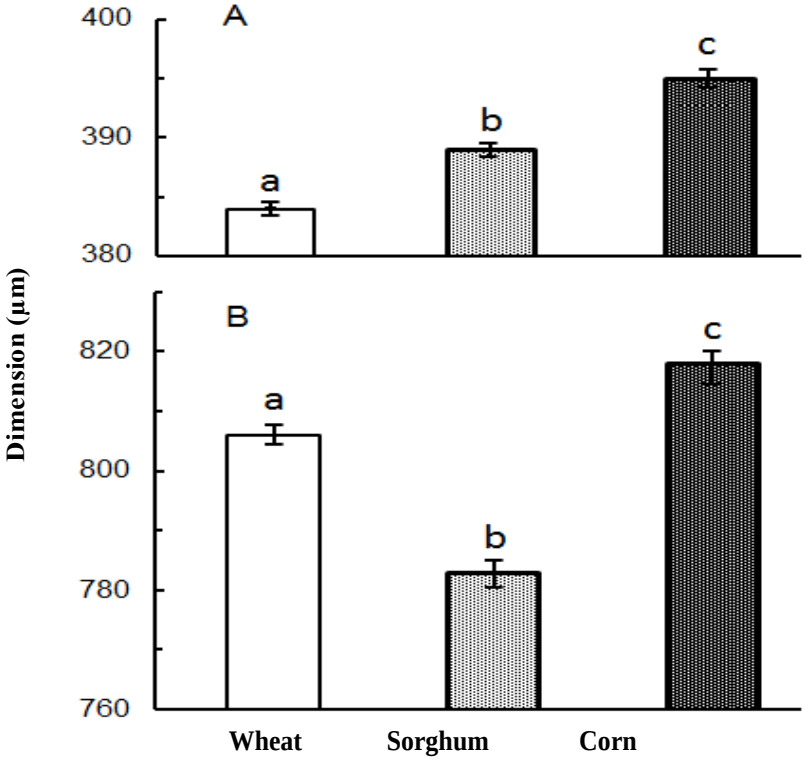

Figure (5): The average width (A) and length (B) of eggs of *Sitotroga cerealella* reared on wheat, sorghum or corn.
N ≥ 200; standard errors are graphically shown; means denoted with different letters are significantly different (ANOVA, for egg width: $F = 8.93$ and $P < 0.001$; for egg length: $F = 22.31$; $P < 0.001$; *Tukey*-test at $P < 0.01$).

1.Effect of host cereal on larval period and larval survival:

Larval period of *S. cerealella* reared on the three cereal types are shown in Figure (6). There was no significant difference between larval period on wheat and its counterpart on sorghum. However, both values of larval period were significantly longer than the larval period on corn (ANOVA, $F = 8.37$; $P < 0.001$; *Tukey*-test at $P < 0.01$).

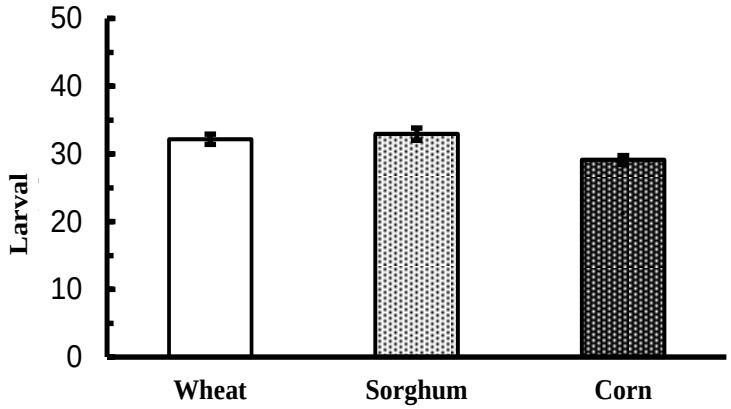

Figure (6): Larval development of *Sitotroga cerealella* reared on wheat, sorghum or corn grains. The food was supplied *ad libitum*; n ≥100; standard errors of mean are graphically shown. Means denoted with different letters are significantly different (ANOVA, $F = 8.37$; $P < 0.001$; *Tukey*-test at $P < 0.01$).

The larval survival was generally low on the three cereal types. The highest survival was observed on wheat, followed by that on corn, after which came the survival on sorghum (Figure, 7). Although the difference between survival on the two former cereals and that on sorghum seemed considerable, the Goodness of Fit test showed that the difference is only weakly significant ($\chi^2 = 4.609$; $P = 0.0998$).

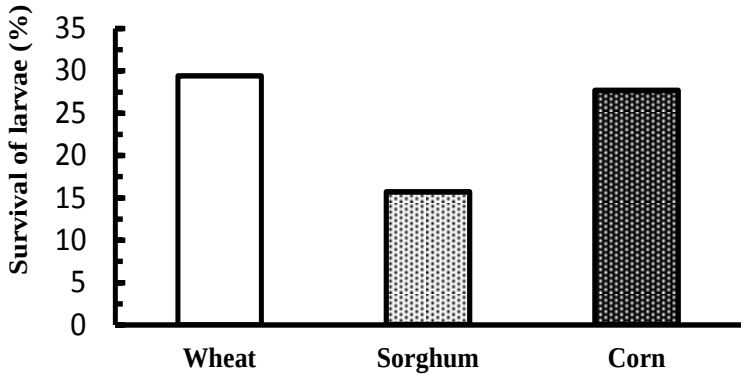

Figure (7): Survival of larvae of *Sitotroga cerealella* reared on wheat, sorghum or corn grains. Differences are not significant (Goodness of Fit test, $\chi^2 = 4.609$; $P = 0.0998$).

2.Effect of host cereal on pupal period and pupal survival:

The length of pupal stage ranged from 6.88±0.18 to 7.08±0.25 days (Figure, 8), with no significant differences between the three cereal types (ANOVA, $F = 0.968$, $P = 0.381$). The highest survival was observed in the pupae that were reared as larvae on corn, and the lowest pupal survival was observed in the sorghum reared insects, but the differences were not significant (Goodness of Fit test, $\chi^2 = 0.637$, $P = 0.727$).

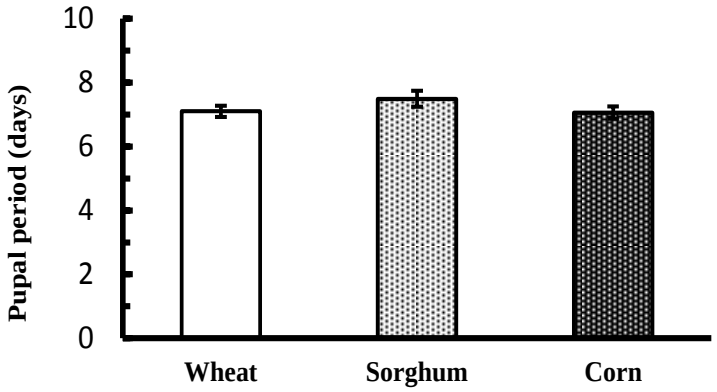

Figure (8): Pupal development of *Sitotroga cerealella* when the larvae were reared on wheat, sorghum or corn grains.
Standard errors are graphically shown. No significant differences were found (ANOVA, $F = 0.968$, $P = 0.381$).

3.Effect of host cereal on adults:

The longevity of adults was not significantly affected by the host cereal. This was the case for both male and female moths. The females lived only slightly longer than the males (Figure, 9). There were also no significant differences between the lengths of pre-oviposition, oviposition, and post-oviposition periods of female moths reared on the three host cereals (Table, 1).

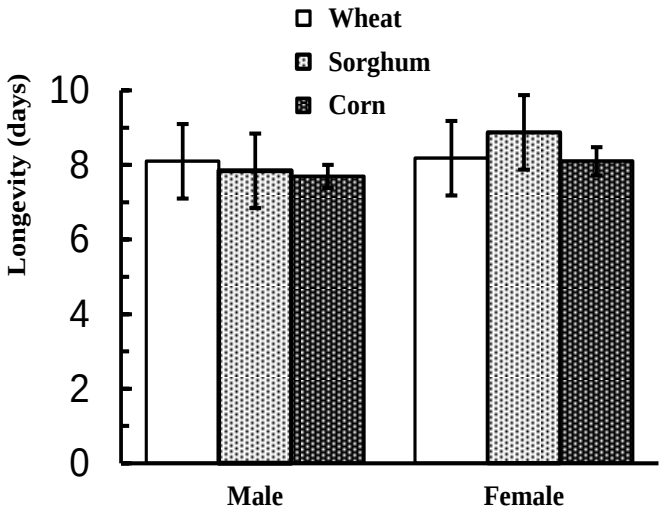

Figure (9): Longevity of *Sitotroga cerealella* moths reared on different host grains.
N ≥ 50; standard errors are graphically shown. No significant differences were found.

Table (1): Length of pre-oviposition, oviposition, and post-oviposition period (in days) of female *Sitotroga cerealella* reared on different host cereals. No significant differences were found.

	Host cereal		
Period	**Wheat**	**Sorghum**	**Corn**
Pre-oviposition	1.78±0.48	1.07±0.46	1.13±0.23
Oviposition	3.68±0.22	4.07±0.49	4.20±0.21
Post-oviposition	1.94±0.34	2.35± 0.34	2.85±0.23
n =	33	14	70

4.Chemical composition of cereals:

Grains were analyzed for their moisture, total carbohydrate, total protein and total crude fat contents. No significant differences in water content were found. However, the three cereals

differed significantly in their carbohydrate contents, with corn having the highest, and sorghum having the lowest content (Table, 2). Protein content was significantly higher in wheat than in sorghum, and the latter was significantly higher than in corn (Table, 2). Crude fats in sorghum did not differ from that in corn, but both were significantly higher than the crude fats in wheat (Table, 2).

Table (2): Percentage of water, carbohydrate, protein and fat in the cereals on which *Sitotroga cerealella* was reared.

	Water*	Carbohydrates*	Proteins*	Fats*
Wheat	5.65 ± 0.004	76.20 ± 0.67^{a}	12.96± 0.026^{a}	3.63± 0.047^{a}
Sorghum	5.84 ± 0.023	74.89 ± 0.42^{b}	10.68± 0.010^{b}	6.57± 0.042^{b}
Corn	5.58 ± 0.003	80.05 ± 0.39^{c}	7.83± 0.008^{c}	5.60± 0.063^{b}
$F_{2,15}$	3.112	7.419	18.436	6.708
P_{ANOVA}	0.074	< 0.001	< 0.001	0.0083

***Data are given as mean ± standard error of mean. N = 6; means denoted with different letters in the same column are significantly different; *F* and *P* values derived from ANOVA are given. For multiple comparisons, *Tukey*-test was applied at $P < 0.01$.**

The obtained results regarding relationship between the three cereal hosts, wheat, sorghum and corn, and the biology of *S. cerealella* under mass-rearing conditions were rather mixed. While the rearing of moths on sorghum yielded the largest amount of eggs, and rearing them on corn yielded the smallest amount, the eggs produced by corn reared moths were both heavier and larger in size, compared to the eggs obtained from wheat or sorghum reared moths. In addition, individual egg weight of wheat reared moths was similar to that of sorghum reared moths but eggs of the former were significantly thinner and longer than those of the latter. However, the weight and size of eggs obtained from the three cultures are largely comparable to the weight and size reported by other researchers (Cônsoli *et al.*, 1999 and Hamed and Nadeem, 2012). Effects of cereal type or even different strains of the same type of cereal on realized fecundity, egg size, and development time of *S. cerealella* have been frequently reported (Ahmed and Raza, 2010; Rizwana *et al.*, 2011 and Hamed and Nadeem, 2012). Such effects are usually attributed to variation among cereals in nutritional quality and physical and chemical characteristics, including cereal morphology, hardness, and moisture content (Khattak and Shafique, 1981 and Khan *et al.*, 2010). The observed high production of eggs in the present study in case of the rearing of moths on sorghum, compared to wheat or corn is consistent with the findings made by Hamed and Nadeem (2012). Obtaining larger amounts of eggs from wheat than from corn reared moths is also consistent with the observations made by Ashraf *et al.* (1994) who studied the rearing of *S. cerealella* on wheat and corn among other cereals. Their results indicated that more progeny was produced when *S. cerealella* was reared on wheat. The distribution of egg production over time in the three cereal hosts suggests a difference in the length of life cycle of the mass-reared insects. Two obvious peak amounts of eggs were observed in case of the sorghum reared culture, while only one peak was observed in case of wheat or corn reared cultures. This result

strongly suggests that the eggs obtained from the sorghum reared culture were produced by two successive generations of moths, and each peak may indicate the time of the largest population size of moths. In contrast, the presence of only one peak amount of eggs in case of wheat reared and corn reared cultures may indicate that the eggs were either the outcome of only one extended generation or two overlapping slow-developing generations. This is consistent with results of other studies showing that sorghum supports faster development rate than other cereals, including wheat and corn (Hamed and Nadeem, 2012), although it is not consistent with the results of developmental experiments in the present study due to factors discussed below. Although grain size was not measured in the present study, it is known that the grain of corn is almost 16 times larger, and that of wheat is about 8 times larger, than the grain of sorghum (Hamed and Nadeem, 2012). It has already been found that the It is therefore plausible to hypothesize that the relatively large size of corn and wheat grains have supported extended larval growth, resulting in only one generation in the same period at which the small grains of sorghum supported two faster developing generations of smaller moths.

When the three cereal types were chemically analyzed for their nutrient contents, the results were rather mixed, with no one type being richer than another in all nutrients. The effects of dietary proteins, fats, and carbohydrates on the life cycle parameters of insects, e.g., growth rate, survival, body weight, adult dispersion, female fecundity and fertility, are relatively well studied in insects (Scriber and Slansky, 1981 and Awmack and Leather, 2002). However, for *S. cerealella* in particular, the development time, longevity and number of progeny produced by a female have not been correlated to the concentration of single type of nutrients. Whereas fecundity has been positively correlated to protein content, it has been negatively correlated to fat content (Rizwana *et al.*, 2011). On the other hand, overall performance of *S. cerealella* has been positively correlated to carbohydrate and water contents but negatively correlated to protein content (Khan *et al.*, 2010). Similarly, trying to link the results obtained in the present study with the chemical structure of grains turned out to be complicated. First, water content did not vary considerably from one of the investigated cereals to another. Sorghum and corn did not differ from each other in terms of fat content, but both had significantly higher fat content than wheat. The three cereals differed significantly from each other only in carbohydrate and protein contents; wheat had the highest content of protein, followed by sorghum, while corn had the lowest content. Meanwhile, corn had the highest content of carbohydrate, followed by wheat, followed by sorghum. Therefore the concentration of any of these nutrient stuffs cannot singly account for the variation in larval survival, larval development rate, female body weight and fecundity of *S. cerealella* observed in the present study. Such intermingled relationships between different nutrient contents and insect performance are common. It is known that nutritional requirements of Lepidoptera change from time to time during larval development. In early instars, for instance, the non-reproductive growth demands diminish and energy storage demands increase, whereas in later instars the opposite is the case, and such changes are typically reflected in

changes in food consumption and feeding behavior (Browne, 1995 and Browne and Raubenheimer, 2003). When offered several types of food that differ in the relative "protein/fat" ratios larva have been shown to shift from one type of food to another depending on their age (Stockhoff, 1993). The difficulty of linking development and body weight to certain nutrient or even certain combination of nutrients in the present study may therefore be due to that larvae were offered only one type of food throughout the larval period. This means that the larvae had no chance to change food with age although they had the chance to alter their rate of ingestion from time to time to balance the ratio of nutrients (Stockhoff, 1993). This conclusion is largely consistent with investigations in which the effects of maize varieties with high protein and low carbohydrate on *S. cerealella* were like the effects of maize varieties with low protein and high carbohydrate contents (Demissie *et al.*, 2015). It is therefore recommended that instead of linking the variation in insect performance to variation in the concentration of one or a few types of nutrients, attention should be focused on the interaction between nutrition, physiology, behavior, and ecology of the different life stages of the insect (Thompson, 1999). As far as the authoress knows, using a mixture of different cereals to rear one culture of *S. cerealella* has not been tried before. It may be useful to carry out such trial to see if larvae can optimize their nutrient acquisition by moving from a grain to another in such setting.

Because sorghum was the food on which the moths produced the largest amount of eggs, it is concluded that it can be better than wheat and corn for the rearing of *S. cerealella*. It is recommended to use sorghum as food for the mass rearing of *S. cerealella* when plentiful eggs are needed for the commercial production of *T. evanescens* or other parasitoids. However, corn may be far better than sorghum for the rearing moth when the target is to obtain large moths and/or large eggs for experimental purposes.

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The blister beetle *Meloe proscarabaeus* (Coleoptera: Meloidae) a dangerous pest threatens field crops in New Valley Governorate, Egypt

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Abstract:

The blister beetle, *Meloe proscarabaeus* L. (Coleoptera: Meloidae) was recorded for the first time as a serious insect pest attacking wheat (*Triticum aestivum*), faba bean , peas, alfalfa, onion and wild weeds in El-Farafra Oasis, Western Desert, Egypt. Beetles feed on foliage and flowers of injured plants causing defoliation and crop loss. The population dynamics and bionomic observations in the wheat fields of *M. proscarabaeus* were studied during two successive seasons (2016/2017 and 2017/2018) in Wheat fields. The pest develops one generation per year. The beetle continued to appear during the first week of December to the first week of April, with the peak of adults at the first week of February. Beetles secrete a cantharidin fluid, a potent blistering agent which burns plant leaves and flowers and at the same time, it is strong poison to all livestock and domestic animals feeding on contaminated plants. The present work shed light on food plants, symptoms of infested crops, adult activity, environmental effects, and sexual behavior of the blister beetle *M. proscarabaeus* under the circumstances of El- Farafra Oasis.

Introduction

Blister beetles or oil beetles are members of the family Meloidae (Coleoptera). This family includes over 300 species in the United States (Selander and Bouseman, 1960; Stebnicka, 1987; Bologna, 1988 and Odegaard and Ligaard, 2000). The genus Epicuta is the largest and contains many species that concern forage producers in semi - arid regions of the western United States. In Egypt, Alfieri (1976) recorded 9 species belonging to family Meloidae collected from different desert localities vicinity to Cairo. Most adults eat only floral parts, but some, particularly those of *Epicuta* spp., eat leaves. El-Sheikh (2007) observed that beetles belonging to *Meloe proscarabaeus* L. (Coleoptera: Meloidae)

eat leaves and flowers of faba bean plants. A few adults are noctural; most are diurnal or show no distinct diel cycle (Selander and Fasulo, 2000 and Bologna and pinto, 2002). However, except for first instar larvae (triunglins) frequenting flowers or clinging to adult bees. So far as known, all larvae are predators. Larvae of most genera enter the nests of wild bees, where they consume both immature bees and the provisions of one or more cells ((Lückmann and Kuhlman, 1997; Klausintzer and Rauch, 2000 and Stebnicka, 1987).

In Central Europe, several types of the Meloidae family have become rare in some regions. The fertility of Meloidae has reached 90% the number of eggs per female is 9500 eggs inside a trench dug by the female (Lückmann, 2001 and Bologna and Pinto, 2001). Blister beetles produce cantharidin, which is toxic to people and animals (Ward, 1985). For centuries, cantharidin was prescribed as a cure for variety of animals. Spanshfly or cantharis, an oreparation of dried beetles, was thought to cure gout, carbuncles, rheumatism and many other medical disorders, in addition to its use as an aphrodisiac (Kinney *et al*., 1998).

The present article sheds light on the occurrence of the blister beetle *M. proscarabaeus*, population dynamics and bionomic observations in the Wheat fields and other legume crops as being recorded for the first time in El-Farafra Oasis.

Materials and methods

1.Study area:

El-Farafra Oasis lies in the western desert, south part of Egypt, located at latitude and longitude (26°49′23.3″N 27°46′33.3″E), belonging to New Valley Governorate; it is 600 km far from Giza. El-Farafra Oasis is rich with submersible water which facilitates reclamation of many thousand hectares. Many different field crops have grown successfully with high yields, especially winter Wheat and legume crops. El-Farafra Oasis is characterized by a very hot desert climate, temperatures during the summer season reach 60 degrees Celsius and decrease significantly in some months during the winter to one degree Celsius. Rain is rare and the average relative humidity is 30-45%. One of the most common cultivated crops there is Wheat (*Triticum aestivum*), its cultivated area amounts approximately 6500 feddans, however, and this crop is threatened by the attack of the blister beetle, *M. proscarabaeus* L., the most serious pest **(El-Sheikh, 2007).**

2.Beetles sampling:

Field observations on the blister beetle, *M. proscarabaeus* L. (Coleoptera : Meloidae) including beetle emergence , distribution, sexual behavior, feeding habits, and dial activity were carried out throughout the years 2016 /2017 and 2017 / 2018 in El-Farafra Oasis, western desert of Egypt. Observations commenced from mid-November till late April; activity period of beetles in the field at about 10 am. The field observations included the study of emergence period, population ecology of the beetles at different times and sexual behavior. This was carried out in two ways: (a) Oviposition sites of the newly emerged female beetles were recognized in the field and marked; these were separated into eight groups, three sites each, according to date of egg laying. Each site contained one egg mass (4000 – 4500 eggs). Wire – wooden cages, 35 x 50 x 50 cm each, were fixed on each site. Daily observations of cages were continued until beetle's emergence. The number emerged beetle of each group was recorded as well as time of

emergence and related environmental prevailing temperature. (b) In El-Farafra as a whole adult *M. proscarabaeus* have been recognized in the months December, January, February, March and April. Freshly emerged beetles were marked with a color code (Whitehead, 1991). This proved that emergence took place in synchronous waves during November and December. Similarly, number of emerged beetles was correlated with prevailing temperature of the environment. Population dynamics of newly emerged beetles and adult abundance in relation to prevailing temperature and relative humidity were conducted in Wheat fields during two successive seasons 2016 / 17 and 2017 / 18. Density of beetles was assessed as direct count in 100 m of Wheat plants. Counting of beetles was carried out in the different directions (north, south, east, and west) of the field at weekly intervals (El-Sheikh, 2019).

The micro-climatic conditions of the air as temperature and relative humidity were also measured. The life – span and the fecundity of adult were determined by confinement freshly emerged beetles in pairs (female & male) on Wheat plants inside wire - wooden cages (35 x 50 x 50 cm) fixed in the field. Daily observations on sexual behavior, feeding habits periodicity and egg laying were started from the first week of December (first beetle emergence) to mid-February (last date of beetle emergence). Data were derived from 24 field cages.

3.Meteorological data:

Daily maximum & minimum air temperatures (˚C), air relative humidity (%) were supplied by the Meteorological Station at New Valley Governorate.

4.Statistical analysis:

To analyze the association between agro-climatic factors prevailing in El-Farafra Oasis and population dynamic of the blister beetle adults through activity time. Weekly number of collected beetles was plotted against the considered agro-climatic factors to establish the relationships between these factors and adult density using correlation and partial regression analysis (α) (Fisher *et al*., 1943).

Results and discussion

1. Food and feeding habits:

Adult beetles *M. proscarabaeus* were feeding on wheat leaves, stem, and spikes, where the beetles are observed moving from places where they were located during their summer (aestivation) dorms towards wheat fields and adults attack in swarms where they disperse and start feeding for up to 80 days. El-Sheikh (2007) explained the blister beetle insect pest infesting crops of beans, alfalfa, onions and wild herbs, which is a pest on flowers, sugar beets and cabbage in Europe (Ozbeck and Szaloki, 1998; Stebnicka, 1987 and Selander and Fasulo, 2000). Date of planting wheat in Farafra Oasis From mid-November, beetles begin to appear when wheat is in the seedling stage about 10-15 days after planting these young plants are the most preferable food for Blister beetles (Figure, 1).

The newly emerged blister beetle are distinguished by strong mandibles and long legs that help them climb the plant, attack wheat seedlings, and feed on leaves and stem, causing completely destroy the whole plants. At risk, the *M. proscarabaeus* secrete a yellow liquid from coxal and antennal joints (Figure, 1). These blister beetles are used to defend themselves, and this liquid causes the leaves to burn and turn brown, eventually the plant dies. Plants that are

under severe attack by blister beetle fail to produce flowers and spikes and consequently the crop is completely lost. Turco (2003) recorded a female *M. proscarabaeus* grazing on *Ranuchus* sp. in Cornwall. Feeding of beetles occurs during the day- light and continues until sunset, and we notice the most general number of beetles on the field side closest to the irrigation canals. Ward (1985) reported that blister beetles feed on plant materials, particularly flowers of such plants as alfalfa, careless weed , peanuts, soybeans and many other species. As shown by Pinto and Selander (1970), the beetles were not recorded on the wheat crop. It appears that the wheat crop is not registered as food for beetles and thus becomes food plant as a diet of *M. proscarabaeus* the first record in Egypt.

2. Seasonal activity of beetles :

In EL-Farafra Oasis, all adult blister beetle, *M. proscarabaeus* were recognized in December, January, February, March and April in 2016 and 2017. We noticed the emergence and dispersion of newly emerging beetles in the fields of wheat. The weekly assessment of the density of adult beetles in different parts of the wheat field indicates that November 3th is the first adult activity and April 2th as the last (Table, 1). Adults emerged from larvae that were in the soil and then evolution into a pupa stage and then an adult insect in first week of November with a few ranged between 12 and 15 beetles / 100 bean plants It was average air temperatures 25.3 - 26 °C and relative humidity 51.5 - 60 % , in 2016 and 2017 respectively (Tables, 1 and 2). The population density of the blister beetle gradually increased with a distinct peak in the first week of February; the average adult density was 115 and 130 beetles / 100 plants in 2016 and 2017 (Tables, 1 and 2).

With the decrease in air temperatures and the increase in relative humidity, the number of blister beetle increased gradually, with the average monthly catch rate of about 39-36 % during the months of January and February in 2016 /17. Adult activity decreased significantly, as the average catch rate in March and April was 8-1% of blister beetle in 2017/18 (Tables, 1 and 2). The study showed that the period from December to February was the highest number of beetles. The weather conditions that prevailed during the two seasons of the study did not differ significantly, accordingly, the variation of *M. proscarabaeus* population for adults mean in 2016/17 (44.8 beetles) and 2017/18 (53.05 beetles). However, climate factors played a large role in the number of beetles in significantly reducing or increasing the population of beetles, and the relationship of temperature was a direct and the relative humidity is an inverse relationship during the two seasons (Tables, 1 and 2). Results from the weekly follow-up of the insect population reveal the persistence of behavior in adults blister beetle *M. proscarabaeus* under EL-Farafra and has only one generation per year. Whitehead (1991) reported that an adult from *M. rugosus* was identified in October and November and that the apparition occurred in simultaneous waves during September and October. However, the frequency of the appearance of adults *M. rugosus* occurs in the same period as *M. proscarabaeus* three waves of one stage have been observed for the present species.

Table (1): Mean numbers of the blister beetle *Meloe proscarabaeus* adult emerged in EL-Farafra Oasis 2016, 2017 in relation to air temperatures and relative humidity.

Inspection date	Mean no. of beetles /100 plants	Climatic factors		% Number of monthly beetles
		Mean air temp. °C	Relative humidity %	
Dec. 3/2016	12	25.3	51.5	14% b
10/2016	17	23.1	66.5	
17/2016	20	24.0	53.5	
24/2016	26	22.5	46	
31/2016	34	21.2	58.6	
Jan. 7/2017	56	19.6	61.3	39% a
14/2017	73	20.1	67.5	
21/2017	89	19.7	71.5	
28/2017	101	18.2	57.5	
Feb. 4/2017	115	17.0	58	36% ab
11/2017	75	16.8	66	
18/2017	61	18.6	55.3	
25/2017	40	20.3	45.2	
March 5/2017	31	22.9	39.4	10% c
12/2017	28	24.4	37	
19/2017	17	26.5	39	
26/2017	8	30.7	32	
April 2/2017	5	35.4	30	1% d
Total	**808**			**100%**
Mean	**44.8**			**25%**

Table (2): Mean numbers of the blister beetle *Meloe proscarabaeus* adult emerged in EL-Farafra Oasis 2017, 2018 in relation to air temperatures and relative humidity.

Inspection date	Mean no. of beetles /100 plants	Climatic factors		% Number of monthly beetles
		Mean air tmp. ^{o}C	Relative humidity %	
Dec. 5/2017	15	26	60	
12/2017	19	24.3	58	
19/2017	25	25.1	56.2	10% b
27/2017	31	23.2	52	
Jan. 3/2018	40	20.6	59	
10/2018	55	18.8	63.2	
17/2018	69	20.7	64.3	42% a
24/2018	95	18.6	70.4	
31/2018	113	17.5	65	
Feb. 7/2018	130	16	60	
14/2018	95	15.8	58	39% ab
21/2018	75	16.4	52	
28/2018	53	18.9	44	
March 7/2018	30	20	42.1	
14/2018	18	23.5	36.5	8% c
21/2018	10	25.7	38.2	
28/2018	7	28.9	32.4	
April 5/2018	5	34.7	31	1% d
Total	**955**			**100%**
Mean	**53.05**			**25%**

3. Mating:

Several pairs of adult's blister beetle were observed in intercourse during the periodic inspection of affected wheat fields, copulation occurred during daylight. Blister beetle, *M. proscarabaeus* showed evidence of mating that reached sexual maturity (50 days post emergence). Certainly those males are attracted to females by sex pheromone emitted by the female. The male begins searching for the female in the early morning and when they meet, courtship may start. Primarily, males touch the female partner's antennae that have faced their side and when the female shows a response, the male touches her abdomen by its antennae. Repeated touches may occur until the female stops quietly, the courtship period lasts 30 minutes. The male jumped on the female side quickly and held the female by the front legs in the thorax area and the back legs in the female's abdominal area (Figure, 1).

Females are taller and larger in size than males; females pull their abdominal segments (telescope movement) to cope male's abdominal end. *Male* abdominal tip flexed below the abdominal tip of the female, the highly chitinized male genitalia protruding and a great part of it was inside the female body, widely opening the female genitalia aperture for the entrance of the apical fleshy part of aedeagus. After this, the male rolled up to the opposite direction (tail to tail position) and mating was carried out see Figure (1). The maximum period of the act of copulation lasted for about one hour. When the male and female are disturbed during copulation, they soon separate from one another. Selander and Pinto (1967) describes sexual behavior in the Meloidae family, as it is very similar in sexual behavior. The male and female meet in the early morning and start feeding, Then the male begins to flirt with the female, and its duration varies from one type to the next,

after the female's approval, copulation takes place. *Turco et al*. (2003) explain that the sexual behavior of the beetle includes strange stages, when the male meets the female, the female controls the male through a sexual pheromone.

4. Oviposition habits:

It was noticed that the female blister beetles after mating begin to search for the preferred location for ovulating and she spent two hours searching for the right place. These blister beetle, *M. proscarabaeus* prefer sandy soil and choose elevated sites from the field near the irrigation canals. Females begin to prepare the egg chamber hole, which is 5 cm in diameter and 6 cm deep in the border strip of the field, and used mandibles, fore and hind legs in excavating the oviposition chamber. After finishing the ovary chamber preparation, it will settle inside and the head and the rest of the body appear inside the chamber, oviposition lasted four hours. The female lays eggs at once, and the eggs are distinguished by a yellow color and arranged in a wonderful way. The female started laying eggs in the last week of January, and after finishing laying the eggs, you then go to the wheat fields for feeding until death see Figure (1).

The results agree with Selander and Fasulo (2000) that a blister beetle, *M. proscarabaeus*, dig an eggs chamber and lay eggs inside. There are some species that lay eggs on the leaves of the plant. Although the female *M. proscarabaeus* laid eggs once in January, the *M. rugosus* females deposit their eggs once in November and twice in December (Whitehead, 1991). The *Meloe* female forms an egg chamber 2 to 3 cm deep in the ground and lays several batches of yellow eggs (Bohac and Winkler, 1964).

5. Significance of the blister beetles:

Field and laboratory research has shown that adult beetles, *M. proscarabaeus* exudates a yellow liquid in abundance from the joints of their legs and Antenna. This fluid is often excreted by reflexive bleeding when an adult beetles are at risk see Fig. (1). This phenomenon is most common in most type's species of Meloid beetles (Ward 1985; Edwards *et al*., 1989 and Whitehead 1991). Like other species of blister beetle, *M. proscarabaeus* contain a large amount of oily, yellow hemolymph that you exude on annoyance. This fluid has been identified as a cantharidin (a bicyclic terpenoid C10 H12 O4). It is found in hemolymph and gonads of beetles. Lytta respiratory (Meloidae) contains more cantharidin than any other member of the family; cantharidin is found mainly in elytra but it has also been shown to exist in the genitalia and the hemolymph (Bohac and Winkler, 1964). Males have the highest levels of cantharidin and they transfer it to females during copulation. Adult blister beetles feed on alfalfa leaves and flowers in the United States, but the real problem lies in the secretion of beetles to the cantharidin substance that toxicity livestock, especially horses, when they are accidentally consumed in feed (Schmitz, 1989).

Many of the common species of blister beetles contain cantharidin (Spanish-fly), a substance that will cause blisters when applied to the skin (Beasley *et al*., 1983). Ward (1985) reported that cantharidin is a stable chemical and long-term health threat to nearly all livestock, particularly horses that are fed contaminated alfalfa hay. Research reports indicate cantharidin toxicosis can be induced in dairy and beef cattle, goats and sheep; other reports include rapist, hedgehogs, mice and dogs (Graziano *et*

al., 1987). Cases of human death also have been reported. However, horses appear to be more susceptible to toxic effects of this potent chemical than other livestock. Although the toxic effects of cantharidin to all livestock and human, blister beetles use this fluid and related analogs as defensive compounds against larger herbivores and predators see Figure (1).

According to the previously mentioned results, the presence of the blister beetle *M. proscarabaeus* as a new insect pest in El- Farafra Oasis may bring us to ring the dangerous bell about the great loss and damage that threat our legume crops and all livestock in such new reclaimed and cultivated areas in Egypt.

Figure (1): a. Blister beetle feeding on wheat plant. b. Courtship period between male and female. c. Mating and copulation behavior (tail to tail position) of the blister beetle. d. Egg laying holes excavated by adult females. e. Egg mass of the blister beetle *Meloe proscarabaeus*. f. Cantharidin fluid excreted by adult beetles on beetle leg.

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Green silver nanoparticles production by and against the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract:

The novel method to produce green silver nanoparticles by *Tetranychus urticae* Koch. (Acari: Tetranychidae) was depicted to provide new biocides. It was started by the exposure of adult females of *T. urticae* to little amounts of silver nitrate AgNO3.Then, table-salt, sodium chloride, was used as an intermediate compound to produce biological spherical silver nanoparticles (AgNPs) by mites. Coating with biogenic amines released from exploded *T.urticae* was detected by monoamine oxidase interaction. Then, such a structure played an important role to penetrate the integument of exposed *T.urticae* easily. Bio-nanoparticles of silver produced from the green form of *T.urticae*, which caused certain mortality percentages over 97% against the same species, while they were more than 90% against the red form of *T.urticae*. In the same trend, the bio product from the red form of *T.urticae*, caused mortality over 88% and 94% in the case of treatments against green and red *T.urticae* forms, respectively. Consequently, determined LC50s of bio AgNPs from green against both forms were 20.58 and 31.81 μLL^{-1} while they were 29.24 and 58.35 μLL^{-1} of bio AgNPs from red *T.urticae* and against the same arrangement of morphs. Therefore, AgNPs resulted from the green form of *T.urticae*, caused sterility with 91.21% and 86.07 % in case of treatments against *T.urticae* green and red forms, respectively. Also, AgNPs from the red form of *T.urticae*, caused sterility with 88.68% and 94.21 % in case of treatments against the same arrangement of morphs, in comparison with control. Revealed data showed that oxidative stress interacted effectively by increase with induced treatments. Reactive oxygen scavengers (ROS) were significantly lower than control ($P < 0.05$). To conclude, the new trend to produce bio AgNPs by mite against the same pest presented a cheap, simple and ecofriendly method to expand the use of nanoparticles in the plant protection field.

Introduction

Pesticides are exceedingly dynamic substances that can debilitate the upholding territorial integrity of certain environments. Because of across the board pesticides to croplands, they have constituted an intemperate danger to all components of biodiversity. So the production of bio nanoparticles through unusual resources would provide a new solution to such dangerous problems. Overall, nanoparticles have advantages as pesticides which presented an evolutionary paradigm. The system of nanoparticle conveyance permits numerous naturally dynamic operators to achieve the coveted site of activity. The upsides of nanotechnology are as per the following: (I) expanded bioavailability (fast disintegration; enhanced entrance/saturation through layers); (ii) minimized required concentrations; (iii) minimized dose-dependent toxicity; (iv) monitored emission; (v) directed bio-distribution; (vi) decrease of the environmental impact on bioavailability inconstancy (Bhushan, 2004; Rao *et al*.,2005; Cheng *et al*., 2015 and Rai *et al*.,2015).

The development, solidness, and movement of nanoparticles depend not just on their shape and size-controlled dissemination at the same time, additionally on their blend root. Numerous procedures and strategies have been embraced for getting ready metallic nanoparticles of different sorts, including polyol techniques (Kim *et al*., 2007), borohydride decrease, dissolvable extraction–reduction (Esumi *et al*.,1991), sonochemical techniques (Mizukoshi *et al*.,1997), photolytic reduction, radiolytic reduction, laser removal, and micro emulsion. Besides, green synthesis of metallic nanoparticles by natural products as reducing and capping agents is represented the easiest methods, ecofriendly, and most effective techniques (Shankar *et al*.,2004; Mondal *et al*.,2011 and Mittal *et al*.,2013). Even the green process could be done in one step by biogenic reduction of the metal ion by plant extracts to get Ag and Au nanoparticles (Mittal *et al*.,2013).

So this paper provided a trial to produce AgNPs from pest against pest and coated them by biogenic amines from exploded resource production. *Tetranychus urticae* was being the resource and the required pest to control at the same time. Thereafter, toxicity and sterility of resulted bio AgNPs from both morphs of *T.urticae*, green and red, were tested against them and almost its mode of action was examined upon oxidative stress.

Materials and methods

1.Maintenance of *Tetranychus urticae* colonies:

The two forms, green and red of *Tetranychus urticae* were gathered from normally cowpea (*Vigna unguiculata*) and strawberry (*Fragaria ananassa*) plants, independently. At that point, maintenance was done on the castor bean leaf discs under laboratory conditions as indicated by Abd El-Wahab (2010) for a half year before treatments.

2. Formation of silk balls:

At that point, single discs of castor bean plants were put separately in cages (30×30×20 cm) under institutionalized conditions (26°C, 33% RH). 500 adult females of each morph were collected by fine brush and permitted to develop on each disc. The pinnacle of the wooden stick (5 cm high, 3 mm diameter) and a square piece of graph paper (2×2 mm) were fixed on the stick to scale the surface of the ball. was watched each hour to recognize the silk

ball's arrangement at the development time. The ball was shaped by 500 mites for all time amassed over the wooden stick which buried in almost 2cm thickness layer of salt. When the first mites landed on the stick, the number of others moving to the highest point of the stick was tallied over a time of 10 minutes, two times a day (at 8h15 and 14h) until the expulsion of the ball at about day 18. Silk ball formed and then harvested occurred at 14 h (TH). To gather the ball, the wooden stick was painstakingly expelled from the plant and from there on the ball was gathered from the stick end with tweezers.

3.Biosynthesis of silver nanoparticles (AgNPs):

Biological silver nanoparticles (AgNPs) were synthesized by reaction with sodium chloride was done for the first time and provided an easier trend to do so. 0.01 mg $AgNO_3$ was spread on the admitted plants just once for by 500 adults of *T.urticae* and the exposure was for 3 hours. Then, the addition of 0.1 mg of NaCl as a stabilizer and as a reduction agent had occurred. Released AgNPs were attached with the silk fibers of *T.urticae* away of salt particles even in the outer or inner layers of fiber balls. Characterization of resulted AgNPs was done by scanning electron microscopy (SEM) as shown in images (1 and 2).

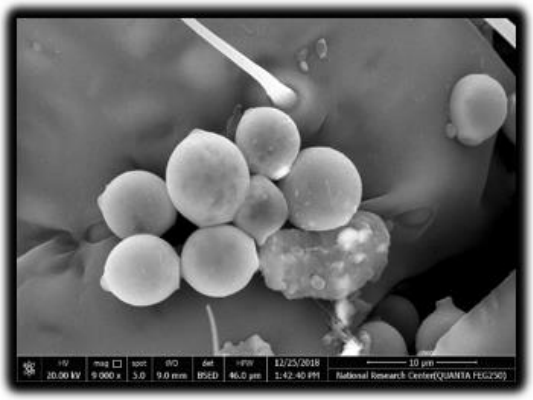


Image (1) Silver nanoparticles (100nm) coated by biogenic amines to produce full capsules

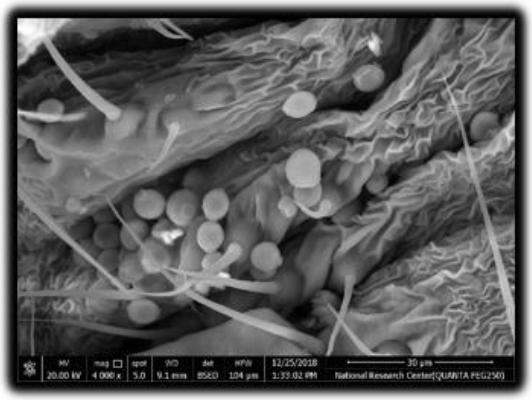
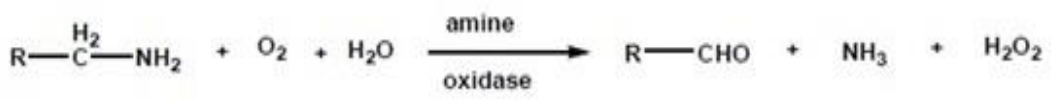


Image (2) Spherical silver nanoparticles (100nm) coated by biogenic amines from exploited bodies of *T.urticae*

4.Monoamine oxidase (MAO-A) Interaction to detect coated biogenic amines:

MAO-A is a flavin adenine dinucleotide (FAD) containing enzyme which is tightly anchored to the mitochondrial outer membrane and responsible for the reaction at equation (1).



Equation (1): Reaction of Biogenic Amines with Mono Amine Oxidase (MAO).

MAO-A potencies were determined in the released homogenates as coatings of AgNPs through each interaction. The rate of the MAO catalyzed oxidation of Kynuramine was measured according to Aiyegoro and Van Dyk (2011). Kynuramine is non-fluorescent until undergoing MAO-catalyzed oxidative deamination and subsequent ring closure to yield 4-

hydroxyquinoline, a fluorescent metabolite. The concentrations of the MAO-generated 4-hydroxyquinoline in the incubation mixtures were determined by comparing the fluorescence emitted by the samples to that of known amounts of authentic 4-hydroxyquinoline at excitation (310 nm) and emission (400 nm) wavelengths. All enzymatic reactions were carried out to a final volume of 500 µL in potassium phosphate buffer and contained kynuramine as substrate, MAO-A (0.0075 mg/mL) and various concentrations of the test inhibitor (treatment). The reactions were carried out for 20 min at 37°C and were terminated with the addition of 200 µL NaOH (2 N). After the addition of distilled water (1200 µL) to each reaction, the reactions were centrifuged for 10 min at 16000 × g. To determine the concentrations of the MAO generated 4-hydroxyquinoline in the reactions, the fluorescence of the supernatant at an excitation wavelength of 310 nm and an emission wavelength of 400 nm was measured (Novaroli *et al*., 2005).

5.Toxicity of biosynthesized AgNPs against the two morphs of *Tetranychus urticae:*

The main used emulsion was prepared from the certain amount 0.01 g/L. Then bioassay was done by leaf discs of castor oil plant which dipped in prepared concentrations (Dittrich, 1962). Three replicates for each treatment with 300 mites/ replicate. Mortality results were taken after 24 hours of exposure and LC50s were estimated (Finney, 1971).

6.Assessment of the sterility effect of biosynthesized AgNPs against the two morphs of *Tetranychus urticae*:

300 mated adult females, 100 individuals/replicate, were treated with LC_{50} of bio AgNPs produced from green *T.urticae* (20.58 and 31.81 $\mu L L^{-1}$) and then red morph (29.24 and 58.35 $\mu L L^{-1}$,resp., based on Finney (1971) by leaf dip technique (Dittrich, 1962). Leaf-discs were placed onto the moistened cotton pad in Petri-dishes after treatments. Both positive and negative control samples which were with 300 mated adult females for each one of both morphs as replicates vis-à-vis both treatments. Treated and untreated individuals allowed to lay eggs for 24 hours according to Abd El-Wahab (2003), then adult females were removed. Eggs were left for hatching and all required biological parameters were determined to calculate sterility percentages (Toppozada *et al*.,1966).

7.Antioxidant of enzyme activities in treated mites by AgNPs:

APX activity was measured by estimating the rate of ascorbate oxidation (extinction coefficient 2.8 mM/cm). The 3 mL reaction mixture contained 50mM phosphate buffer (pH 7.0), 0.1 mM H2O2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of enzyme extract. The change in absorbance was monitored at 290 nm and enzyme activity was expressed as the unit's min/mg protein (Nakano and Asada, 1981).

8.Data analysis:

The statistical software SPSS for Windows 16.0 was used to perform T-test. Values of $p<0.05$ and $p<0.001$ were considered as statistically significant values. Gained data through perceptions were not in every case typically dispersed. As long as, both parametric and non-parametric tests were utilized in this research. Linear regression was used to define the relation between treatments and the non-linear squares was utilized in nonlinear relationships. A paired t-test was utilized to decide the distinction

between endurance paces of mites inside balls.

Results and discussion

1.Detection of biogenic amines:

Mono Amine Oxidases (MAO) was used to detect the presence of biogenic amines in the coatings of AgNPs produced by green and red morphs of *T.urticae* (Figure, 1). With lower MAO activity, the more accumulation of biogenic amines specifically with the presence of AgNPs. The specific activity as shown in Figure (1) of MAO was higher in the case of both morphs released the AgNPs than control. Affected MAO activity with 2.9 and 1.5 mOD min-1 mg-1 proteins in comparison with control (5.1 and 3.7 mOD min-1 mg-1 proteins) in green and red forms of *T. urticae*, respectively. Partial correlation between biogenic amines presented in the coatings and affected by MAO recorded .624*. Paired Samples Correlations (.247) and Paired Samples Test (t=2.410) between two morphs and resulted from ratios of MAO in comparison with control showed a non-significant difference at 95% (Sig. (2-tailed) =.028) which means that the main difference depended mainly on the certain morphs. Kendall's tau_b Correlation Coefficient between Nanoparticles and MAO =.894*, Spearman's rho=.917** and Pearson Correlation=.677* were calculated to confirm results. Moreover, R=.747*, R^2=.793, Adjusted R^2=.658. ANOVA showed that F=12.74** which showed a significant relation between MAO and certain morphs released AgNPs coated with biogenic amines.

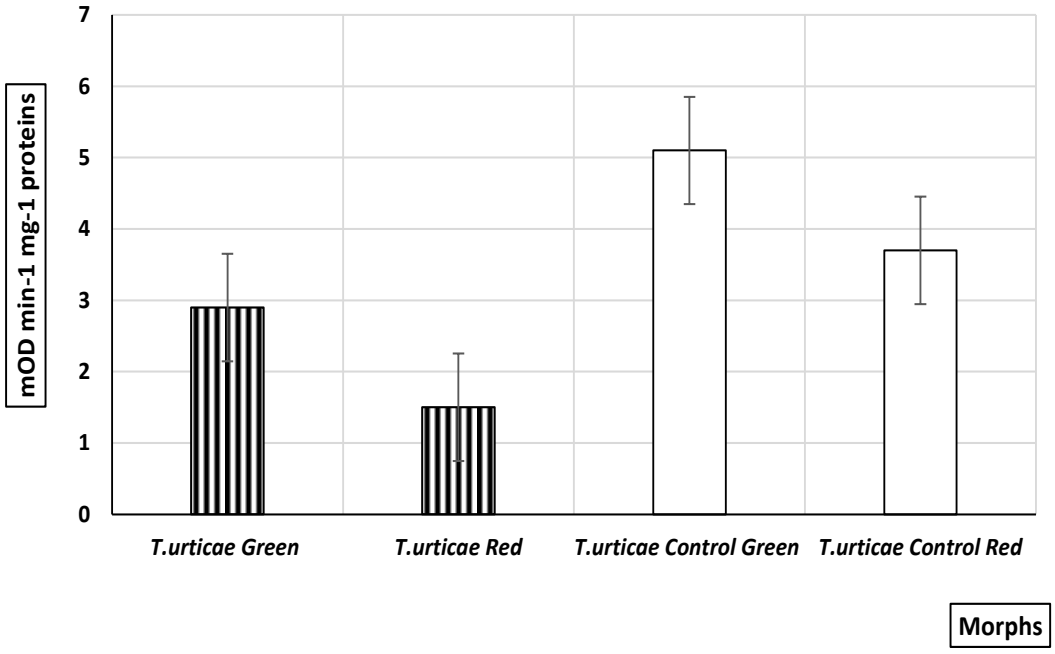


Figure (1): Monoamine oxidase (MAO) interaction to detect coated biogenic amines of AgNPs produced by certain *Tetranychus urticae* morphs .

Biogenic amines have a wide variety of functions in both the central and peripheral nervous systems of insects. They can act as neurotransmitters, neuromodulators and even circulating neurohormones. Knowledge of the pharmacology of the receptors that mediate the actions of biogenic amines in insects is increasing, there was only one known example of a pesticide that activates biogenic amine receptors. The knowledge of the mode of action of insect biogenic amine receptors is mediated through second messenger systems. Accumulation of biogenic amines played a specific role in both *T.urticae* forms and mainly red morph. Furthermore, it's proved recently that biogenic amines affected mosquito fertility. Subsequently, egg melanization was regulated by adrenergic signaling, whose disruption caused premature melanization specifically through the action of tyramine (Fuchs *et al*., 2014). Also, mosquito locomotion and survival were affected negatively by the strong cumulative of biogenic amines. Dopaminergic and serotonergic antagonists such as amitriptyline and citalopram recapitulated this effect.

Hereinafter, biogenic amines catabolism was assessed in hemolymph and saliva of *Amblyomma hebraeum* Koch. Rapidly conversion occurred of Dopamine (DA) and 5-hydroxytryptamine (5-HT) to dihydroxy phenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) respectively, indicating that monoamine oxidase (MAO) constituted a major catabolic pathway for biogenic amines in this species (Kaufman and Sloley,1996). Moreover, Deprenyl was about 44-72 times more potent an inhibitor of MAO than clorgyline when either DA or 5-HT was offered as substrate, suggesting that this MAO was of the MAOB type. Therefore, inhibition of MAO would lead to the accumulation of biogenic amines with their effects. Ceaselessly, the most widely recognized procedure is to utilize stabilizing agents that can be retained onto the AgNPs surface, maintaining a strategic distance from their agglomeration (Bai *et al*., 2007). Coating agents/surfactants could be used mainly to avoid agglomeration certainly. It can be done even by electrostatic or steric repulsion (Pillai and Kamat,2004; Oliveira *et al*.,2005 and El-Nour *et al*., 2010). Even though, any modulation of coatings, average size and distribution were affected self-assembly and stability of AgNPs (Lee and Jun, 2019).

2. Effect of Ag-Bio nanoparticles :

2.1. As miticides:

Results showed that used bio AgNPs at 0.01 g quantity which produced from the green form of *T.urticae*, caused mortality against both forms of the two-spotted spider mite, *T.urticae* in Figure (2). Mortality recorded 97.74% and 93.1% in the case of green and red forms of *T.urticae* in comparison with control (7.24 and 10.77%), respectively. Non-parametric tests were used to show the effect of the difference morphs of *T.urticae* responses to nanoparticles. Through runs test, Z =.612* was significant at 5%. Also, Wald-Wolfowitz Test was used to calculate Z for the effect of certain treatments on morphs and it was highly significant at 1% (Z=1.837**).

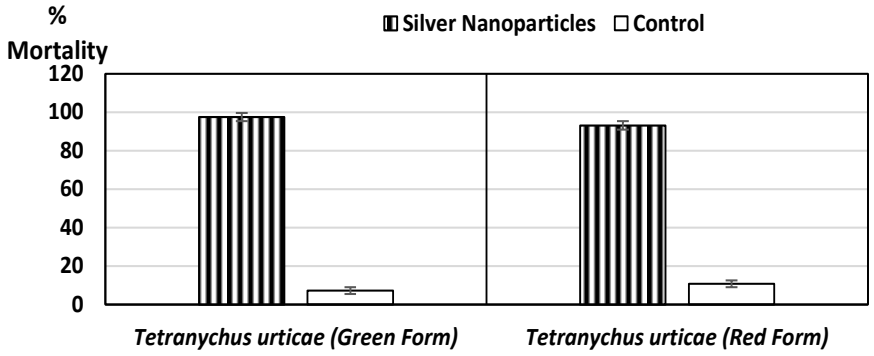


Figure (2): Effect of AgNPs from *Tetranychus urticae* (green form) against certain morphs of *T. urticae* .

Furthermore, results showed that used bio AgNPs at 0.01 g quantity which produced from the red form of *T.urticae*, caused mortality against both forms of the two-spotted spider mite, *T. urticae* in Figure (3). Mortality recorded 89.74% and 95.21% in the case of green and red forms of *T.urticae* in comparison with control (15.29 and 8.07%), respectively. Non-parametric tests were used to show the effect of the difference morphs of *T.urticae* responses to nanoparticles. Through runs test, Z =.612* was significant at 5%. Also, Wald-Wolfowitz Test was used to calculate Z for the effect of certain treatments on morphs and it was highly significant at 1% (Z=1.837**).

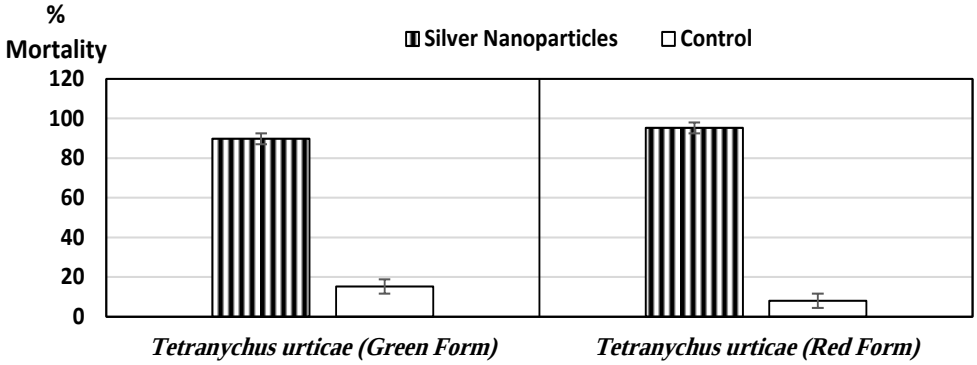


Figure (3): Effect of AgNPs from *Tetranychus urticae* (red form) against certain morphs of *T. urticae* .

2.2. As sterilants:

Produced bio AgNPs by both morphs of *T.urticae* showed a general quietly severe effect against both of them. Furthermore, results showed that used LC50s of bio AgNPs by green morph caused sterility against both forms of *T. urticae* in Figure (4) with 91.21% and 86.07% in the case of green and red forms of *T.urticae* in comparison with control (8.04 and 12.57%), respectively. Superficially, paired-samples correlations showed that between treatments, there was a highly significant difference at 1% (Std Error Mean=28868**). Kendall's Coefficient of Concordance (Kendall's W^a) = .857* and Chi-Square recorded 6.857*. Besides, Friedman Test showed a significant difference at 5% (Chi-Square=6.86*).

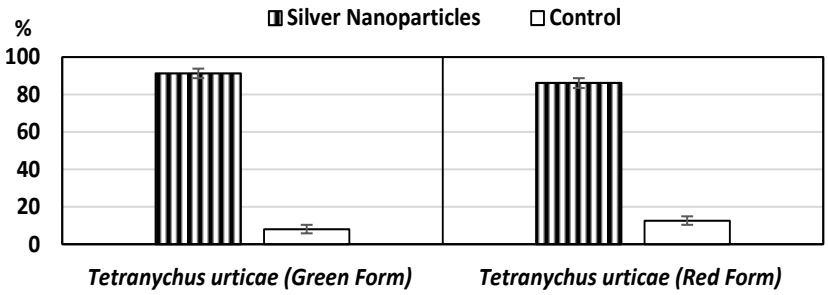


Figure (4): Sterility effect of AgNPs from *Tetranychus urticae* (green form) against certain morphs of *T. urticae* .

Consequently, results showed that used LC50s of bio AgNPs by red morph caused sterility against both forms of *T. urticae* in Figure (5) with 88.68% and 94.21% in the case of green and red forms of *T.urticae* in comparison with control (10.25 and 12.87%), respectively. Paired samples correlations showed that between treatments, there was a highly significant difference at 1% (Std Error Mean=28868**). Apparently, reliability Statistics showed Cronbach's Alpha[a]=.036 and ANOVA with Tukey's Test for Nonadditivity recorded the highest significant difference at 1% between the sterility of treatments and control (F=8014.542**) while F=4.631* at 5% for differences between both green and red morphs of *T.urticae*.

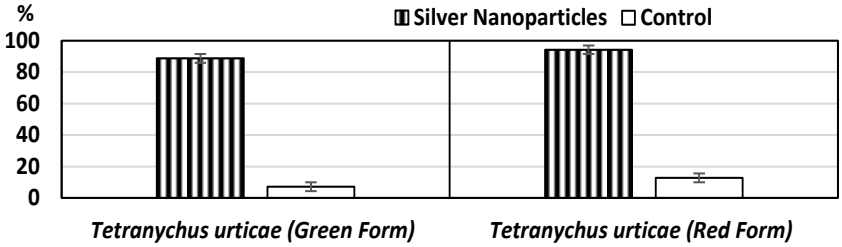


Figure (5):Sterility effect of AgNPs from *Tetranychus urticae* (red form) against certain morphs of *T. urticae* .

Nanoparticles showed repellency, acaricidal, and ovicidal effects against *Tetranychus urticae*. Acaricidal and ovicidal activities appeared clearly with nano encapsulated carvacrol and linalool, however, free compounds were more effective as repellents (Campos *et al*., 2018). Subsequently, Silver particles from cellulose/silver nanocomposites are powerful to decrease microbial development in contact with natural product exudates (Llorens *et al*., 2012). Moreover, the production of silver nanoparticles biologically by *T.urticae* was resulted to facilitate the penetration of them to other individuals after direct exposure. So problems related to physical-substance factors (e.g., ionic strength and surface charge heterogeneity) prevented either remobilization of the nanoparticles or further arrival of the active ingredient to play at any rate somewhat apart (Schweizer, 2014). Forwardly, Tetranychid mites build a typical web to ensure the settlements of their individuals. At the point when plants progress toward becoming stuffed and nourishment assets turn out to be rare, people accumulate at the plant peak to shape a ball made out of mites and their silk strings. This ball is a structure

encouraging gathering dispersal by wind or creature transport (Clotuche *et al*., 2011), and the consequent dispersal of produced bio nanoparticles of silver. Furthermore, the reinforcement of more silk production is expanding the effect of AgNPs potentially.

The green nanoparticles production process offers many advantages when the comparison was being with classical chemical and physical methods since it needn't bother with the utilization of exceptionally dangerous synthetic concoctions, nor high vitality inputs (Govindarajan *et al*., 2016a, 2016b and Teimouri *et al*., 2018). The general procedure is modest, simple to do and prompts the creation of a wide exhibit of nanoparticles, including gold (Murugan *et al*., 2015a and Balalakshmi *et al*., 2017), silver (Rajakumar and Rahuman, 2012; Govindarajan and Benelli, 2016; Murugan *et al*., 2015b; Azarudeen *et al*., 2017; Aziz *et al*., 2018 and Alyahya et al., 2018), titania (Jinu *et al*., 2018), zinc oxide (Kirthi *et al*., 2011 and Ashokan *et al*., 2017), iron (Murugan *et al*., 2018), palladium (Jayaseelan *et al*., 2018) and carbon (Rajaganesh *et al*.,2016).

Fundamentally, AgNPs like reduced fertility of treated insects and their movement ability was precipitously dropped (Armstrong *et al*., 2013). That was because of AgNPs ability to penetrate pest integument, and affected its life span, feeding, physiological and behavioral manifestations. Then mortality occurred (Sap-Iam *et al*., 2010), even it was detected that contact with AgNPs was more entomotoxic potential than feeding (Sedighi *et al*., 2019).

3. Reactive oxygen scavengers (ROS) :

Reactive oxygen scavengers (ROS) in treatments were significantly lower decrease than control ($P < 0.05$). Table (1) showed that Superoxide dismutase (SOD) in control was higher than treatments. Subsequently, decreased ratio percentages of SOD than control recorded 25.30 and 41.1 % by bio AgNPs at 0.01 g quantity which produced from the green form of *T.urticae* , against green and red forms, resp. In the same arrangement, but with bio AgNPs from red form, SOD ratio decreased in treatments than control with 19.1, and 26.40%.

Table (1): Reactive oxygen scavengers (ROS) ratio during Nano-metals treatments with control comparison .

Treatments	Against Green Morph		Against Red Morph	
	[1]ROS (Reactive Oxygen Scavengers) (SOD)	[2]Decreased Ratio %	[1]ROS (Reactive Oxygen Scavengers) (SOD)	[2]Decreased Ratio %
AgNPs From Tetranychus green morph	14.25±0.36a	25.30	25.07±2.71b	41.1
AgNPs From Tetranychus red morph	10.74±1.05a	19.1	16.11±1.88b	26.40
Control (green morph)	56.33c			
Control (red morph)	61.02c			

[1]ROS (Reactive Oxygen Scavengers) SOD-Superoxide dismutase (unit/mg protein).
Values are expressed as the means ±SE. Mean
[2] Decreased Ratio % = ROS ratio of the tested strain / ROS ratio of the control strain*100

Furthermore, the mode of action against insects is being through oxidative stress by a significant impact on detoxifying enzymes and antioxidants, which lead to cell death. Also, AgNPs were able to reduce the activity of acetyl-cholinesterase. Besides, Ag nanoparticles up- and downregulate key insect genes, reducing protein synthesis and gonadotrophin release, leading to developmental damages and reproductive failure. Silver nanoparticles influenced certain proteins, which are liable for neutralization of ROS, in the interacted cells. Fiery reaction and irritation are started by the gathering of reactive oxygen species (ROS), which likewise incite devastation of mitochondria and cell apoptosis (Sharma *et al*., 2015).

Crucially, silk is classified as an informative material that can provide a conspecific' turnout. It is being used as a social tool that has its impact on microhabitat, group behavior and the response of individuals (Clotuche *et al*.,2013). Three elements that could possibly impact living place decisions were controlled: the strain, number, and the phase of mites. Three factors are demonstrated their impact on the decision of microhabitat (Clotuche *et al*.,2013). The inclination of whether to settle on a silk-secured region was affected by the beginning of mites (strain impact). Grown-up females demonstrated a higher propensity to settle on a territory secured with the silk laid by various congeners (number impact). Also, hatchlings appeared to be more receptive to the nearness of silk than grown-ups (stage impact). Upon, the population of mites was able to work together to make silk balls which contained bio-AgNPs coated with biogenic amines released from exploded mites. Then prompted toxic effects of NPs have increased both chemical reactivity and penetration in cells because of small size and large surface area (Medina *et al*.,2007 and Pan *et al*.,2009). Finally, nanotechnology can alter farming and can give an answer to pest management. Bio silver nanoparticles with its properties as biocides can be a solution for even the pesticide resistance (Alif and Thangapandiyan, 2019).

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Elicit effects of potassium phosphite versus to emamectin benzoate on the defensive response of cotton seedlings against *Spodoptera littoralis* (Lepidoptera: : Noctuidae)

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Abstract:

This study was investigated on a novel rule of potassium phosphite as promising elicitors comparing to emamectin benzoate against *Spodoptera littoralis* (Boisduval) **(** Lepidoptera: **:** Noctuidae) . The toxicity on the 2nd instar larvae showed that LC_{25}, LC_{50} and LC_{90} values of emamectin benzoate (0.005, 0.012 and 0.248 mg L^{-1}, respectively) had more toxic than potassium phosphite (1326.2, 5302.4 and 73757.2 mg L^{-1}, respectively) after 96 hrs post-treatment. Gas chromatography–mass spectrometry **(**GC-MS) analysis identified the induced VOCs from untreated cotton seedling compared to those induced by potassium phosphite and emamectin benzoate. Induce Volatile organic compounds (VOCs) by potassium phosphite were featured by dibutyl phthalate, ß-caryophyllene, ethyl palmitate, ethyl linoleate and methyl linolenate, docosane and benzaldehyde, 3-phenoxy-. Major VOCs induced by emamectin benzoate were dibutyl phthalate, bis (2-ethylhexyl) phthalate, squalene and methylprednisolone. Biological tests at LC_{25} and LC_{50} values of emamectin benzoate showed pupal weights (150.4 and 95.2 mg, respectively) < potassium phosphite (262.3 and 224.3 mg, respectively) compared to 309.2 mg in the control. Adult emergence percentages of emamectin benzoate at LC_{25} and LC_{50}s were 31.7 and 18.3%, respectively < potassium phosphite were 72.3 and 53.8%, respectively compared to 86.8% in the control. Emamectin benzoate significantly prolonged the larval durations (16.4 and 17.2 days, respectively) > potassium phosphite (14.3 and 15.9 days, respectively) compared to the control (13.8 days). While no significant changes in pupal durations in both treatments. Significant decreases in pupation percentage revealed at LC_{50}s of emamectin benzoate (21.5%) and potassium phosphite (57.3%) compared to 90.3% in the control. Olfactometer dual choice tests on 2nd instar larvae showed preferable response to untreated cotton seedling versus to each of the two treatments at LC_{90} and LC_{50}s. Choice tests between the two treatments showed surpasses of potassium phosphite in orienting responses of larvae. Finally, these olfactory and biological assessments could enroll potassium phosphite as a novel elicitor against *S. littoralis*.

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a destructive and polyphagous insect pest causing great losses in quantity and quality for most of the injured crops (Matthews and Tunstall, 1994). The extensive uses of synthetic insecticides lead to harmful effects to environment and beneficial organisms (Pavela *et al*., 2008). Emamectin benzoate is a second-generation avermectin analog with exceptional activity against lepidopteran pests. It modulates specific glutamate-gated anion channels in synapse and muscle cells thereby increasing the influx of chloride ions. Furthermore, emamectin benzoate has a lack of cross-resistance compare to other synthetic insecticides (White *et al*., 1997 and Dunbar *et al.*, 1998). Therefore, more safe compounds should be employed alternatively and complementarily with the synthetic pesticides to realize safer pest management strategy for the environment (Bahlai *et al*., 2010).

In these respects, the last evaluations of potassium phosphite applications drew more attention towards strengthening plant vigor, health and tolerance against a variety of pathogens and environmental stress (Costa *et al*., 2018). Potassium phosphite applications provide efficient phosphorus for plant that enhances assimilation to activate its defense (Ogoshi *et al*., 2013). Foliar spray of potassium phosphite on potatoes plants after 48 hours brought out tubers with high significant contents of phytoalexins, phenols and some enzymatic activities that defend the crop against late blight injuries (Mohammadi *et al*., 2019). Moreover, a specific role had been discussed for phytoalexins in plant defense against herbivores. This discussion, reviewed that phytoalexins may include isoflavonoids, terpenoids, alkaloids glucosinolates and benzoxazinoids, which mediate the release of various biocidal aglycone metabolites to motivate the defensive responses of plant against insects attack (Morant *et al*., 2008 and War *et al*., 2012).

The defensive mechanisms could be exploited as an important tool for minimizing insecticides quantities for pest control and to predict the herbivores behavior affected by the induced responses (War *et al*., 2012). The defense compounds like allelochemicals in the form of secondary metabolites and volatile organic compounds (VOCs) possessed defense mechanisms through repellency, reduce digestibility or even toxic against the insect herbivores injuries (Dicke and Baldwin, 2010 and Dong *et al*., 2016). Many evidences showed that feeding behavior of herbivores could elicit the injured plant-defense through induction of repellant VOCs signals (Alborn *et al*., 1997; War *et al*., 2011; Zhou *et al*., 2013 and Krempl *et al*., 2016) and vice versa these signals may attract the natural enemies of herbivores (Turlings *et al*., 1990; D'Alessandro and Turlings, 2005 and Erb *et al*., 2009). Recently, many techniques of olfactometer choice tests were investigated to study insect response to different odors and volatile compounds (Avila *et al*., 2017; Papenberg *et al*., 2019 and Dory *et al*., 2019).

The main targets of our study were directed towards: (1) Investigation of the toxicity and sub-lethal effects of potassium phosphite on some biological aspects compared to emamectin benzoate against *S. littoralis* larvae. (2) Simulation method for extracting induced VOCs

from elicited plant by the tested compounds preluding to be identified by Gas chromatography–mass spectrometry (GC-MS). (3) Evaluation of olfactometer dual choice test for the responses towards the induced VOCs by the tested compounds. (4) Reviewing discussion on the capabilities of the tested compounds to regulate *S. littoralis* larvae behavior.

Materials and methods

1. Insect rearing:

A susceptible strain of *S. littoralis* was reared on fresh castor leaves (*Ricinus communis*), under controlled conditions according to the method of Eldefrawi *et al*. (1964).

2. Tested compounds:

Two compounds were submitted in this study as follows:

2.1. An inducer compound for plant defense response known by potassium phosphite (Quelagrow Iberica –Spain; applied dosage rate of 170 ml/ 100 L).

2.2. Semi-synthetic insecticides known by emamectin benzoate (El-Helb pesticides & chemical Co – Egypt; applied dosage rate of 40 ml/ 100 L).

3. Larvicidal bioassay technique:

Toxicity of emamectin benzoate and potassium phosphite was determined by using the leaf dipping method. Six sequential concentrations of each tested compound were freshly prepared in distilled water. Treated castor leaf pieces with each concentration were dried at room temperature before being placed to newly ecdysed 2^{nd} instar larvae. Untreated larvae were fed on castor leaf pieces immersed in distilled water only. Each treatment was replicated four times with 10 larvae per replicate. Mortality percentages were recorded after 96 hrs post-treatment and subjected to probit analysis according to (Finney, 1971). Sub-lethal concentrations of the tested compounds were calculated with their 95% confidence limits.

4. Extracting and sampling of emitted volatile organic compounds (VOCs) in static headspace:

VOCs emissions were trapped and extracted by static headspace method from cotton seedlings (3 weeks old) in pots (dia. 25 cm) under laboratory conditions (Figure, 1). These emitted VOCs induced by foliar spray treatments of emamectin benzoate and potassium phosphite at concentrations equivalent to their applied doses as well as distilled water in control. The treated cotton seedlings were enclosed under an inverted glass tube (dia. 10 cm X L. 25 cm) immersed in freshly agar layer poured as isolated barrier above the soil surface and sealed the emitted volatiles against leakage. The emitted VOCs were trapped in darkness overnight and then extracted by injecting ethanolic solvent in the static headspace through a lateral opening in the glass tube. Then the obtained ethanolic solvent samples were stored in a sealed glass bottle below 0ºC preluding for GC-MS analysis (Rohloff and Bones, 2005 and Tholl *et al.*, 2006). The emitted VOCs in head state were classified by World Health Organization (WHO) according to their evaporation activity based on initial boiling point into three class; very volatile organic compounds (VVOCS), volatile organic compounds (VOCs) less or equal 260º and Semi- volatile organic compounds (SVOCs) ranged from 260 up to 400 ºC (WHO, 1989).

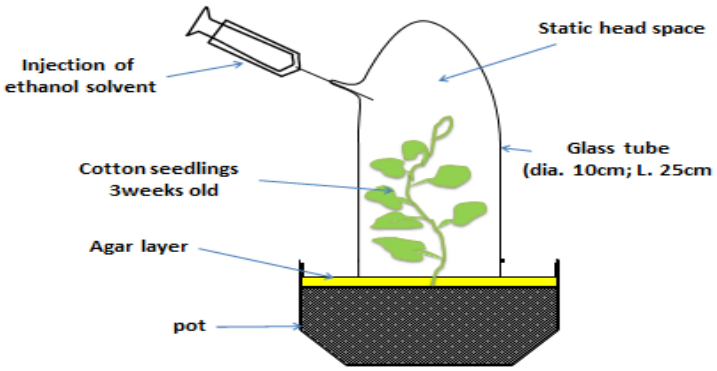


Figure (1): Extracting and sampling of emitted volatile organic compounds in static headspace.

5. Gas chromatography–mass spectrometry (GC-MS) analysis:

The chemical composition of the obtained ethanolic solvent samples eluted VOCs of treated seedlings was performed using Trace GC-ISQ Q mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C / min to 200 °C, hold for 2 min followed by increasing to the final temperature 300°C by 30°C / min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270 and 260°C, respectively. Helium was used as a carrier gas at a constant flow rate of 1 ml/ min. The solvent delay 3 min and diluted samples of 1 µl was injected automatically using Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–550 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with mass spectral database of WILEY 09 and NIST 11.

6. Effect of tested compounds on some biological aspects of *Spodoptera littoralis*:

The sub-lethal effects of potassium phosphite and emamectin benzoate at their equivalent concentrations of LC_{25} and LC_{50} on some biological aspects of *S. littoralis* were evaluated. Each treatment was replicated four times. Each replicate had one hundred newly ecdysed 2nd instar larvae. These larvae were fed on treated and untreated castor leaves with the tested compounds and distilled water in control, respectively. Surviving larvae were transferred to jars containing sufficient portions of untreated fresh leaves after 96 hrs of exposure and observed daily for larval and pupal development durations (days), larval and pupal weights (mg), pupation and adult emergence percentages.

7. Olfactometer dual choice test:

A simulated still-air olfactometer illustrated by (Weeks *et al*., 2011) made from a tube container (dia. ٥ cm; L. ١٠ cm) to insert the 2nd instar 24 hrs pre-starved larvae of *S. littoralis*, which exposed over 3 hrs to VOCs emitted by each of untreated and treated cotton seedlings that previously incubated in darkness over 24 hrs under glass tube (dia. 10 cm; L. 25 cm). The VOCs passed via short junctions from one lateral opening of the inverted glass tube upon cotton seedlings to the lateral hole of the tube container (dia. 2.5cm). A tube

container was sealed to prevent larvae escape and external foreign odors that contaminate the test environment. Thus, the exposed larvae were allowed to express their preference for the VOCs emitted from each treatment at intervals of times 1, 2 and 3 hrs. Each treatment was replicated 3 times with 10 larvae per replicate. The dual choice tests were design to evaluate the preferable ability of larvae to choose separately between each of treated and untreated cotton seedling as well as choice between each of the two treated cotton seedling (Figure, 2). The determination of olfactometer responses by equation mentioned by Del Socorro *et al*. (2010):

$$\%\, Total\ response = 100 \times (T + C)/N$$

Where; T= number of larvae entering the test chamber, C= number of larvae entering the control chamber and N= total number of larvae in the olfactometer.

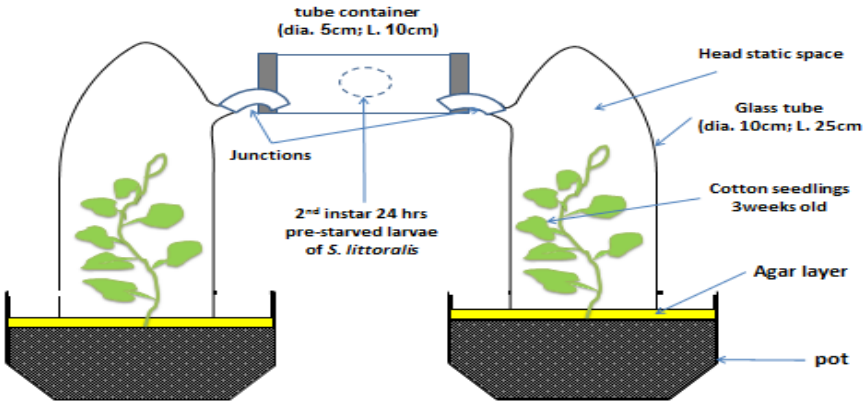


Figure (2): Olfactometer dual choice test.

8. Statistical analysis:

The data were analyzed using one-way analysis of variance (ANOVA). Means were determined for significant differences using SAS software (2002) (LSD at P < 0.05). Olfactometer responses were determined by using Paired T-test.

Results and discussion

1. Toxicity of emamectin benzoate and potassium phosphite against of *Spodoptera littoralis*:

Toxicity of tested compounds against 2nd instar larvae of *S. littoralis* were presented in Table (1). Emamectin benzoate was more toxic than potassium phosphite. LC_{25}, LC_{50} and LC_{90} values were recorded after 96 hrs post-treatment for emamectin benzoate (0.005, 0.012 and 0.248 mg L^{-1}) and potassium phosphite (1326.2, 5302.4 and 73757.2 mg L^{-1}), respectively.

Table (1): Toxicity of emamectin benzoate and potassium phosphite against 2nd instar larvae of *Spodoptera littoralis* after 96 hrs post-treatment.

Compounds	Conc. (mg L^{-1})		Confidence limits (mg L^{-1})	Slope ± SE*
Emamectin benzoate	LC_{25}	0.005	0.004-0.006	1.78 ± 0.13
	LC_{50}	0.012	0.011-0.014	
	LC_{90}	0.248	0.16-0.38	
Potassium phosphite	LC_{25}	1326.2	962-1824	1.12 ± 0.18
	LC_{50}	5302.4	3560-7285	
	LC_{90}	73757.2	30630.9-38304-E	

***SE means Standard Error**

2. Gas chromatography–mass spectrometry analysis:

GC-MS analysis of the ethanolic extract of the VOCs emitted by untreated cotton seedling identified main compounds of 1,2 benzenedicarboxylic acid (25.1%), Linoelaidic acid (16.2%), Phthalic acid, butyl hex-3-yl ester (5.32%) and 1,2-benzenedicarboxylic acid, dibutyl ester (4.24%) out of ten identified compounds (Table, 2). On the other hand, VOCs emitted from elicited cotton seedling by potassium phosphite at equivalent concentration to dosage rate of 170ml/100L revealed presences of seven identified compounds of docosane (7.43%), Dibutyl phthalate (3.68%), 12, 15-Octadecadienoic acid, methyl ester (2.24%), Benzaldehyde, 3-phenoxy- (1.27%), Hexadecanoic acid, ethyl ester (1.21%), ethyl (9z, 12z)-9, 12-octadecadienoate (0.86%) and ß Caryophyllene (0.84%) (Table, 3). Eventually, induced VOCs from cotton seedling by emamectin benzoate at equivalent concentrations to 40ml/100L were distinguished by eight compounds representing majorities of squalen (9.6%), Bisn (2-ethylhexyl) phthalate (9.2%), Methylprednisolone (5.65%) and Dibutyl phthalate (3.48%) (Table, 4).

Table (2): Gas chromatography–mass spectrometry chemical profile of volatile organic compounds emitted by untreated cotton seedling.

Compound	Identified Groups	Retention time	Area %	Molecular weight	VOC's class*
1,2-benzenedicarboxylic acid, dibutyl ester	-	15.95	4.24	278	SVOC
Phthalic acid, butyl hex-3-yl ester	-	16.87	5.32	306	SVOC
Linoelaidic acid	Monoterpens	17.80	16.2	280	VOC
4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-d ihydroxy-7-methoxy	Aroma	18.27	1.02	344	SVOC
Isochiapin B	-	19.37	1.13	346	VOC
N-butylboronate of methyl 9,10-dihydroxy-stearate	-	19.56	1.17	396	VOC
1H-cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a (1ah)-pentol, 3-[(acetyloxy)methyl] -1b,4,5,7a,8,9-h exahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate	-	19.73	1	534	VOC
Flavone 4'-oh,5-oh,7-di-o-glucoside	-	19.84	2.11	594	VOC
1,2 benzenedicarboxylic acid	-	20.07	25.1	390	VOC
4H-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-d i-á-d-glucopyranosyl-5,7-dihydroxy	-	21.32	1.30	610	SVOC

***The identified emitted organic compounds were classified for their volatility according to WHO, 1989; where: VOCs = volatile organic compounds and VOCs = semi volatile organic compounds.**

Table (3): Gas chromatography–mass spectrometry chemical profile of induced volatile organic compounds from cotton seedlings by potassium phosphite.

Compound	Identified Groups	Retention time	Area %	Molecular weight	VOC's class*
ß Caryophyllene	Sesquiterpene	12.95	0.84	204	VOC
Benzaldehyde, 3-phenoxy-	-	20.2	1.27	198	SVOC
Hexadecanoic acid, ethyl ester	Ethyl palmitate fatty acid	20.66	1.21	284	SVOC
Dibutyl phthalate	Phthalic acid	22.44	3.68	278	SVOC
Ethyl(9Z,12Z)-9,12-Octadecadienoate	Ethyl linoleate	22.86	0.86	308	SVOC
12,15-Octadecadienoic acid, methyl ester	Methyl linolenate	23.59	2.24	294	SVOC
Docosane	Higher alkane	27.04	7.43	310	SVOC

***The identified emitted organic compounds were classified for their volatility according to WHO, 1989; where: VOCs = Volatile organic compounds and VOCs = Semi volatile organic compounds.**

Table (4): GC-MS chemical profile of VOCs from elicited cotton seedlings by emamectin benzoate

Compound	Identified Groups	Retention time	Area %	Molecular weight	VOC's class*
Dibutyl phthalate	Phthalic acid	16.85	3.48	278	SVOC
Cyclopropane	-	18.62	3.54	302	VOC
Methylprednisolone	Corticosteroid	18.85	5.65	374	VOC
1,3-Dioxolan-2-one,5-methyl-4-(4,4-dimethyl-2,3- di methyl enecyclohexyl)	Alkyl-amides	19.52	2.73	236	VOC
2-[1-(adamantan-1-ylamino)-2,2,2-tri fluoro-ethylidene]-malononitrile	-	19.69	2.5	295	SVOC
1-Heptatriacotanol	Alcoholic compound	19.52	1.45	536	SVOC
Bis(2-ethylhexyl) phthalate	Phthalates	20.06	9.20	390	SVOC
Squalene	Triterpene	20.75	9.6	410	VOC

***The identified emitted organic compounds were classified for their volatility according to WHO, 1989; where: VOCs = volatile organic compounds and VOCs = semi volatile organic compounds.**

3. Effect of the tested compounds on some biological aspects of *Spodoptera littoralis*:

Sub-lethal effects of emamectin benzoate and potassium phosphite were manifested on some biological aspects of 2nd instar larvae of *S. littoralis* in Tables (5 and 6). Treatments at LC_{50}s affected the average weights of 2nd instar larvae as emamectin benzoate had the lowest values of 96.4, 125.3, 56.2 and 28.4 mg while potassium phosphite significantly surpassed with values of 118.2, 173.4, 248.2 and 358.2 mg compared to the highest values of 167.5, 347.2, 889.7 and 1247.3 mg in the control at 3, 6, 9 and 12 days post-treatment, respectively. Symmetrically, treatments at LC_{25}s had lower significant effects on the larval average weights. Longest average durations were recorded for treated larvae with LC_{25} and LC_{50} values of emamectin benzoate (16.4 and 17.2 days, respectively) and significantly decreased in those treated with potassium phosphite (14.3 and 15.9 days, respectively) whereas control treatment had the shortest duration time of 13.8 days (Table , 5).

Table (5): Sub-lethal effects of tested compounds on the 2nd instar larvae of *Spodoptera littoralis* via larval mean weight after sequent days of treatment and larval duration.

Treatments	Conc. (mgL^{-1})	Larval mean weight (mg) ± SE				Larval duration (days) ± SE
		3-days	6-days	9-days	12-days	
Control	-	167.5^{a} ± 2.4	347.2^{a} ± 3.5	889.7^{a} ± 3.2	1247.3^{a} ± 5.2	13.8^{c} ± 1.6
Emamectin benzoate	**0.005**	124.6^{c} ± 1.3	154.5^{c} ± 2.6	84.8^{d} ± 2.4	64.2^{d} ± 3.8	16.4^{b} ± 1.2
	0.012	96.4^{d} ± 1.2	125.3^{d} ± 2.2	56.2^{e} ± 2.1	28.4^{e} ± 2.4	17.2^{a} ± 1.3
Potassium phosphite	**1326.2**	148.2^{b} ± 1.9	265.7^{b} ± 2.4	574.3^{b} ± 2.8	893.8^{b} ± 4.6	14.3^{c} ± 1.8
	5302.4	118.2^{c} ± 1.6	173.4^{c} ± 2.8	248.2^{c} ± 2.3	358.2^{c} ± 3.2	15.9^{b} ± 1.3

***Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$.**

Data showed highest declinations in the mean weights of pupae treated with LC_{25} and LC_{50} values of emamectin benzoate (150.4 and 95.2 mg, respectively) followed by potassium phosphite (262.3 and 224.3 mg, respectively) compared to 309.2 mg in the control treatment. However, pupal duration did not change significantly in all treatments compared to the control. Significant decreases in pupation percentage revealed in the LC_{50}s of emamectin benzoate (21.5%) followed by potassium phosphite (57.3%) compared to 90.3% in the control treatment. Reduction in the adult emergence percentage was significantly exhibited in all treatments. Adult emergence percentages treated with LC_{25} and LC_{50} values were 31.7 and 18.3% for emamectin benzoate besides 72.3 and 53.8% for potassium phosphite, respective compared to 86.8% in the control treatment (Table, 6).

Table (6): Sub-lethal effects of tested compounds on 2nd instar larvae of *Spodoptera littoralis* via mean weight and duration of pupa, pupation and adult emergence percentages:

Treatments	Conc. (mgL^{-1})	Pupal mean weight (mg) ± SE	Pupal duration (days) ± SE	Pupation (%) ± SE	Adult emergence (%) ± SE
Control	-	309.2^{a} ± 2.8	10.3^{a} ± 0.6	90.3^{a} ± 2.5	86.8^{a} ± 2.1
Emamectin benzoate	**0.005**	150.4^{d} ± 3.4	10.6^{a} ± 0.3	36.4^{d} ± 1.2	31.7^{d} ± 1.3
	0.012	95.2^{e} ± 2.6	10.8^{a} ± 0.2	21.5^{e} ± 2.3	18.3^{e} ± 1.8
Potassium phosphite	**1326.2**	262.3^{b} ± 3.2	10.4^{a} ± 0.3	78.6^{b} ± 1.7	72.3^{b} ± 1.6
	5302.4	224.3^{c} ± 2.4	10.6^{a} ± 0.5	57.3^{c} ± 1.4	53.8^{c} ± 2.2

***Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$.**

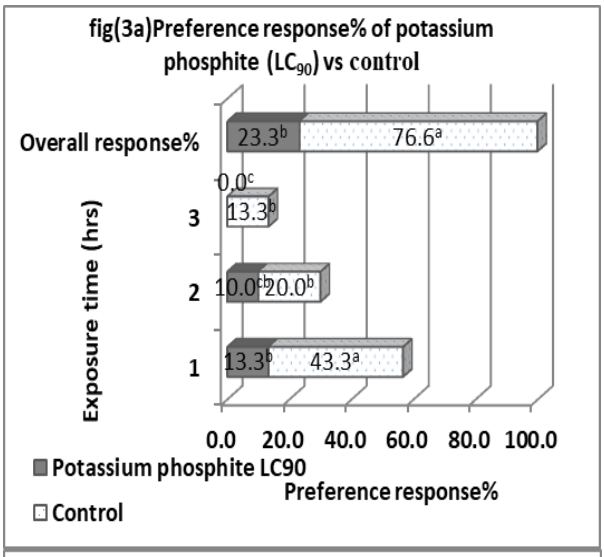
4. Olfactory response (%) choice tests:

Data of the overall preference response percentage of the 2nd instar 24 hrs pre-starved larvae of *S. littoralis* that exposed over 3 hrs to treated and untreated cotton seedlings after incubating in darkness over 24 hrs (Figures, 3a, 3b, 4a, 4b, 5a and 5b).

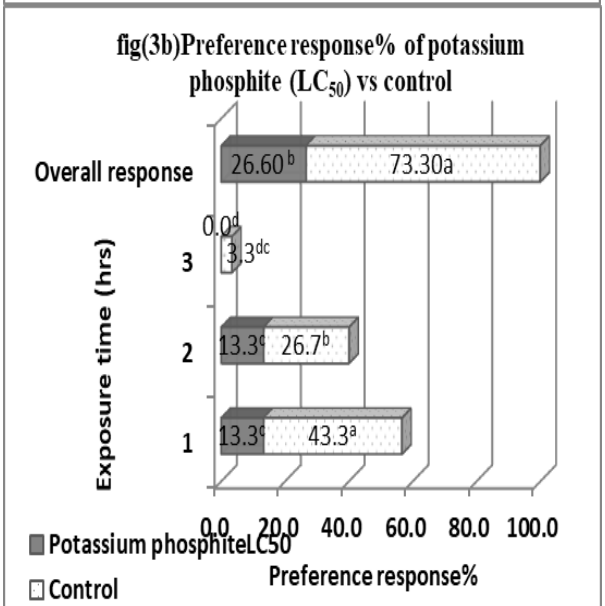
In the first dual choice test, high significant overall preference response percentage to control treatment (76.6%) were arisen compared to potassium phosphite treatment at LC_{90} (23.3%). Precisely, the exposed larvae via 1st, 2nd and 3rd hrs of exposure showed high significant preference response percentage to control (43.3, 20.0 and 13.3%, respectively) versus to potassium phosphite at LC_{90} (13.3, 10.0 and 0.0%, respectively) (Figure, 3a). Moreover,

high significant of the overall preference response percentage in potassium phosphite treatment at LC_{50} (26.60%) was occurred versus to the control treatment (73.30%). Particularly, high significant preference response percentage was revealed in the control treatment over potassium phosphite at LC_{50} via 1st, 2nd and 3rd hrs of exposure (Figure, 3b).

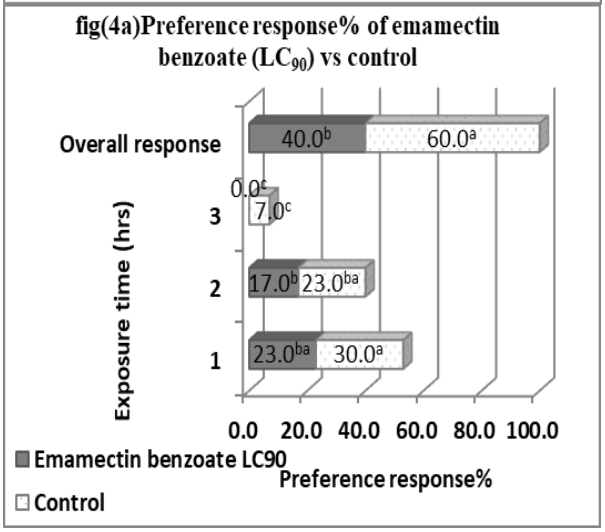
The second dual choice test showed that emamectin benzoate at both of LC_{90} and LC_{50} caused the same lower overall preference response percentages of 40.0 % compared to control treatments (60.0 %). Particularly, the exposed larvae after 1st, 2nd and 3rd hrs showed high significant preference response percentage to control (7.0, 23.0 and 30.0 %, respectively) versus to emamectin benzoate at LC_{90} (0.0, 17.0 and 23.0 %, respectively) (Figure, 4a). Otherwise, preference response percentage was revealed in the control treatment (26.7 and 33.3 %) over emamectin benzoate at LC_{50} (16.7 and 23.3 %) via 1st, 2nd hrs of exposure, respectively (Figure, 4b). The results of the third dual choice test between the two tested compounds at LC_{90} and LC_{50} showed significant overall preference response percentages to emamectin benzoate (66.6 and 66.7 %, respectively) more than potassium phosphite (33.4 and 33.3 %, respectively). Predominately, the exposed larvae to sub-lethal concentrations of LC_{90} and LC_{50} throughout the three hours of exposure showed significant preference response percentages to emamectin benzoate more than potassium phosphite treatments (Figures, 5a and 5b).



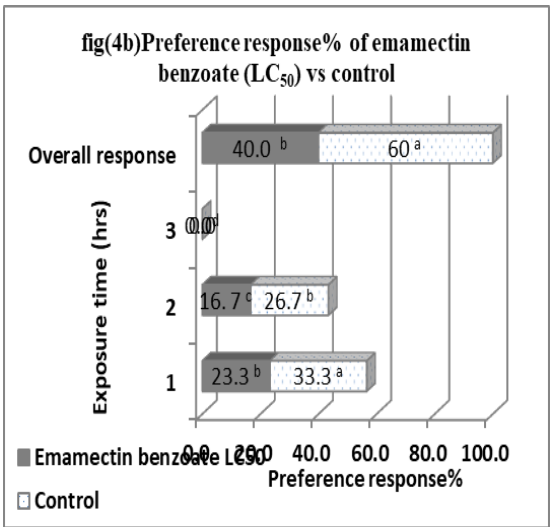
fig(3a)Preference response% of potassium phosphite (LC_{90}) vs control

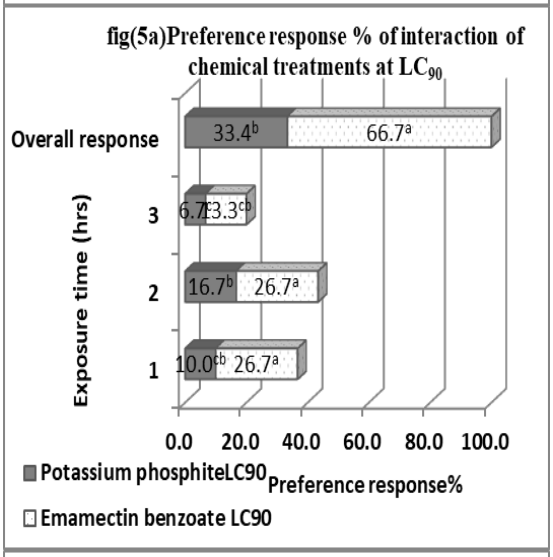


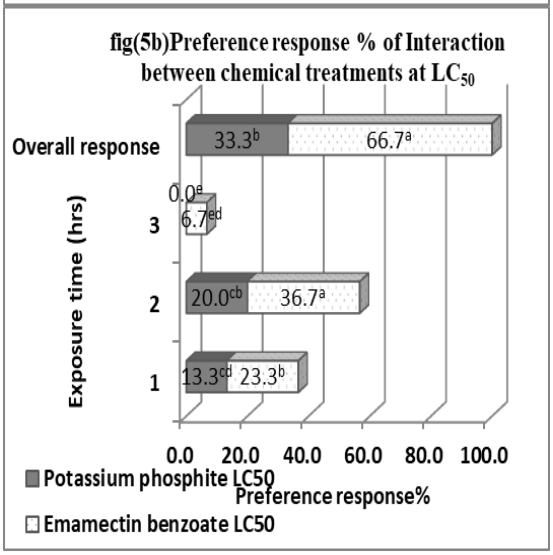
fig(3b)Preference response% of potassium phosphite (LC_{50}) vs control



fig(4a)Preference response% of emamectin benzoate (LC_{90}) vs control







- **Means of preference response % based on treatments and time (hrs) interactions with the same letter are not significantly different.**
- **Means of overall response % over 3 hrs of exposure with the same letter are not significantly different.**

Many studies have been investigated the role of potassium phosphite to induce the synthesis of plant defense and resistance against pathogens and environmental stress (Babu *et al.*, 2003; Rios *et al.*, 2014; Araujo *et al*., 2015 and Nascimento *et al*., 2016). Plant defensive mechanisms to curb herbivores attacks are still limited. Needs for more acquaintance about these mechanisms could develop pest control management and regulation of herbivores responses (War *et al*., 2012). Therefore, these evidences motivate our study to explore new defensive and biological activity for potassium phosphite against *S. littoralis* versus to emamectin benzoate one of the most common and environmentally safe semi-synthetic insecticide (Dunbar *et al*., 1998).

The obtained results of leaf-dip bioassay on the sub-lethal concentrations of emamectin benzoate was more toxic than potassium phosphite at 96 hrs post-treatment against 2nd instar larvae of *S. littoralis*. These results came in accordance to the toxicity tests of emamectin benzoate which seemed to be more fit and sensitive with leaf-dip bioassay against different larval stages of *S. Iitura* due to its stomach poison and contact mode of action (Birah *et al*., 2008). Meanwhile, the toxic effect of sub-lethal concentrations of potassium phosphite on cotton seedlings may be related to the phenolic derivative of benzaldehyde, 3-phenoxy- that could produce direct toxins that deter the insect's feeding (Chen *et al.*, 2009 and War *et al*., 2012).

The obtained data by GC-MS analysis for induced VOCs by untreated cotton seedling (control) were distinguished by majorities of phthalic acid, butyl hex-3-yl ester, linoelaidic acid (monoterpen), 1,2 benzenedicarboxylic acid and 1,2-

benzenedicarboxylic acid dibutyl ester out of ten identified compounds. Exclusively, the induced VOCs by potassium phosphite were featured by dibutyl phthalate, ß-caryophyllene (sesquiterpene), fatty acid derivatives (ethyl palmitate fatty acid, ethyl linoleate and methyl linolenate), docosane and benzaldehyde, 3-phenoxy-. Whereas, the identified VOCs by emamectin benzoate were differentiated by major components of dibutyl phthalate, bis (2-ethylhexyl) phthalate, squalene (triterpene) and methylprednisolone besides other minor groups. From the previous results, the differences in the emission patterns of the green leaf volatiles in the entire pathway in plant results of treatment modification of existing pathways via up-/ down-regulation of biochemical steps or by blocking the competing pathways. The concurrent temporal changes in activities of enzymes responsible for the final steps of VOC formation, enzyme protein content, and the expression of corresponding structural genes suggest that the developmental biosynthesis of volatiles is regulated largely at the level of gene expression (Dudareva *et al*., 2000; McConkey *et al*., 2000 and Muhlemann *et al*., 2012).

Data of biological tests showed that LC_{25} and LC_{50} values of emamectin benzoate had the highest significant declinations on the larval and pupal average weights and pupation percentage of the 2nd instar larvae more than potassium phosphite but normally increased in control treatment. In addition, sub-lethal concentrations of emamectin benzoate significantly prolonged the larval durations more than potassium phosphite and control treatment while no significant changes in pupal durations in both treatments compared to the control. These results were agreed with the data of biological aspect of emamectin benzoate that significantly decreased the consumption index; relative growth rate and efficiency of converting ingested and digested food in body tissue, while significantly did not affect the approximate digestibility of survived larvae of *S. littoralis*. Emamectin benzoate significantly prolonged the larval duration and decreased the pupal duration, pupal and larval means weight, pupation and adult emergence percentages of *S. littoralis* compared with control treatment (El-Dewy, 2017 and El-Sayed *et al*., 2017). However, the obtained data by GC-MS analysis showed that VOCs came out from cotton seedling in response to potassium phosphite particularly contain benzaldehyde, 3-phenoxy- that may probably possessed negative adverse against the larvae of *S. littoralis*. This allegation was supported by many reviews carried out on the direct toxic and deterrent effect of phenoxy derivatives and other oxidative radicals on the insect's feeding by reducing the plant digestibility, increasing nutrient deficiency and reducing growth and development of insects (Zhang *et al*., 2008; Chen *et al*., 2009 and War *et al*., 2012). Eventually, cotton seedlings VOCs induced by potassium phosphite were distinguished by high potent of long carbon chain double bonded-fatty acid derivatives (ethyl palmitate fatty acid ($C_{18:1}$), ethyl linoleate ($C_{20:2}$) and methyl linolenate ($C_{19:2}$)) which may play larvicidal effects on larvae of *S. littorals* compared to only linoelaidic acid ($C_{18:2}$) in control treatment. These data were justified by the observations concerning the defensive and toxic activity of fatty acids that may related by the increase of unsaturated bonds in carbon chain against the 4th instar larvae of *S. littoralis* as well

as their inhibitory action on the growth of some bacteria (US EPA, 2002; Marounek *et al*., 2002; Maia *et al*., 2010 and Abay *et al*., 2013). These toxic activities might be due to the relative abilities of the fatty acids to involve either the site of acetyl cholinesterase or octopaminergic receptors (Perumalsamy *et al*., 2015 and Hikal *et al*., 2017). In the way, phytoalexins originate in cotton plant; family Malvaceae is commonly existed found in the form of Terpenoids, naphthaldehydes and/or gossypol (Sunilkumar *et al*., 2006 and Jeandet *et al*., 2014). Consequently, naphthalene compounds were supposed to mediate in the formation of dibutyl phthalate and bis (2-ethylhexyl) phthalate which has been detected in our study by GC-MS analysis for the treatments of potassium phosphite and emamectin benzoate, respectively. These detected derivatives of phthalate might cause toxic effects. This supposition were supported by many reviews and investigations compiled on the natural formation of phthalic acid (Heudorf *et al*., 2007; Husein *et al*., 2014 and Przybylińska and Wyszkowski, 2016).

The designed dual choice tests in this research showed preferable ability of 2nd instar larvae to untreated cotton seedling versus to each of potassium phosphite and emamectin benzoate at concentrations of LC_{90} and LC_{50}, separately. Preference toward the volatiles blends emitted by untreated plants over the blends emitted by treated plants by tested compounds these preferences could be attributed to amounts of linoelaidic acid in untreated plants. However, choice tests between the two treatments showed surpasses of potassium phosphite in orienting responses of larvae over emamectin benzoate at all concentrations. These preferences could be attributed to amounts of ethyl linoleate and methyl linolenate emitted by cotton seedling treated by potassium phosphite. This result agreed with (Carlsson *et al*., 1999 and Shelton and Badenes-Perez, 2006) which demonstrated that either linalool or geraniol could serve as olfactory attractants to 3rd instar of *S. littoralis*. In addition; ß-caryophyllene emitted by cotton seedling in response to potassium phosphite could probably play a role in regulating the response behavior of *S. littoralis*. This thought was emphasized by the phenomena of attracting nematodes of *Heterorhabditis megidis* by ß-caryophyllene released by maize roots injured by larvae of the beetle *Diabrotica virgifera* in the soil (Rasmann *et al*., 2005 and Kant *et al*., 2015). Furthermore, the presence of high amounts of terpenoids in GC-MS analysis from treated cotton seedlings by emamectin benzoate were identified as squalene (triterpene) while potassium phosphite induced ß-caryophyllene (sesquiterpene). Moreover, many findings of that terpenoids (*β*-myrcene, (*E*)-*β*-ocimene, DMNT and (*E*)-*β*-caryophyllene) induced by cotton plant VOCs were considered as direct repellents for *S. exigua, Helicoverpa zea* and *Lygus Hesperus* as well as attractive for predators and parasitoids (Röse *et al*., 1998; Manrique *et al*., 2005 and Huang *et al*., 2015).

Eventually, the olfactometer and biological assessments in this study enrolled potassium phosphite as a novel inducer compound for plant defense against *S. littoralis*. Potassium phosphite treatment on cotton seedlings was distinguished by induced active blends of VOCs included benzaldehyde, 3-phenoxy- and fatty acid derivatives that elucidate the latent toxic, defensive and biological activities besides ß-

caryophyllene and dibutyl phthalate, which had a latent role in regulating larval responses. These observations, may leads to employ potassium phosphite amongst the applications of synthetic insecticides in the control of *S. littoralis*.

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Population dynamics of certain mites infesting sugar beet at Beheira and Sharkia Governorates in Egypt

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Abstract:

The population dynamics of the spider mite *Tetranychus cucurbitacearum* (Sayed) (Acari: Tetranychidae) and the predaceous mite *Amblyseius swirskii* Athias-Henriot (Acari:Phytoseiidae) associated with sugar beet plants, *Beta vulgaris* L. were recorded at Nobaria and Diarb- Nigm districts in Beheira and Sharkia Governorates, respectively, in Egypt during two seasons 2016-2017 and 2017-2018. The population of the mite *T. cucurbitacearum* had five peaks at Sharkia and four peaks at Beheira during both seasons, showing a highly infestation on sugar beet at Sharkia compared to Beheira district. The predaceous mite *A. swirskii* had one peak in both Governorates during the two seasons. Statistical analysis showed significant correlation coefficient between the population of *T. cucurbitacearum* and temperature during both seasons at two Governorates and it was highly significant at the second season at Sharkia Governorate.

Introduction

Despite the newness of sugar beet in Egypt (Leilah *et al*. 2005 and Fouad, 2011), it ranks the second important sugar crop after sugar cane, *Saccharum officinarum* L. producing annually about 48.1 % of sugar production all over the world (Anonymous, 2012). So, the Egyptian Government policy aims to encourage the farmers to increase its cultivation to conserve water and for its high concentration of sugar. This crop attacks by several numbers of insect pests causing the considerable damage in the yield (Bassyouny, 1987; Shaheen, 1992; Shalaby, 2001 and El-Zoghbey *et al.*, 2003). The total loss in the yield caused by the insect pests recorded 8.2 % in 1954 and 12.4 % in 1965 (Kolbe, 1967 and Sherief *et al*., 2013). The spider mite *Tetranychus cucurbitacearum* (Sayed) (Acari :Tetranychidae) has recently become a more serious pest for sugar beet plants (Al-Habshy *et al*., 2014). The predators of mites and spiders are the

most important elements to reduce the number of different pests (Kalmosh *et al*., 2018).

So, it was felt necessary to throw light on mites associated with sugar beet in Sharkia and Beheira Governorates in Egypt. The population dynamic of mites infesting sugar beets and their relationship with climatic factors were studied.

Materials and methods

The present work was carried out on sugar beet (*Beta vulgaris* L.) at Diarb-Nigm district, Sharkia Governorate and Beheira district, Beheira Governorate during 2016/2017and 2017/2018 seasons. Samples were weekly taken from (October 2016 to February 2017) during the two seasons from fields of the crop to study mites associated with sugar beet. One fedden (4200m^2) was chosen and divided into three plots. The crop was planted in the fourth week of August during the two seasons. Samples started after month of sowing and continued at weekly intervals until the end of season. The normal agricultural practical was followed, and no pesticides treatments were applied during the whole experiment period.

Samples were randomly collected from diagnosis of the inner sugar beet area of each experimental plot for counting mite species. Samples were collected weekly in early morning. Each sample consisted from 20 leaves, collected randomly. Samples were directly transferred to the laboratory and examined carefully using a stereoscopic binocular microscope and the number of phytophagous mite and its predator were counted. Effects of certain weather factors such as temperature and atmospheric relative humidity on the population dynamic of mites were studied. Number of mites was counted per square inch (4 square / leaf). The daily means of the two factors were provided by the Meteorological Central Laboratory for Agricultural Climate, Agricultural Research Center during the whole period of the two seasons (2016/2017 and 2017/ 2018).

The obtained data were statistically analyzed according to Snedecor and Cochran (1980) using Costat (2004) statistical analysis software, microcomputer program.

Results and discussion

The present study recorded five mite species (*T. cucurbitacearum, Amblyseius swirskii* Athias-Henriot (Acari:Phytoseiidae), *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae), *Tydeus californicus* (Banks) (Acari :Tydeidae) and *Tydeus* sp.) belonging to four genera and three families during 2016/2017 and 2017/2018 growing seasons. The common species were *T. cucurbitacearum* and *A. swirskii*

1. Population dynamics of *Tetranychus cucurbitacearum* at Beheira Governorate:

During the first season, 2016/2017 the infestation with mite was started at 13th of October 2016 with 11.33 mites/ square. The population was increased gradually to record the first peak at 27th of October. Then after that the population was decreased till 13th June to reach 1.66 individual/square inch; after that the number was increased gradually to reach the second peak recording 29.8 mite/ square inch on 11th of February. In the second season 2017/2018 the infestation also started on 13th of October with 13.56 mite/square inch. The population was increased gradually forming two peaks on 17th of October and 11th of February, with a mean number of 29.43 and 27.9 mite/ square inch, respectively; then the

population was decreased until the end of the season (Figure, 1).

2. Population dynamics of *Amblyseius swirskii* at Beheira Governorate:

The species appeared for the first time during the first season 2016/2017 on 11th of November 2016 with mean number of 4.2 mite/ square inch. The population was increased gradually to record one peak on 2nd of December with mean number of 19.4 mite/ square inch. Afterwards the population was declined to reach 2.6 mite/square inch on 30th of December 2016, then it dropped to zero on 6th and 13th of January 2017. The population was increased gradually to record 12.6 mite/square inch on 18th Feb. 2017 (Figure 1).

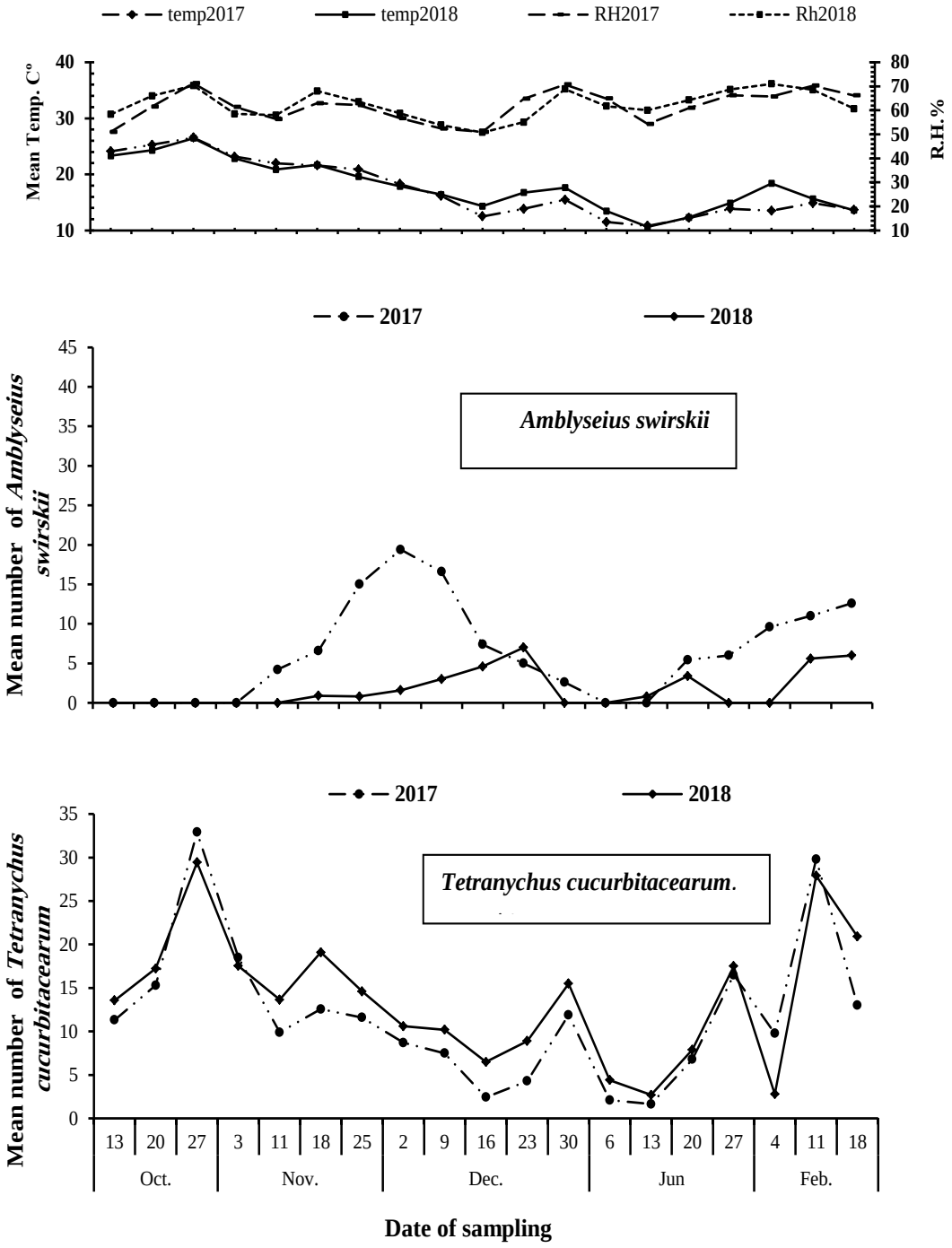

Figure (1): Population dynamics of *Tetranychus cucurbitacearum* and *Amblyseius swirskii* on sugar beet at Beheira Governorate during 2016/2017 and 2017/2018.

Regarding the second season 2017/2018 the predator appeared for the first time on 18th Nov.2017 with mean number of 0.9 individual/square inch. The mean number increased gradually to reach the first peak at 23rd December 2017 and the second peak at 20th Jan. 2018 with mean number of 7.00 and 3.4 individual/square inch, respectively. The population was dropped to zero two times during the growing season, after that the population was increased gradually at the end of the season on 18th Feb. 2018 reaching 6.00 mite/square inch.

3. Population dynamic of *Tetranychus cucurbitacearum* at Sharkia Governorate:

Results in Figure (2) cleared that the infestation with spider mite, *T. cucurbitacearum* occurred from 11th October to 16th February during the two seasons, respectively. During the season, the infestation of mites started after 35 days of sowing at 11th October. the population increased gradually to record 5 peaks during the two sowing seasons at 18th October., 23th November; 28th December; 11th January; and 2nd February with mean number of 28.56, 22.3, 21, 19 and 22.3 mite / square inch; respectively. Afterwards the population was fluctuated to reach value of 21.2 mite/ square inch at the end of the season 16th February 2017.

The same trend was observed during the second season 2017-2018 recorded five peaks at 18th October.; 16th November; 14th December; 28th December; and 2nd February;2018 with mean number of 32.45, 30.9, 30, 32.9 and 37.5 mite/ square inch; respectively. Afterwards, the population was increased until the end of the growing season recording 31.2 mite/ square inch on 16th February (Figure, 2).

4.Population dynamic of *Amblyseius swirskii* at Sharkia Governorate:

Data presented in Figure (2) indicated that predator mite appeared for the first time in 9th November 2017 with mean number of 2.6 mite/ square inch. The population was increased gradually to record one peak on 28th December 2016 with mean number of 16.4 mite/square inch. Afterwards the population was declined abruptly to reach 4.2 mite/square inch on 9th February 2017, then increased gradually to record 14.4 mite/square inch at the end of growing season at 16th February.

On the other side, in the second season predator recorded for the first time on 9th November 2017 with mean number of 0.9 mite/ square inch. The population was fluctuated to record 2.8 mite/ square inch in 30th November, then declined abruptly to reach zero in 7th December and being increased to record 1.4 mite / square inch on 21st December; then population was declined abruptly to zero on 28th December. Afterwards the population was increased quickly showing one peak on 18th January 2018 with mean number of 9.6 mite/ square inch; after that the population decreased till 9th February 2018 with population was 4.2 mite/ square inch, then increased to record 6.6 mite/ square inch at the end of the season on 16th February.

From the previous data it is cleared that *T. cucurbitacearum* infested sugar beet at Sharkia more than at Beheira. This observation may be due to that Sharkia is older than Beheira distract in the date of reclamation. Al-Habshy *et al*. (2014) recorded the same conclusion when studied the seasonal abundance of *T. cucurbitacearum* and *A. swirskii* on sugar beet.

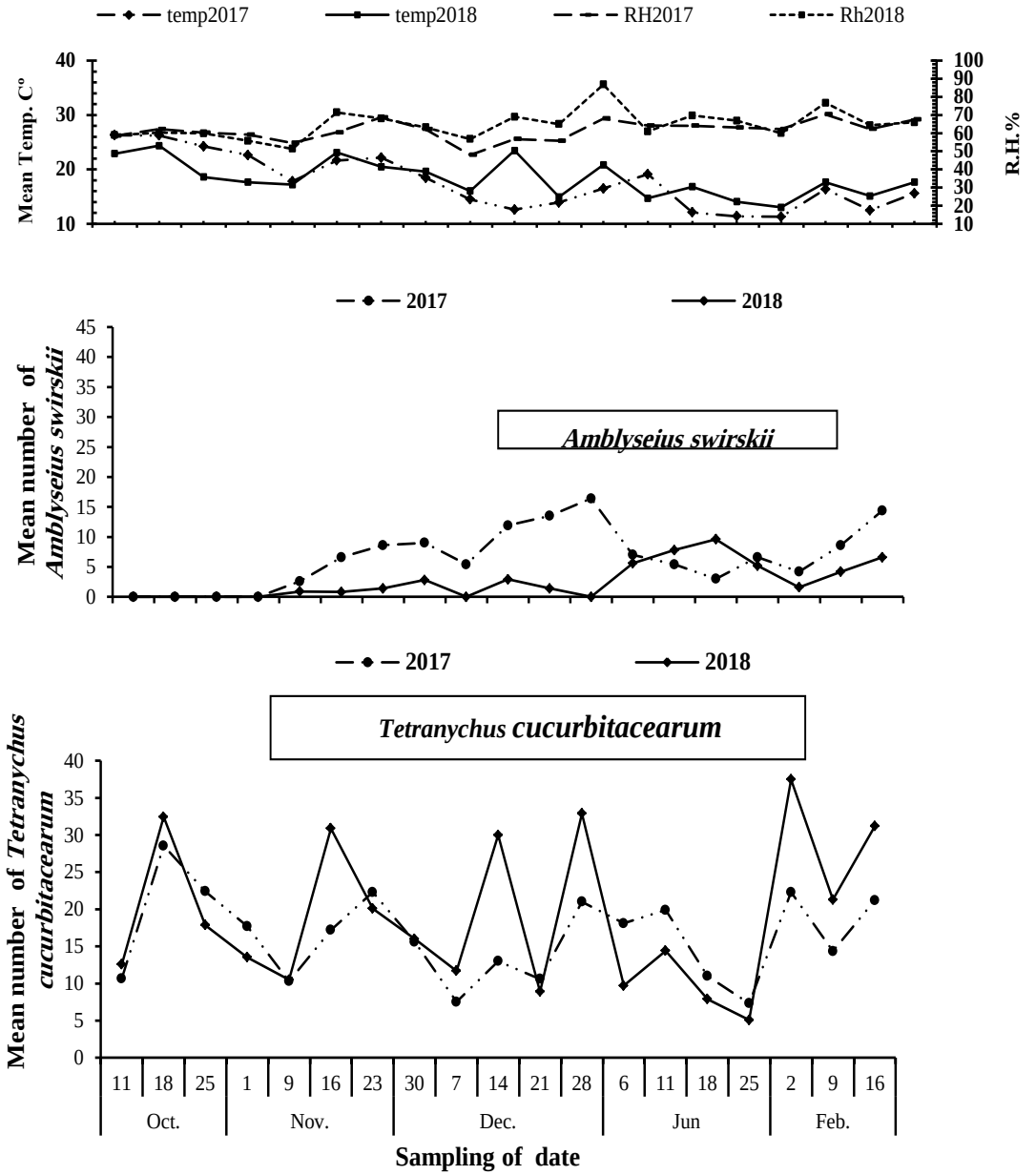

Figure (2): Population dynamic of *Tetranychus cucurbitacearum* and *Amblyseius swirskii* on sugar beet at Sharkia Governorate during 2016/2017 and 2017/2018

5. Interrelation between mite species and temperature and relative humidity:

As shown in Table (3), data and statistical analysis cleared that the correlation between *T. cucurbitacearum* with temperature at Beheira during the two seasons and at Sharkia during the first season was significant, but it was highly significant during the second season at Sharkia Governorate. Also, the correlation between the population of *T. cucurbitacearum* and relative humidity was highly significant during the first season at Beheira and during the two growing seasons at Sharkia, while it was non-significant at the second season at Beheira.

Table (3): Matrix correlation and regreation between *Tetranychus cucurbitacearum* and *Amblyseius swirskii* on sugar beet with temperature and relative humidity at Beheira and Sharkia Governortes during 2016/2017 and 2017/2018 seasons.

Species	Locality	Beheira						Sharkia					
	Season	2016/2017			2017/2018			2016/2017			2017/2018		
	Reliable	r	b	P	R	b	p	r	B	p	r	b	P
Tetranychus cucurbitacearum	Mean temp.	0.54	0.33	*	0.51	0.29	*	0.48	0.41	*	0.63	0.21	**
	Mean R.H.	0.58	0.45	**	0.45	0.34	ns	0.65	0.6	**	0.62	0.49	**
	Combined effect %	0.649			0.367			0.643			0.648		
Amblyseius swirskii	Mean temp.	-0.21	-0.11	ns	-0.47	-0.26	*	-0.51	-0.51	*	-0.54	0.62	*
	Mean RH.	-0.1	-0.11	ns	-0.39	-0.97	ns	0.23	0.25	ns	0.09	0.26	Ns
	Combined effect %	0.059			0.306			0.238			0.346		

The correlation between the populations of *A. swirskii* with temperature it was significant during the second season at Beheira and during the two growing seasons at Sharkia, although it was non- significant at the first season at Beheira. But regarding to the correlation between the populations and relative humidity, it was non- significant during the two growing seasons at both distracts.

6.Predator – pest mite interrelation:

Data given in Table (4) indicated that it was non- significant correlation between *T. cucurbitacearum* as a pest and its predator mite *A. swirskii* during the two growing seasons at both distracts.

Table (4): Matrix correlation between *Tetranychus cucurbitacearum* with *Amblyseius swirskii* at Beheira and Sharkia Gvernorates during 2016/2017 and 2017/2018 seasons.

locality	Beheira		Sharkia	
seasons	2016/2017	2017/2018	2016/2017	2017/2018
r	-0.069 ns	0.05 ns	-0.044 ns	-0.261ns
b	-0.051	0.051	-0.037	-0.076

r = simple correlation of coefficients b = regression of coefficient p = significant probability.
Ns= non-significant *significant and ** highly significant

These results with those obtained by Mohamed (2004), who studied the population of *T. cucurbitacearum* on sugar beet at El- Salheia and San-Alhagar during 1999- 2001. Also, he studied the simple correlation between the population dynamic with some climatic factors and found that, *T. cucurbitacearum* recorded a highly infestation on sugar beet at San-Alhagar more than El- Salheia. Legrand *et al*. (2000) declared that T. urticae was very occasional in sugar beet crop. Muchembled (1999) discussed the conditions which favor that development of *T. urticae* in sugar beet crop and used acaricides for the control of this pest are presented. Al – Habshy *et al*. (2014) recorded the same conclusion when studied some ecological studies on sucking pests infesting sugar beet crop and their associated natural enemies in Sharkia Governorate.

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Efficacy of two plant oils and their mixture on two species of *Tetranychus* spp. (Acari: Tetranychidae)

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Abstract:

The biological effects of ginger oil (*Zingiber officinale*), castor oil (*Ricinus communis*) and their mixture were studied under laboratory conditions against adult female of carmine spider mite *Tetranychus cinnabarinus* (Boisduval) and the two spotted spider mite *Tetranychus urticae* Koch. (Acari: Tetranychidae). Also, LC_{50} of each treatment was established and the obtained results revealed that the mixture of ginger and castor essential oils was the most effective in the two species. Ginger oil was more effective than castor oil which has very low effect on the two species. LC_{50} was 322.54, 682.65 and 17305.99 ppm for the mixture, ginger oil and castor oil, respectively, for *T. cinnabarinus*. However, the LC_{50} was 429.71, 1517.39 and 23587 ppm for the mixture, ginger oil and castor oil, respectively, for *T. urticae*. The results indicated that, the essential plant oils were more effective on *T. cinnabarinus* than *T. urticae.*

Introduction

The environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and public in recent years. It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually. The reasons for this are twofold: (1) the high toxicity and non-biodegradable properties of pesticides and (2) the residues in soil, water resources and crops that affect public health. Thus, on the one hand, one needs to search the new highly selective and biodegradable pesticides to solve the problem of long term toxicity to mammals and, on the other hand, one must study the environmental friendly pesticides and develop techniques that can be used Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment.one of the most important natural products is essential oils (Koul *et al.*, 2008).

The move toward green chemistry processes and the continuing need for developing new crop protection tools

with novel modes of action makes discovery and commercialization of natural products as green pesticides an attractive and profitable pursuit that is commanding attention. Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give distinctive odor, flavor or scent to a plant. Essential oils are usually obtained via steam distillation of aromatic plants, specifically those used as fragrances and flavorings in the perfume and food industries, respectively, and more recently for aromatherapy and as herbal medicines.

Red spider mites, *Tetranychus* spp. (Acari: Tetranychidae) are associated with more than 120 host plants of economic importance worldwide, including cotton, strawberry, ornamental plants, deciduous fruit trees, tomato, eggplant, and other vegetables, with a wide distribution in different parts of the world (Çakmak and Demiral, 2007). Red spider mites can complete their life cycle from egg to adult in one to two weeks under favorable conditions (Bolland and Valla, 2000 and Biswas *et al*., 2004).

Commercially available synthetic acaricides are usually expensive and may be needed to be imported for use by farmers. They also tend to have detrimental effects on the environment and can be hazardous to humans. These negative effects have resulted in an increasing interest for natural plant-based pesticides which are assumed to be safer than the synthetic pesticides (Yanar *et al*., 2011). Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms (Sharma *et al*., 2006 and Habashy *et al*., 2015). Moreover, botanical insecticides usually contain a mixture of several active substances which exert different mechanisms of action as a rule and thus may be able to effectively prevent the emergence of resistant pest populations (Rattan, 2010 and Pavela, 2014). Ginger (*Zingiber officinale*) is a perennial and rhizome producing plant that is known to contain resins and a volatile oil (Zahir *et al.*, 2011). The castor bean *Ricinus communis* (Euphorbiaceae) has shown a great potential as a source of insecticidal molecules against several insects (Rossi *et al.*, 2010), including species of *Spodoptera* (Ramos-López *et al*., 2012).

The two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is the most economically important plant feeding mite pest in the world, it attacks broad range of crops. Due to its wide host range, its high reproductive capacity and its ability to rapidly develop resistance to pesticides, hence *T. urticae* is difficult to control. To reduce these negative effects, alternative methods for the control of *T. urticae* are being tested, including the use of essential oils. Essential oils are promising agents for the control of agricultural pests.

The present work was aimed to evaluate the biological aspects of ginger oil and castor oil and their mixture against two species of *Tetranychus*.

Materials and methods

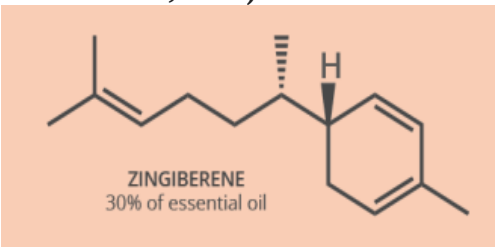
1.Rearing mites:

T. urticae and *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) were collected from unsprayed castor bean plants and reared at 25± 2° C and 60± 5%RH.

2.The tested plant oils:

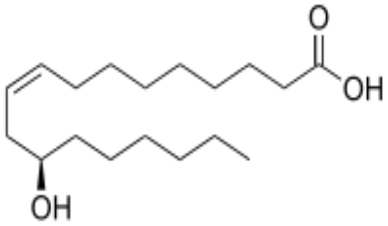
- Ginger oil and castor oil were bought from Essential oil Extracts Center, National Research Center.

- Ginger oil, is extracted from fresh ginger roots, primarily consisting of zingiberene **(An *et al.*, 2016)**



(An *et al.*, 2016)

- Castor oil is extracted from castor beans and consisting primarily of ricinoleic acid **(Thomas, 2005)**.



Ricinoleic acid formula **(Thomas, 2005)**

- Mixture of the oils made by adding proportion of 1:1 of each essential plant oil.

3. Preparing the stock solution the tested plant extracts:

Convenient stock concentrations of each plant oil were prepared on basis of the tested plant weight and the volume of the distilled water (w/v) in the presence of tween 80(0.1%) as emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for each plant oil were used to draw the LC-P lines. Three replicates were used for each concentration.

4.Toxicity test:

The toxicity of ginger oil, castor oil and their mixture was evaluated against adult females of *T. cinnabarinus* and *T. urticae*.Thirty newly emerged adult females were transferred to the lower surface of castor leave discs (2.5 cm diameter) placed separately on moist cotton wool in Petri dishes. Each petri dish contains three replicates, ten individuals in each replicate. Each acaricide had four concentrations which were sprayed on the individuals. Mortality was recorded for 7 days after treatment. The mortality percentage was estimated and corrected according to the Abbott's formula, 1925. LC_{50} values were determined using probit analysis statistical method of Finney, 1971.

Equation: Sun, 1950 (to determine LC_{50} index**)**

$$\text{Toxicity index for } LC_{50} = \frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

Results and discussion

1. Bio efficacy of ginger plant oil, castor plant oil and their mixture on adult female of carmine spider mite *Tetranychus cinnabarinus* (Boisduval):

The data in Table (1) demonstrated that, the mixture of ginger and castor oils caused the highest mortality proportion on *T. cinnabarinus* in all tested concentrations. Then, the ginger oil caused high mortality proportion. While castor oil caused very low mortality proportion. These results agreed with Isidia *et al.* (2010) who proved that ginger oil has high toxic effect on cowpea insects.

Table (1): Corrected mortality % of carmine spider mite *Tetranychus cinnabarinus* treated with ginger, castor oils and their mixture under laboratory conditions 25±2 ˚C and 60±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Ginger oil	1000	26.67	13.33	10	6.67	56.67
	5000	33.33	13.33	20	3.33	70
	10000	40	20	13.33	6.67	80
	15000	43.33	20	16.67	6.67	86.67
Castor oil	1000	-----	6.67	-----	3.33	10
	5000	13.33	-----	3.33	3.33	20
	10000	20	3.33	6.67	6.67	36.67
	15000	23.33	13.33	10	6.67	53.33
Mixture of ginger and castor oils	1000	30	23.33	6.67	10	70
	5000	46.67	20	16.67	-----	83.33
	10000	70	13.33	10	-----	93.33
	15000	70	16.67	10	-----	96.67

However, Table (2) and Figure (1) indicated that, the mixture of ginger and castor oils was more effective than each essential oil alone against *T. cinnabarinus* with LC_{50}: 322.54 ppm. Also, ginger oil alone was effective with $LC_{50:}$ 682.65 ppm, but castor oil was not effective and $LC_{50:}$ 17305.99 ppm. The toxicity index was 100% for the mixture while it was 47.25&1.86 for ginger oil &castor oil, respectively. The slope values indicated that , ginger oil had the lowest value was 0.740 followed by 0.962 and 1.16 for the mixture and castor oil, respectively. Also, the obtained results proved that, castor oil alone has weak effect on *T. cinnabarinus* but when added to ginger oil to form mixture, it increases its toxicity against pests. Abd Allah and Marouf, 2015 proved that the mixture of two plant extracts was more effective in toxicity than each extract alone.

Table (2): Efficacy of ginger and castor oils and their mixture against *Tetranychus cinnabarinus*.

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90}/LC_{50}	R	P
Ginger oil	1000	56.67	682.65	36760.44	0.740± 0.15	47.25	53.85	0.970	0.475
	5000	70							
	10000	80							
	15000	86.67							
Castor oil	1000	10	17305.99	220346.44	1.16± 0.18	1.86	12.73	0.954	0.087
	5000	20							
	10000	36.67							
	15000	53.33							
Mixture of ginger and castor oils	1000	70	322.54	6921.57	0.962± 0.17	100	21.46	0.962	0.360
	5000	83.33							
	10000	93.33							
	15000	96.67							

R: Regression **P: Probability**

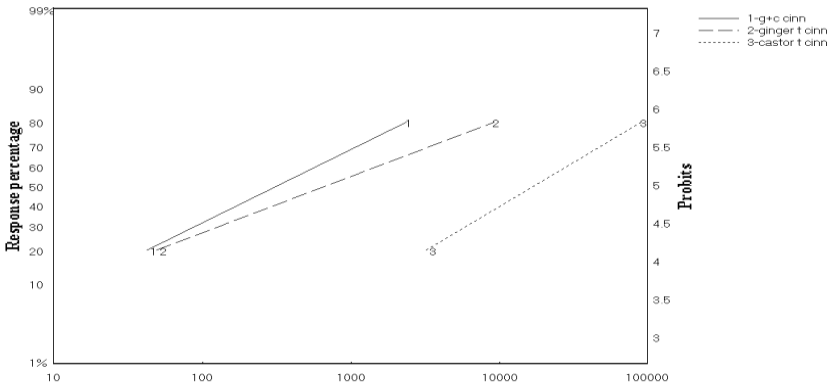


Figure (1): LC-P lines for ginger oil, castor oil and their mixture against adult female of *Tetranychus cinnabarinus.*

2. Bio efficacy of ginger plant oil, castor plant oil and their mixture on adult female of two spotted spider mite *Tetranychus urticae:*

Data given in Table (3) revealed that, the mixture of ginger and castor oils caused higher mortality proportion than ginger oil alone on *T. urticae,* while castor oil alone caused very low mortality proportion. Mohammed *et al*., 2018 proved that, castor oil has moderate mortality proportion against *T. urticae* with high concentrations.

Data in Table (4) and Figure (2) showed that, the mixture of ginger and castor oils and ginger oil were more effective than castor oil with LC_{50} : 429.71 ppm, 1517.39 ppm& 23587 ppm, respectively. However, the toxicity index was 100% for the mixture of two oils, 28.32 % for ginger essential oil while was 1.82% for castor essential oil. The slope values indicated that the ginger oil had the lowest value which was 0.751 followed by 0.762 and 1.067 for the mixture and castor oil, respectively. These results agreed with Abd Allah and Marouf, 2015 and Mohammed *et al*., 2018. The results proved that *T. cinnabarinus* was more effective to plant extracts than *T. urticae.* Habashy *et al*., 2015 proved that also.

Table (3): Corrected mortality % of two spotted spider mite *Tetranychus urticae* treated with ginger and castor oils and their mixture under laboratory conditions 25±2 ◦C and 65±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Mortality after treatments %
		One day	Three days	Five days	Seven days	
Ginger oil	1000	23.33	6.67	10	6.67	46.67
	5000	13.33	16.67	20	10	60
	10000	26.66	6.67	23.33	16.67	73.33
	15000	30	10	23.33	16.67	80
Castor oil	1000	6.67	-----	3.33	-----	10
	5000	6.67	10	------	-----	16.67
	10000	16.67	3.33	6.67	3.33	30
	15000	23.33	10	10	6.67	50
Mixture of ginger and castor oils	1000	23.33	23.33	6.67	10	63.33
	5000	30	20	16.67	6.66	73.33
	10000	53.33	16.67	10	6.67	86.67
	15000	53.33	20	10	6.67	90

Table (4): Efficacy of ginger and castor oils and their mixture against *Tetranychus urticae.*

Treatments	Conc.	Corrected mortality %	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90}/ LC_{50}	R	P
Ginger oil	1000	46.67	1517.39	77038.39	0.751± 0.145	28.32	50.77	0.969	0.414
	5000	60							
	10000	73.33							
	15000	80							
Castor oil	1000	10	23587	375043.09	1.067± 0.184	1.82	15.9	0.916	0.022
	5000	16.67							
	10000	30							
	15000	50							
Mixture of ginger and castor oils	1000	63.33	429.71	20614.37	0.762± 0.155	100	47.97	0.951	0.239
	5000	73.33							
	10000	86.67							
	15000	90							

R: Regression **P: Probability**

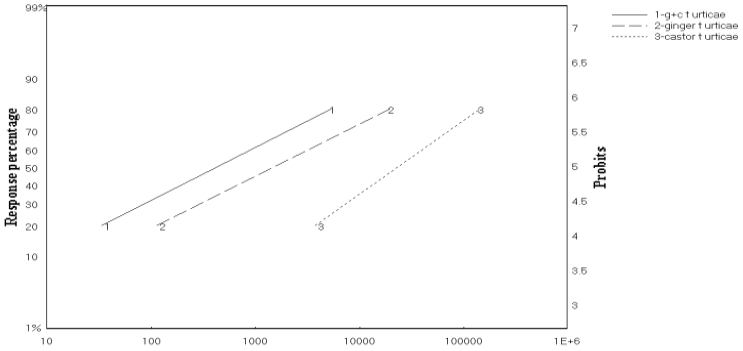


Figure (2): LC-P lines for ginger oil, castor oil and their mixture against adult female of *Tetranychus urticae.*

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Laboratory evaluation of different host plants and *Taxodium distichum* ethanolic extract on *Nezara viridula* (Hemiptera: Pentatomidae)

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Abstract:

The different host plants and ethanolic extract of *Taxodium distichum* were evaluated on mortality and some biological parameters of green sting bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) a major pest of some economic crops. The data revealed that the adult survival on okra pod, cabbage and lettuce were 52.94, 30 and 27.27% with longevity 9.56, 7.66 and 6.8 days, respectively. In addition, the insecticidal activity of ethanolic extract of *T. distichum*, it had a more potent on controlling *N. viridula* where LC_{50} and LC_{90} were 7.49 and 14.41% after 72h post-treatment. The ethanolic extract induced noted a decline in the longevity of male (4.42, 3.14) and female (5.57, 2.71) at 5% and 10% conc., respectively in comparison to control 14.71 and 22.57 day for male and female. Finally, *T. distichum* ethanolic extract is evidence that it is a good efficient for green stink bug control.

Introduction

The green sting bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) is a serious economic polyphagous pest to most of the crops in Egypt due to its stylet penetrate the plant tissues causing damage to all developmental stage of the plant and so it is difficult to control. A few studies are known concerning feeding techniques of *N. viridula* , although *N. viridula* biology and ecology are extensively recorded (Huang and Toews, 2012). There was a limited attempt to improve the Menusan's green bean method in evaluating laboratory rearing of green sting bug on *Phaseolus vulgaris* (L.) as a diet (Gonzales and Ferrero, 2008 and Silva *et al*., 2011). The green sting bug reared on different seed and plant tissue combination as green snap beans, raw-shelled peanuts, immature radish fruits and immature soybean pods caused shorter to duration period from egg to adult (Panizzi and Saraiva, 1993; Noda and Kamano, 2002 and Gonzales and Ferrero, 2008). In Integrated Pest Management, the resistances of plant studies are techniques take part in insect population reduction (Souza *et al*., 2013).

The using increase of chemical insecticides or alternate one chemical product leads to more problems in controlling sting bugs (Musser *et al*. 2011) which aid in developing sting bug resistance to chemical insecticides and environment pollution (Hart and Pimentel, 2002 and Sosa-Gomez and Silva, 2010). Consequently, the alternative uses of plant extracts as natural pesticides are more remarkable because they have toxic bioactive compounds that low toxicity on mammalian.

Taxodium distichum (Cupressaceae) exhibited antioxidant, antitumor, cytotoxic, antiviral, antibacterial and antifungal activities (Ibrahim *et al*., 2006 and Kusumoto *et al*., 2010) because of its enrichment in glycosides flavonoids, monoterpenes, diterpenes, and sesquiterpenes. The insecticidal activity of *T. distichum* extracts was less evaluated.

The present work aims to estimate survival activity observed for *N. viridula* reared on the different host plant. Also, the toxic effect of *T. distichum* fruit ethanol extract on some biological parameters of *N. viridula* adult and determined its LC_{50} and LC_{90} were evaluated.

Materials and Methods

1.Insect source:

Adults of *N. viridula* were obtained from a colony reared on fresh green bean, *Phaseolus vulgaris* L. in the laboratory with fixed temperature of 27± 2 ^{o}C , 65±10% RH and12:12 (L:D) h. photoperiod at Pest Physiology Research Department, Plant Protection Research Institute. The ethanolic extracts of *Taxodium distichum* were dissolved in ethanol.

2. Extraction of *Taxodium distichum*:

The fruit of *T. distichum* collected from El-Orman garden Giza, Egypt and then dried under vacuum at 30 oc until and crashed into powder. About 500g of *T. distichum* powder was steeped at room temperature in ethanol for one week. The extract was then filtered, concentrated to dryness in a rotary evaporator at 50 oc.

3.Bioassay:

3.1. The effect of different host plants:

The adult of *N. viridula* fed on cabbage, *Brassica oleracea* (Brassicaceae); lettuce, *Lactuca sativa* (Asteraceae); castor leaves, *Ricinus communis* (Euphorbiaceae); okra leaves and pods, *Hibisicus esculentus* (Malvaceae) under laboratory conditions in glass jars. The food source was renewed daily. The survival percent and longevity of adult green sting bug were determined.

3.2. The effect of *Taxodium distichum* ethanolic extract:

Two concentrations of *T. distichum* ethanolic extract 5 and10% were prepared with distal water. 5µL of each concentration were applied on the tergites of each adult with the topical micro applicator and 5µL distal water for control then one pair of male and female was put in glass jar lined with filter paper and covered with organza and feeding with okra pods according to (Costa *et al*., 1998). Seven replicates for each treatment were used to evaluate the mortality percent and longevity of male and female adults, the number of deposited eggs per female and hatchability. All bioassays were established at 27°C± 2°C, RH of 65%±10 %, and photoperiod of 12 hours. The mortality data after 48, 72 and 96h were corrected according to Abbott's formula (1925).

4.Statistical analysis:

Differences among mean were analysed using Duncan's analysis of variance (ANOVA), the least significant difference by computer statistical software Costat® (2005). The significance test at probability value $p < 0.05$ was considered significant. The toxicity line of *T. distichum* ethanolic extract were analyzed with Biostat version 5 using Probit-analysis.

Results and discussion

1.Effect of different host plants on adult *Nezara viridula* survival:

Data in Figure (1) illustrated the effect of different host plants as cabbage, *B. oleracea*; lettuce, *L. sativa*; castor leaves, *R. communis*; okra leaves and pods, *H. esculentus* on survival percent of *N. viridula*, green sting bug. All host plants caused 100% survival on the first day after feeding except castor leaves caused 37.3%. While on a ninth day the adult survival on okra pod, cabbage and lettuce were 52.94, 30 and 27.27%, respectively. In the same trend, regarding Table (1) the longevity of adult green sting bug was 9.56, 7.66 and 6.8 days on okra pods, cabbage and lettuce host plants on the contrary, the lowest longevity of adult 2.5 days was recorded on castor leaves. There were highly significant differences in the longevity of *N. viridula* adult feeding on the various host plants.

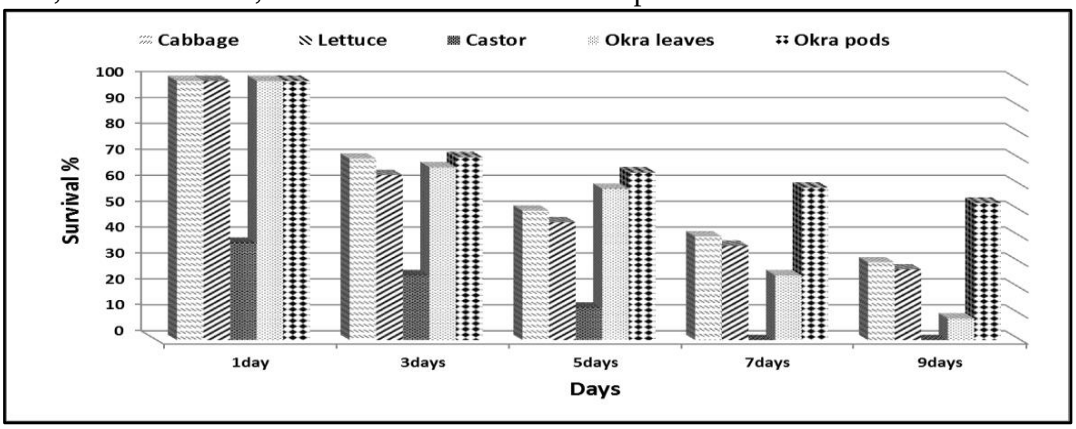

Figure (1): Survival percentage of adult *Nezara viridula* reared on different host plants.

Table (1): Effect of different host plants on *Nezara viridula* adult longevity.

Host plants	Longevity (days)
Cabbage leaves	$7.66^{b}\pm0.12$
Lettuce leaves	$6.8^{bc}\pm0.15$
Castor leaves	$2.5^{d}\pm0.28$
Okra leaves	$5.53^{c}\pm0.28$
Okra pods	$9.56^{a}\pm0.79$
LSD	1.28
P	0.0000***

L.S.D. means low significance differences at P< 0.05

2. Effect of *Taxodium distichum* ethanolic extract on mortality of *Nezara viridula* adult:

The efficacy of two concentrations (5 and 10%) *T. distichum* ethanolic extract were observed in Table (2). The mortality percent of adult green sting bug at 5% concentration was slightly decreased after 48h (7.14) then increased after 96h reached to 50%. On the other hand, the second concentration 10% exhibited more toxic after 48, 72 and 96h

and the mortality % were 35.71, 71.43 and 100%, respectively on comparison to control. The mortality of green sting bug adults increased with increasing concentrations of *T. distichum* as well as experimental duration.

Table (2): Mortality % of *Nezara viridula* adult infected with ethanolic extracts of *Taxodium distichum* under laboratory conditions.

Treatment	Conc. (%)	Mortality%		
		48h	72h	96h
Taxodium distichum	5%	7.14	21.42	50
	10%	35.71	71.43	100
Control		0.00	0.00	0.00

3.Susceptibility of *Nezara viridula* adult to *Taxodium distichum* ethanolic extract:

Regarding Table (3) and Figure (2) the lethal concentration of T. *distichum* ethanolic extract caused 50% and 90%; LC_{50} and LC_{90} mortality to green sting bug adults were 7.49 and 14.41% with slope 4.50 after 72h post-treatment. The mortality of *N. viridula* increased with increasing concentrations of *T. distichum* ethanolic extract.

Table (3): The lethal concentrations of *Taxodium distichum* ethanolic extract against *Nezara viridula.*

Ethanolic extract of	Lethal concentration (%)		Lower limit	Upper limit	slope
Taxodium distichum	LC_{50}	7.49	5.29	11.64	4.50
	LC_{90}	14.41	10.06	18.27	

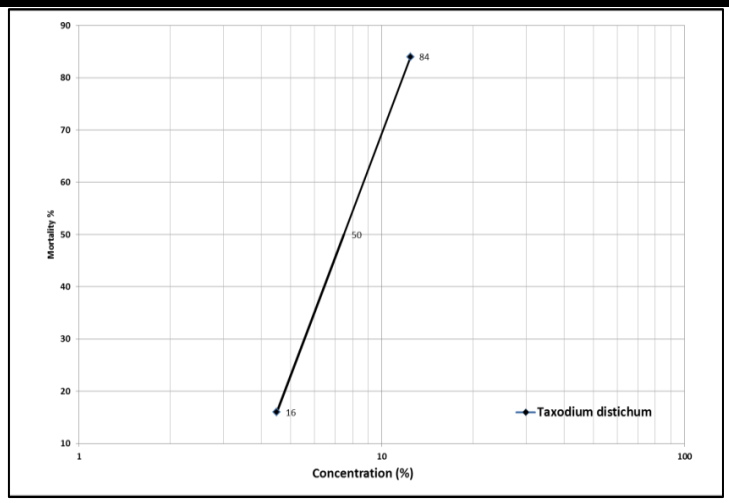

Figure (2): Probit-analysis of toxicity lines of *Taxodium distichum* ethanolic extract on *Nezara viridula* adult.

4.Effect of *Taxodium distichum* ethanolic extract concentrations on some biological parameters of *Nezara viridula* adult:

The obtained results in Table (4) and Figure (3) illustrated the longevity of male and female, mean no. of deposited egg per female and hatchability% of green sting bug. There were highly significance differences between all treatments in various observed biological parameters. The male longevity was 4.42, 3.14 and 14.71day at 5%, 10% and control, respectively. In the same context, the female longevity was 5.57, 2.71 and 22.57 days at 5%, 10% and control, respectively. There was no egg deposited at 10 % concentration subsequently, there was no hatchability. While at 5% there were 11.28 eggs with 7.49% hatchability in comparison with control was 55.71 eggs with 93.27% hatched. In conclusion, all tested biological parameters were highly decreased than the control.

Table (4): Biological parameters of *Nezara viridula* adult after treatments with *5*&10% *Taxodium distichum* ethanolic extract.

Treatments	Longevity(days) Mean±SE		Mean no of deposited Egg/female Mean±SE	Hatchability% Mean ±SE
	Male	Female		
5%	4.42^{b}±0.29	5.57^{b}±0.61	11.28^{b}±4.13	7.49^{b}±3.08
10%	3.14^{b}±0.34	2.71^{c}± 0.28	0.00^{c}±0.00	0.00^{c}±0.00
Control	14.71^{a}±0.91	22.57^{a}±0.84	55.71^{a}±2.73	93.27^{a}±1.39
LSD	1.75	1.85	8.50	5.8
P	0.0000***	0.0000***	0.0000***	0.0000***

Same letters mean non-significant effect
Different letters mean significant effect at p<0.05.

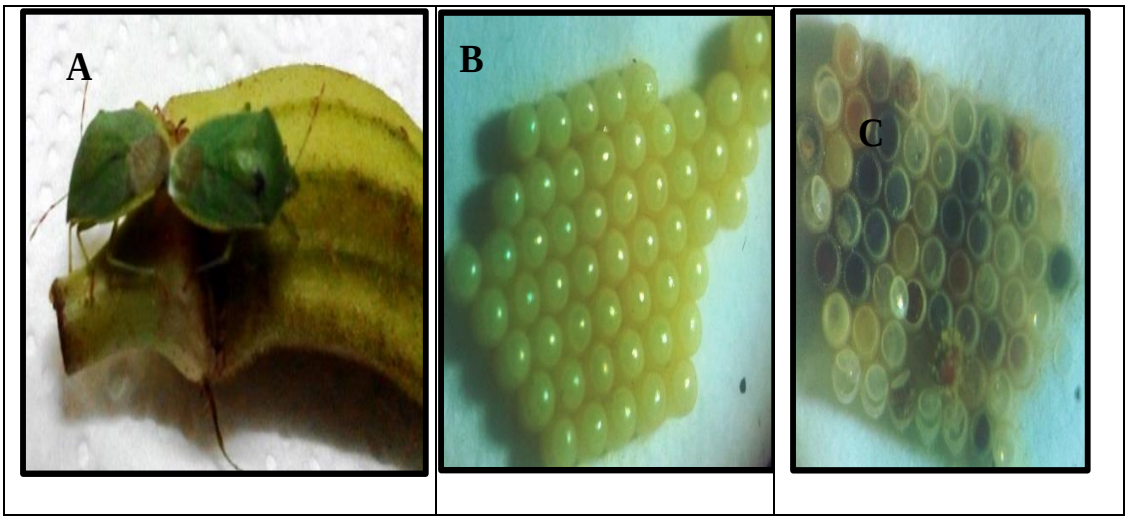

Figure (3): Effect of ethanolic extract of *Taxodium distichum* on egg deposit.

A. Mating of male and female after treatment.
B. Normal deposit egg in control.
C. Unviable egg in treatment with 5% conc.

The different host plants under investigation are effects on survival percent of *N. viridula*, green sting bug. The survivorship of adult on okra pod, cabbage and lettuce were 52.94, 30 and 27.27% and the longevity of adult green sting bug were 9.56, 7.66 and 6.8 days, respectively on contrary the castor leaves not a preferred host plant to of adult 2.5 days was recorded on castor leaves. There were highly significant differences in the longevity of *N. viridula* adult feeding on various host plants. These results are in harmony with (Panizzi *et al.*, 2000) stated that the low nymphal mortality of green sting bug reared on the artificial diet and soybean pods was 30%. In the same trend, the adult of green sting bug survival was 97.3 and 74.67% on fresh and dry yolk chicken egg-based diet, respectively (Portilla *et al.*, 2015). Also, the duration of *N. viridula* reared on immature soybean pods was 90.6 d (Gonzales and Ferrero, 2008).

The concern of rearing techniques of green sting bug on different host plants is not only to investigate the population dynamics in the laboratory but also to design its control strategies. The obtained results indicated that the ethanolic extract

of *T. distichum* has insecticidal activity on *N. viridula* adult where LC_{50} and LC_{90} were 7.49& 14.41% with slope 4.50 after 72h post-treatment. These results are in the same context as the results of (Sabry, 2018) revealed that the LC_{50} and LC_{90} were 10490 ppm and 27890 ppm with slope 3.007 on 4th instar larvae of *Spodoptera littoralis* after 72 hours of treatment. The contact and the stomach poison activity of petroleum ether and acetone extracts of leaves from bald cypress (*T. distichum*) were tested on adults *Tribolium castaneum* Herbst, mortality percentage increased with concentration and exposure time increasing (Shoukry *et al*., 2017).

The ethanolic extract of *T. distichum* fruits caused highly decreased in all tested biological parameters; longevity, deposited egg/female and hatchability than the control, there was no egg deposited at 10 % concentration while at 5% there was 11.28 egg with 7.49% hatchability in comparison with control was 55.71 egg with 93.27% hatched. These results are supported by Piton *et al*. (2014) evaluate the contact toxicity of the leaves acetonic extract of *Piper aduncum* (L.) on brown stink bug, *Euschistus heros* developmental stages, all concentrations tested reduced significantly the survival and reproduction in the adult bioassay. In the effect on egg contact bioassay the 8% concentration caused 19% mortality. In the same frame of reference Carneiro *et al*. (2013) found that ovicidal action of *Annonaceae* extract on the chorion of *Rhodnius neglectus* (Lent.) (Hemiptera: *Reduviidae*) eggs caused unviability of 90% of the eggs.

The mortality, low longevity and fertility of *N. viridula* adults may be related to the presence of the bioactive compounds in *T. distichum* ethanolic extract which responsible for its toxicity. (Sabry, 2018) recorded that Ferruginol, (Di-(2-ethylhexyl) phthalate), piperine, 3alpha, Didecyl phthalate and octadecane, 1-[2-(hexadecyloxy) ethoxy] are the major active compounds. Furthermore, the existence of taxodione compound; a quinone methide diterpene that possesses insecticidal activity and DNA-binding effects (Fraga *et al*., 2005 and Zaghloul *et al*., 2008). Besides, it acts as cholinesterase inhibitor (Kuźma *et al*., 2016 and Liu *et al*., 2014) and cytotoxic activities, causing apoptosis in several tumor cell lines as well as Phthalate compound is a more efficient as larvicidal activity Khatiwora *et al.* (2013).

It is concluded that the okra pod plant can be used in rearing techniques of green sting bug under laboratory conditions. The castor leaves not a preferred host plant to *N. viridula* so it can be cultivated surrounding the economic crop. The insecticidal activity and the latent effect of the biological parameter of *N. viridula* adult may be regarding the active chemical compounds of *T. distichum* ethanolic extract. These promising results are encouraged to schedule *T. distichum ethanolic* extract into insect management programs.

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Impact of nanoparticle materials on the control of seedling pests in the Egyptian cotton.

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Abstract:

Nanotechnology opens a large scope of novel application in the fields of biotechnology and agricultural industries, because nanoparticles often have unique physical and chemical properties, i.e. high surface area, high reactivity, tunable pore size, and particle morphology. So, this study aims to assess the effects of nanoparticles on cotton seedling pests under field conditions. Cotton seeds were treated with five nanoparticles (NPs) ; titanium dioxide (TiO_2), zinc oxide (ZnO), iron oxide (FeO), silicon dioxide (SiO_2) and Copper oxide (CuO) at three concentrations; high 1000 ppm, middle 500 ppm and low 250 ppm in a field experiment during 2017 and 2018. Our results demonstrated that cotton plants cultivated among 25cm distance treated with five nanoparticles affect the seedling pest infestation. Both CuO and TiO_2 nanoparticles were the most effective treatments against the Jassid pest *Empoasca lybica* (De Berg.) (Hemiptera:Cicadellidae) at high concentration during the two tested years. While, the ZnO had the most potent effect in decreasing the whiteflies populations *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) at the three tested concentrations during both 2017 and 2018 years. Also, TiO_2, SiO_2 and CuO induced the most potent effect against thrips pest *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) at high concentrations during the two tested years, also ZnO and FeO reduced thrips pest populations to zero at 1000 ppm during 2017 year. On the other hand, Tio_2 nanoparticles caused the highest powerful effect against the red spider pest *Tetranychus telarius* (L.) (Acari: Tetranychidae) in during the two years. TiO_2 and ZnO nanoparticles treatments caused the most competent action against aphids *Aphis gossypii* Glover (Hemiptera: Aphididae) during 2017 and 2018 respectively. Thus, treatment of cotton plants with these nanoparticles extremely contributed in lessening insect populations and so improving cotton crop production.

Introduction

Cotton plants from the genus Gossypium are one of the major sources of fiber (Trapero *et al*., 2016). Besides its fibers, cotton plants also produce a large amount of seeds (1.65 kg seeds per kg lint) (Cai *et al*., 2010). The seeds are rich in protein and are a valuable source of oil and fodder (Watkins and Waldroup, 1995

and Bertrand *et al*., 2005). Cotton crop is infested by a wide range of insect pests at various growth stages (Uthamasamy, 1994). The insect pest's spectrum of cotton is quite complex and about 1326 species of insect pests have been listed on this crop throughout the world (Shivanna *et al*., 2011). Among these insects, Jassid *Amrasca devastans* (Distant) (Hemiptera: Cicadellidae) , thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) are very serious affecting the yield and quality of this cultivar (Ali, 1992). Thrips are minute plant feeding insects that produce scars on leaves, flowers and fruit surface (Mahesh *et al*., 2010). Cotton aphids *Aphis gossypii* Glover (Hemiptera: Aphididae) injure cotton plants by continually feeding on fluids in plant phloem tubes. This feeding can stimulate foliar alterations, delay of the plant growth, fewer fruit setting, lower fruit retention and reduced cotton lint weight (Raboudi *et al*., 2002). Cotton jassids are known as standard sucking pest of cotton crop. Cotton yield becomes lesser, as low due to the increasing population of jassid which contrasted with different cotton yields (Ahmad, 1999 and Sahito *et al*., 2011). Cotton whitefly has very old history of infestation on cotton even before the introduction of modern insecticides (Hussain and Trehan, 1933). It is a polyphagous insect pest of many agricultural crops and cosmopolitan in distribution. In addition to direct damage to cotton crop, it inhibits photosynthetic activity and impairs fiber quality of the cotton. It is also well known vector of various viral diseases on many economic crops (Henneberry *et al*., 1999). Two spotted spider mite (TSSM) *Tetranychus urticae* (Koch.) (Acari: Tetranychidae) is a polyphagous and cosmopolitan pest of many field and horticultural plants (Hoy, 2011). TSSM is the 5th most damaging pest of cotton (Williams, 2016).

In the agricultural systems, nanotechnology has a great potential in providing a novel and improved solutions for many challenges. Nanotechnology improves safety of products, increases the efficiency of the production and decreased the pollution through the using of controlled delivery of pesticides, herbicides and fertilizers (Mehrazar *et al*., 2015). The Application of nanotechnology in crop protection have promising the future in management of the insects and pathogens, through controlled and targeted delivery of agrochemicals and as a tool for early detection (Pavitra *et al*., 2018). The toxic effects of nanoparticles (NPs) can be attributed to the small size and large surface area, thereby increasing chemical reactivity and penetration in the living cells (Gojova *et al*., 2007; Medina *et al*., 2007 and Pan *et al*., 2009). Shaker *et al*. (2017a) demonstrated that TiO_2 NPS are effective against the survival of the 2nd and 4th instar larvae of *Spodoptera littoralis*. Also, Shaker *et al*. (2018) indicated the efficacy of titanium dioxide (TiO_2) (NPs)+ copper oxide (CuO) (NPs) mixture against the same insect. Seed treatment is one of the highly progressive and demandable technologies in integrated pest management (IPM) for controlling various crop pests (Taylor *et al*., 2001 and Magalhaes *et al*., 2009). Thus, this study was designed to evaluate the beneficial effects of titanium dioxide (TiO_2), zinc oxide (ZnO), iron oxide (FeO), silicon dioxide (SiO_2) and Copper oxide (CuO) NPs on decreasing the populations of cotton jassids, aphids, thrips, white fly and two-spotted spider mite under field conditions during two seasons (2017 and 2018).

Materials and methods

Experiments were conducted in 2017 and 2018 at the Sids research station farm in Beni suef. Prior to planting, seeds were treated with the five nanomaterials, TiO_2, ZnO_2, FeO_2, SiO_2 and CuO NPs tested at three concentrations, High 1000ppm, Middle 500ppm and low 250ppm.Trials in 2017 year were planted on 15 Mars and on 10April at 2018 year. The cultivated area divided into several plots, each plot exceed 13.6 meter, in addition to that of control. Five replicates was utilized for each treatment of the five treatment in addition to three replicates or plots used as control to estimate the five NPs treatments impact on the seedling pests populations

Analytical grade titanium tetrachloride, sodium hydroxide Precursor zinc nitrate (Zn (NO3)2. 6H2O), precipitating agent KOH, Copper (II) chloride dehydrates and sodium hydroxide pellets were covered. Explanatory reagent graded chemicals were utilized within the analysis without further purification. Deionized water was utilized for washing purposes. All Nanoparticles were synthesized and characterized according to our previous work Shaker *et al*. (2017a and b)

Results and discussion

1. Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and Copper oxide nanaoparticles treatments on jassid count on cotton plants during 2017 and 2018:

Data shown in Table (1) illustrated the effect of treatments of cotton seeds cultivated among 25cm distance with TiO_2, ZnO, FeO, SiO_2 and CuO NPs at three tested concentrations (1000, 500, 250 ppm) on jassid pest infestation on cotton crop during 2017 and 2018. Treatments with the five tested NPs in all concentrations showed highly significant ($P<0.01$) effect on diminishing the jassid count on cotton plants during 2017 and 2018. Treatments with the high concentration of TiO_2 and CuO NPs seemed to have the highest significant ($P<0.01$) effect on decreasing jassid infestation during 2017 and 2018 years. Also, treatment FeO NPs in the three concentrations highly significantly ($P<0.01$) decreased the mean numbers of the Jassid/25 leaflets in 2017 and 2018 to average 39.9, 49.7, 62.9 and 35.1, 39.9, 55.8, respectively, as compared to controls.

Table 1: Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on jassid count on cotton plants during 2017 and 2018.

Treatments	Concentrations	Insect count (Mean±SE)	
		2017	2018
TiO_2	H.Conc.	10.8±0.7**	7.9±0.3**
	M. Conc.	56.8±0.7**	49.9±4.1**
	L. Conc.	69.7±5.8**	65±2.1**
ZnO	H.Conc.	53.3±0.9**	49.8±4.1**
	M. Conc.	62.2±0.4**	54.9±2.1**
	L. Conc.	76.6±0.9**	69.9±4.1**
FeO	H.Conc.	39.9±5.9**	35.1±2**
	M. Conc.	49.7±5.8**	39.9±4.1**
	L. Conc.	62.9±0.7**	55.8±1.9**
SiO_2	H.Conc.	53.2±0.7**	49.9±4.1**
	M. Conc.	61.2±0.7**	64.5±1.8**
	L. Conc.	77.3±0.9**	69.9±4.1**
CuO	H.Conc.	11±0.6**	8.9±0.4**
	M. Conc.	23.2±1**	27.9±0.4**
	L. Conc.	28.2±0.4**	36.7±0.5**
Untreated (Control)		140.2±1	130.1±0.7
P-value		0.000155	0.00155
F-value		60643.57	21912.93
at 0.05		9.546667	7.46
at 0.01		20.31333	13.68667

Data are expressed as Mean±Standard error (SE) ****= Highly significant (P<0.01)**

2. Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and Copper oxide nanaoparticles treatments on the whitefly on cotton plants during 2017 and 2018:

Data illustrated in Table (2) showed illustrated the effect of treatments of cotton seeds with TiO_2, ZnO, FeO, SiO_2 and CuO NPs at three tested concentrations (1000, 500, 250 ppm) on White fly count on cotton plants during 2017 and 2018. Treatments with the five tested NPs in all concentrations showed highly significant (P<0.01) effect on lessening the white fly count on cotton plants during 2017 and 2018. ZnO NPs treatment was the most potent in lowering the white fly count on cotton crop followed by TiO_2 and SiO_2 NPs treatments during the two tested years. Whereas, the plants treated with the FeO NPs had the least significant effect in the mean numbers decrease of the white fly/ 25 leaflets with the three concentrations. It averaged during the two tested years; 3.2, 4.8, 6.2 and 3.98, 4.8, 7, respectively as compared to 12.8 and 9.6 of the untreated plants.

Table (2): Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on the whitefly on cotton plants during 2017 and 2018.

Treatments	Concentrations	Insect count (Mean±SE)	
		2017	2018
TiO_2	H.Conc.	1.8±0.1**	1.5±0.2**
	M. Conc.	2±0.09**	1.8±0.2**
	L. Conc.	2.95±0.4**	2.98±0.3**
ZnO	H.Conc.	1.6±0.2**	1.4±0.2**
	M. Conc.	1.8±0.2**	1.8±0.1**
	L. Conc.	2.2±0.3**	2.3±0.4**
FeO	H.Conc.	3.2±0.3**	3.98±0.7**
	M. Conc.	4.8±0.1**	4.8±0.9**
	L. Conc.	6.2±0.1**	7±1.1**
SiO_2	H.Conc.	1.8±0.2**	1.9±0.2**
	M. Conc.	3±0.3**	3.1±0.3**
	L. Conc.	3.4±0.3**	4±0.7**
CuO	H.Conc.	2.6±0.2**	2.8±0.3**
	M. Conc.	3.6±0.5**	4±0.3**
	L. Conc.	4.2±0.7**	5±0.4**
Untreated (Control)		12.8±0.7	9.6±0.6
P-value		0.00176	0.00718
F-value		161.0667	1.35
at 0.05		2.706667	2.31
at 0.01		4.86	4.19

Data are expressed as Mean±Standard error (SE) **** = Highly Significant (P<0.01)**

3. Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on thrips during 2017 and 2018:

The effect of treatments of cotton seeds with TiO_2, ZnO, FeO, SiO_2 and CuO NPs at three tested concentrations (1000, 500, 250 ppm) on thrips count on cotton plants during 2017 and 2018 is demonstrated in Table (3). All treatments induced highly significant (P<0.01) effect on diminishing the thrips count on cotton plants during 2017 and 2018. TiO_2, SiO_2 and CuO treatments in high concentration recorded the most potent effect by decreasing the thrips count to zero in both years. Also, treatment with ZnO and FeO caused marked effect causing thrips count to be zero in 2017 compared with 8.9 of controls.

Table (3): Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on thrips during 2017 and 2018.

Treatments	Concentrations	Insect count (Mean±SE)	
		2017	2018
TiO_2	H.Conc.	0±0**	0±0**
	M. Conc.	0±0**	0.1±0.03**
	L. Conc.	0±0**	0.3±0.06**
ZnO	H.Conc.	0±0**	0.4±0.07**
	M. Conc.	0.8±0.1**	0.8±0.2**
	L. Conc.	1.1±0.3**	1±0.2**
FeO	H.Conc.	0±0	0.8±0.1**
	M. Conc.	0.5±0.04**	0.7±0.1**
	L. Conc.	0.8±0.1**	0.8±0.1**
SiO_2	H.Conc.	0±0**	0±0**
	M. Conc.	0.8±0.1**	1.1±0.3**
	L. Conc.	1±0.3**	1.2±0.2**
CuO	H.Conc.	0±0**	0±0**
	M. Conc.	0±0**	0.4±0.07**
	L. Conc.	0.6±0.1**	0.5±0.2**
Untreated (Control)		8.9+0.4	10.9+0.4
P-value		0.000013	0.000151
F-value		1010	1149.94
at 0.05		1.111111	1.34
at 0.01		2.027778	2.466667

Data are expressed as Mean±Standard error (SE) **** = Highly Significant (p<0.01)**

4.Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on the red spider during 2017 and 2018:

As depicted in Table (4) treatments of cotton seeds with TiO_2, ZnO, FeO, SiO_2 and CuO NPs at three tested concentrations (1000, 500, 250 ppm) caused a remarkable highly significant (P<0.01) effect on spider count on cotton plants during 2017 and 2018. Spider count in cotton plants decreased from 4.9 and 9.9 in controls of the two tested years to be 0.8 and 1.1 in high concentrated TiO2 treated plants. TiO_2 treatment induced the most potent effect followed by ZnO in 2017 and 2018.

Table (4): Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on the red spider during 2017 and 2018.

Treatments	Concentrations	Insect count	
		2017	2018
TiO_2	H.Conc.	0.8±0.04**	1.1±0.2**
	M. Conc.	1.1±0.3**	1.3±0.2**
	L. Conc.	1.2±0.3**	1.5±0.3**
ZnO	H.Conc.	1±0.4**	1.2±0.2**
	M. Conc.	1.6±0.3**	1.6±0.3**
	L. Conc.	1.8±0.4**	1.7±0.2**
FeO	H.Conc.	2±0.2**	3±0.4**
	M. Conc.	2.6±0.4**	3.6±0.2**
	L. Conc.	3±0.6**	3.9±0.4**
SiO_2	H.Conc.	1.8±0.4**	2.2±0.4**
	M. Conc.	2.2±0.4**	2.6±0.2**
	L. Conc.	2.8±0.5**	2.95±0.2**
CuO	H.Conc.	1.6±0.2**	2±0.4**
	M. Conc.	1.95±0.5**	2.7±0.4**
	L. Conc.	2.4±0.4**	3±0.4**
Untreated (Control)		4.9+1	9.9+0.4
P-value		0.021	0.00095
F-value		15.5	251.4067
at 0.05		2.84	1.72
at 0.01		5.21	3.193333

Data are expressed as Mean±Standard error (SE) **** = Highly Significant (p<0.01)**

5. Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on the aphid during 2017 and 2018:

Data showing the effect of treatments of cotton seeds with TiO_2, ZnO, FeO, SiO_2 and CuO NPs at three tested concentrations (1000, 500, 250 ppm) on aphids count on cotton plants during 2017 and 2018 are presented in Table (5). Treatments with these tested NPs in all concentrations showed highly significant ($P<0.01$) effect on reducing the aphid count on cotton plants during 2017 and 2018. TiO_2 and CuO Nps treatments induced the most potent against aphids in 2017 while ZnO NPs treatment was the most remarkable one in 2018.

Table (5) : Effect of titanium dioxide, zinc oxide, iron oxide, silicon dioxide and copper oxide nanaoparticles treatments on the aphid during 2017 and 2018.

Treatments	Concentrations	Insect count	
		2017	2018
TiO_2	H.Conc.	10.9±0.3**	1.1±0.2**
	M. Conc.	56.9±0.3**	1.9±0.6**
	L. Conc.	70±4.2**	3±0.3**
ZnO	H.Conc.	53±1.6**	0.9±0.1**
	M. Conc.	62±2.7**	0.8±0.2**
	L. Conc.	77.8±3**	1.1±0.3**
FeO	H.Conc.	40±3.2**	1.2±0.2**
	M. Conc.	50±3.2**	1.8±0.5**
	L. Conc.	63±0.9**	1.4±0.2**
SiO_2	H.Conc.	53.3±2.3**	2.9±0.5**
	M. Conc.	61±0.5**	3.1±0.3**
	L. Conc.	77±2.4**	5±0.9**
CuO	H.Conc.	11±0.3**	3.3±0.3**
	M. Conc.	22.6±0.8**	5.8±0.9**
	L. Conc.	27.5±0.5**	9.5±0.7**
Untreated (Control)		139.8	13±1.6
P-value		0.0000092	0.0102
F-value		81407.83	51.9
at 0.05		5.744667	4.95
at 0.01		10.54667	9.11

Data are expressed as Mean±Standard error (SE) ** = Highly Significant (p<0.01)

Thiamethoxam and Imidacloprid pesticides were examined against *B. tabaci* on cotton seeds. The data revealed that the used pesticides have a great effect on the control of *B. tabaci* up to 45 days under laboratory and greenhouse conditions, and up to 2 months under field conditions (Zhang *et al*., 2011). Also maize seeds treated with imidacloprid show resistance against soil pests, aphids, leafhoppers and the first generation of corn borers (Pons and Albajes, 2002). Treatments of cotton seeds with TiO_2, ZnO, FeO_2, SiO_2 and CuO at 1000, 500, 250 ppm in the current study reduced the cotton seedling pests; *T. tabaci*, *E. lybic*a, *B. tabac*i, *A. gosspiila*, *T. telarius*, as respect of that of control. Rouhani *et al*. (2012a) indicated that Ag and Ag-Zn NPs synthesized through a solvothermal method at different concentrations induce insecticidal activities aganist *Aphis nerii*. They recorded that LC_{50} value for imidacloprid, Ag and Ag-Zn NPs were 0.13 µL mL-1, 424.67 mg mL-1, and 539.46 mg mL-1, respectively. They showed that Ag NPs can be used as a valuable tool in the pest management programs of *A. nerii*. However; Rouhani

et al. (2012b) estimated the efficacy of silica NPs against the larvae and adults of *Callosobruchus maculatus*. They showed that the silica nanoparticles were very effective against both larvae and adults.

Vinutha *et al.* (2013) reported that nanotechnology played a very important role in the pest control of *Helicoverpa armigera* through biological control of its life cycle. Osman *et al*. (2015) mentioned that the nano-silica was the most effective compound followed by nano-Zinc oxide, then effective microorganisms (EMs), in causing high toxicity against *S. littorals*. They reported that all tested materials exhibited latent effect via producing high reduction in pupation and adult emergence rates, decreasing both larval and pupal weight of this pest and reducing estimated enzymes activity, except phenol oxidase. Also, these NPs decrease both total carbohydrates and proteins suggesing that using silica, ZnO NPs as well as EMs would be useful eco-friendly components for controlling *S. littoralis*. Moreover, Araj *et al.* (2015) used five sources of silver NPs and sulfur NPs in different concentrations on the larval, pupal, and adults of the fruit fly *Drosophila melanogaster* under laboratory conditions. They found that Ag NPs were most effective against the larvae, pupae, and adults' mortality and egg suppression. In addition, Routray *et al*. (2016) proved that Application of nanotechnology in the crop protection holds a significant promise in management of insects and pathogens, by controlled and targeted delivery of agrochemicals. They found that the nanoparticles had insecticidal properties well studied on the stored grain insects (*Tribolium castaneum, Martianus dermestoides, Callosobruchus maculatus, Sitophilus oryzae, Corcyra cephalonica, Rhyzopertha dominica*), crop pests (*Spodoptera litura, Aphis nerii, Bactrocera dorsalis*) and other pests. They supposed that nanotechnology will revolutionize agriculture including pest management in the near future.

Khooshe-Bast *et al*. (2016) demonstrated high mortality rates of *Trialeurodes vaporariorum* after treatment with with ZnO NPs. Also, Shaker *et al*. (2017a and b) recorded that treatments with TiO_2 NP tested against the larvae of *Spodoptera littoralis* at all concentrations used 1000, 500, 250, 125, 62.5 and 31.25 ppm indicated higher toxic action for the 2nd instar parallel with concentrations than of the 4thone. Athanassiou *et al. (*2018) mentioned that NPs can be used successfully as insecticides alone and several types of NPs are produced by natural resource-based substances used them promising green alternatives to the use of traditional pest control.

It is concluded that cotton seed treatments withTiO2, ZnO, FeO, SiO_2 and CuO NPs induced potential effects against seedling insect population which were evidenced by decreasing jassid, aphids, thrips, whitefly and red spider counts in cotton plants.

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Direct toxicity effect of *Beauveria bassiana* and emamectin benzoate on *Pectinophora gossypiella* eggs (Lepidoptera: Gelechiidae) and *Tetranychus urticae* and their indirect effect on *Euseius scutalis* (Acari: Tetranychidae: Phytoseiidae)

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Abstract:

Two experiments were carried out to study the toxicity of the entomopathogenic fungus, *Beauveria bassiana* (10%) and the bioinsecticide, emamectin benzoate (2.15% EC) on eggs of the cotton pink bollworm, *Pectinophora gossypiella* (Saund.) (PBW) and two spotted spider mites *Tetranychus urticae* (Koch) (Acari: Tetranychidae) and their indirect effect on some biological parameters in addition to the feeding capacity of the predacious phytoseiid mite, *Euseius scutalis* (El-Badry) (Athisa-Henriot) (Acari: Phytoseiidae), under the laboratory conditions of 26 ±1°C and 65±5% RH. The results revealed that the two pests were highly susceptible to Emamectin benzoate than *Beauveria bassiana* as the LC_{50s}values were 0.484 and 0.179 ppm on PBW eggs and *T. urticae*, respectively, when treated with emamectin benzoate, while they were 43.3 and 11.07 ppm with *Beauveria*. The incubation period of *P. gossypiella* eggs prolonged to (4.66 days), when treated with *B. bassiana* and increased to (6.7 days), with emamectin, compared with (3.3 days) in the untreated (control). Feeding predacious mite, *E. scutalis* on *P. gossypiella* eggs and moving stages of *T. urticae* treated with emamectin benzoate and *B. bassiana***,** showed a considerable prolongation in total immature stages to (6.1 and 7.4 days) on PBW than (5.4 days) in the control and (7.2 and 8.8 days) than (6.0 days) for control, when fed on *T. urticae* treated with Emamectin benzoateand *B. bassiana*, respectively. Treatment with emamectin benzoate caused a higher reduction in the total food consumption of the predatory mite than that with *B. bassiana*.

Introduction

The pink bollworm (PBW), *Pectinophora gossypiella* (Saund.)(Lepidoptera: Gelechiidae), is a significant pest of cotton plants in Egypt (Abd El-Mageed *et al*., 2007). It lays its eggs on different parts of the cotton plant; squares, flowers and green bolls. The eggs hatch in 3-4 days and larvae

penetrate flower orthe squares or the green bolls to complete their development (Amer, 2006). The two spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae), is a polyphagous mite and a serious pest world-wide (Nauwen *et al*., 2001). The importance of this mite pest is not only cussed the direct damage to the plants but also it decreases the photosynthesis and transpiration of the plant leaves causing low yields (Golam, 2002). Many trials all over the world have succeeded using bio-pesticides in controlling mite pests in different orchards and field crops, such as the studies by Aucejio *et al*. (2003) and Aimee and Oscar (2007).

Insect pests and/or spider mites problems usually increase, when their natural enemies are destroyed by applications of broad spectrum pesticides (Mainul *et al*., 2010). Spider mites are rapidly developed resistance to a series of acaricides (Van Leeuwen *et al.,* 2004) and have recently assumed a new aspect of multiple resistances (Kim *et al.,* 2006). A large numbers of commercial pesticides have a negative impact on the environment as well as natural enemies. Therefore, it is necessary to minimize the dependence on using chemical control and encouraging the use of biocides (El-Saiedy *et al*., 2015).The development of microbial control technology can help in developing its application in control programs; on other hand the laboratory evaluations of the effectiveness of the potential microbial control agents are necessary (Wraight *et al.* ,1998) ,the bio-pesticides; emamectin benzoate is a derivative of the natural Avermectin family produced by fermentation of soil microorganism *Streptomyces avermitilis* (Schallman *et al.*, 1987).

Beauveria bassiana is a virulent, entomopathogenic fungus with a very wide range of insect pests and it is a resident of soil (Klingen *et al.*, 2002) and has semelparous life history with a single reproductive episode. This entomopathogenic fungus considerable a novel foliar insecticides of lepidopteron and other groups biological control agent against insect pests or mites (Lacey and Gottel, 1995).

Several laboratory methods are designed to evaluate the effects of pathogens by exposing predatory mites to pathogen (Zhang *et al.,* 2015 and Dogan *et al.,* 2017). The predatory mite *Euseius scutalis* (El-Badry) (Athisa-Henriot) (Acari: Phytoseiidae) is considered the most common predator on cotton and other economic crops in Egypt (Fouly *et al*., 2013). Other studies reported that the predatory mite *E.scutalis* attacks many species of preys such as *T. urticae* (Osman *et al*., 2013), whitefly (Mainul *et al.*, 2010) and reared under laboratory condition on *T. urticae* and PBW eggs (Sholla *et al.*, 2017).

The objective of the present study was to evaluate under laboratory conditions the direct effects of *B. bassiana* and emamectin benzoateon pink bollworm eggs and moving stages of *T. urticae* and their indirect effects on some biological aspects, when the predacious mite, *E.scutalis* was allowed to feed on pink bollworm eggs and moving stages of *T. urticae.*

Materials and methods

1.Biopesticides used:

Two bio-pesticides were evaluated:

1.1. Common name: Emamectin benzoate
Trade name: (Emacte 2.15 %EC). Rate of application: 150 cm^3 / 100 L.

1.2. Common name: *Beauveria bassiana*
Trade name: Biover 10%Rate of application: 200 g / 100 L

2.Tested insect:

Laboratory strain of the pink bollworm (PBW), *P. gossypiella*, reared for several generations at Bollworms Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt under the laboratory conditions of 26±1°C and 65±5 RH% on an artificial diet previously described by Rashad and Ammar (1985).

2.1. *Tetranychus urticae:*

Castor bean leaves, infested with the two spotted spider mite *T. urticae* was collected from Giza Governorate, Egypt; and transferred to the laboratory for mass rearing of the mite. Adult females of *T. urticae* were left to lay eggs on leaf discs of *Acalypha marginares* and kept on a moist cotton pad in a petri dish (15 cm in diameter), where suitable moisture was supplied daily to keep the leaf discs fresh for longer time and for collecting the deposited eggs easily.

2.2. Collection and rearing of *Euseius scutalis* predator:

The predacious mite *E. scutalis* (different immature stages) were collected from the leaves and flowers of Egypt cultivated cotton (during 2017) at Qaluobia Governorate and then transferred to the laboratory. The adult females of *E. scutalis* were provided by *T. urticae* and/or eggs of *P. gossypiella* as food sources and incubated at 26±1°C and 65±5% RH. The newly deposited eggs were singly transferred from the culture to the leaf discs, kept on moist cotton pads in (15 cm petri dishes) to estimate the incubation periodand hatchability of *E. scutalis* for used in the experiment..

3.Preparation the pesticides used:

Two preparations (*B. bassiana* and *E. benzoate*) for tested against PBW eggs and moving stages of *T. urtic*ae. Five concentrations / eachcompound were prepared as follow: (1.98, 0.99, 0.495, 0.242 and 0.0.121 ppm) for emamectin and (200,100, 50, 25, 12.5 and 6.25 ppm) for *B. bassiana*.

4. Experimental techniques:

4.1.Toxicity of tested compounds to *Pectinophora gossypiella* eggs and *Tetranychus urticae*:

4.1.1.Toxicity on *Pectinophora gossypiella* eggs:

The toxic of two tested bio-chemicals; against the *P. gossypiella* eggs, were evaluated by the dipping technique; Three replicates from *P.gossypiella* eggs for each concentration for *B. Bassiana* and *E. benzoate* were used, each replicate contained 150-200 eggs (0-2 day old), deposited on piece of paper. The strips with attached eggs were dipped in each tested concentration (*B. bassiana* or *E. benzoate*) for 10 sec., and then left to dry. Another three replicates (100-150 eggs, deposited on a piece of paper), were dipped in water as check. Treated eggs were placed in a clean tube (3x10 cm.) until hatching under the previous conditions. Afterwards the hatched and unhatched eggs were recorded for each treatment; also the incubation periods were estimated.

4.1.2.Toxicity on *Tetranychus urticae*:

The toxic of two tested bio-chemicals; against the two spotted spider mite *T. urticae*, were evaluated by thespray technique; 150 individuals of moving stage (immature of the spotted spider mites) were divided into two groups, each group75 individuals and each group was divided into three (replicates), each replicate (25 individuals), placed on discs of *Acalypha marginares* and kept on a moist cotton pad in a Petri dish (15 cm in Diameter). The first group was sprayedby *B. bassiana* and the 2nd group was sprayed by Emamectin. The mortality rate after 24h to 3 days was estimated. Data were corrected according to Abbott's formula (1925), the LC_{20}, LC_{50} and

LC_{90} valuesfor each compound were calculated, using the LDP line program. The potency levels and the toxicity index were also calculated, according to (Sun, 1950).

Toxicity index = LC50 or LC90 of the most toxic compound/ LC50 or LC90 of the tested compounds x 100.

Relative Potency = LC50 of the least toxic compound/ LC50 of the tested compounds.

4.2. Some biological aspects and food consumption of *Euseius scutalis* when fed on treated *Tetranychus urticae* and *Pectinophora gossypiella*:

Newly hatched larvae of *E. scutalis* were divided into six groups; each group replicates three times, each replicate (20 individuals). The everyone from each group, concluded the predator of *E. scutalis* were confined singly on the strip with *P. gossypiella* eggs were dipped in each LC_{50} values for *B. bassiana* or emamectin tested compounds as following:

-The first group fed on *P. gossypiella* eggs (from 0-2 days eggs age) dipping in LC_{50}values of *B. bassiana.*

-The second group fed on *P. gossypiella* eggs (from 0-2 days age) dipping in LC_{50}values of Emamectin.

- The third group fed on eggs of *P. gossypiella* untreated as a control.

-The fourth group, predator of *E. scutalis* was confined singly on the leaf discs after spring the moving stages of *T. urticae,* after spraying by LC_{50} of *B. bassiana* for food

-The fifth group, predator of *E. scutalis* were confined singly on the leaf discs after spraying the moving stages of *T. urticae,* by LC_{50} of emamectin. At the same time and the 6^{th}group was fed on untreated immature stages of *T. urticae*.

The treated or untreated of *T. urticae* (immature stages) or *P. gossypiella* eggs were provided every day as a food source for predatory mites, the numbers of introduced prays increased (20 individuals) daily until thepredacious mite*E. scutalis* completing different immature stages. All experiments observed daily to recorded some biological parameters of *E. scutalis* such as; developmental time of different immature stages, food consumption /day, percent of mortality, life cycle and life span of the predator, data were daily recorded.

5. Statistical analysis :

All biological parameters of the predatory mite, *E. scutalis* were analyzed by Costat statistical program software, 1990 and Duncan's multiple range test (Duncan, 1955) at 5% probability level to compare the differences among time means.

Results and discussion

1.Toxicity effects of emamectin benzoate and *Beauveria bassiana* on *Tetranychus urticae* and *Pectinophora gossypiella.*

Based on all LC values data in Table (1) showed that, the effect of emamectin benzoate was greater than that of *B. bassiana* on both *P. gossypiella* eggs and moving stages of *T. urticae.* The LC_{50} values for emamectin treatments were 0.484 and 0.179 ppm for PBW eggs and moving stages of *T. urticae,* respectively, while for *B. bassiana* LC_{50} values were 43.35 and 11.07 ppm for PBW eggs and moving stages of *T. urticae,* respectively.

Table (1): Effect of *Beauveria bassiana* and emamectin benzoate on *Tetranychus urticae* moving stages and *Pectinophora gossypiella* eggs under laboratory conditions.

Treatment		PBW eggs				Susceptibility index based on		Potency levels based on	
		LC_{25}	LC_{50}	LC_{90}	Slop	LC_{50}	LC_{90}	LC_{50}	LC_{90}
P .gossypiella	***Beauveria***	18.47	43.35	219.17	1.82	1.12	1.11	1	1
	Emamectin	0.207	0.484	2.435	1.83	100	100	89.57	90.01
Treatment		**Moving stages**				**Susceptibility index based on**		**Potency levels based on**	
		LC_{25}	LC_{50}	LC_{90}	Slop	LC_{50}	LC_{90}	LC_{50}	LC_{90}
T. urticae	***Beauveria***	3.04	11.07	121.17	1.19	1.62	1.08	1	1
	Emamectin	0.0628	0.179	1.31	1.48	100	100	61.8	92.51

2.Susceptibility index and potency levels:

The data revealed that the PBW eggs and *T. urticae* moving stages were highly susceptible to emamectin benzoate treatment than *B. bassiana* with high potency of emamectin compound which is declared by (Sun, 1950) formulas of susceptibility index and potency level. At the level of LC_{50} Susceptibility index for *B. Bassiana* recorded 1.12 and 1.62 compared to 100 for emamectin benzoate for PBW eggs and *T. urticae* moving stages treatments, respectively.

These data indicated that the *T. urticae* moving stages high toxicity and high susceptibility to two compounds than *P. gossypiella* eggs. Amer (2004) found that spintor (natural compound) was potent against *P. gossypiella* (LC_{50} was 0.131 ppm). Al-Shannaf and Kandil (2005) recorded that the LC_{50} of spinosad for one and two day's old eggs of *Helicoverpa armigera* (Hb.) were 2.56 and 1.31 ppm, respectively. Sahab and Sabbour (2011) recorded that the LC_{50} values of *B. bassiana* was (179×10^4spores/ml) for PBW treated.

3.Effect two compounds on hatchability and incubation period of *Pectinophora gossypiella* eggs:

B. bassiana and emamectin benzoate, at LC_{50} level, reduced the percent of hatchability of PBW eggs to (56.0 and 49.6%), respectively, compared to (94%) in the control (Table, 2). In *B. bassiana* treatment, most of the egg hatchability percent (69.6%) occurred after 3-4 days post treatment, while in Emamectin benzoate treatment the most hatchability percent (71.0%) occurred after 4to 8 days post treatment. This different in hatchability may be due to the mode of action and penetration of these compounds into the eggs. However, the eggs were the most sensitive to emamectin benzoate than *B. bassiana*. Also, the percentages of egg hatchability recorded in Table (2) indicated that eggs were more sensitive to Emamectin benzoate treatment than *B. bassiana*. The incubation period of pink bollworm eggs was high affected by LC_{50} treatment of Beauveria and emamectin (Table, 2).

Table (2): Effect of *Beauveria bassiana* and emamectin benzoate on some parameters of *Pectinophora gossypiella* eggs.

Treatments (LC_{50})	Eggs hatchability % (after ---days)			Mean of Incubation period (Days±SE.)
	%	3-4 days post treatment	4-7 days post treatment	
B. bassiana	56.0	69.6	30.4	4.66±0.40
E.benzoate	49.6	29.0	71.0	6.7±0.54
Untreated	94.0	90.0	10.0	3.3±0.33
LSD				

The time required for incubation period estimated by 4.66 days/eggs when eggs treated with *B. bassiana* and highly increased to 6.7 days when treated with emamectin benzoate compared with 3.3 days with control with (approximately1 to 2 times).Other researchers have also reported ovicidal activities are due to fungal species as well as host species (Erler *et al*., 2013 and Dogan *et al.*, 2017).

4. Developmental periods of *Euseius scutalis*:

As shown in Tables (3 and 4), the incubation periods of eggs were (2.3 and 2.7 days), when *E. scutalis* was reared on *P. gossypiella* and *T. urticae,* respectively.

The total developmental period of the immature stages of *E. scutalis* was high significant affected by different food sources, treated with *B. Bassiana* or emamectin. The two tested compounds prolonged the duration of all immature stages than the control.5.4 and 6 days were required from larvae to develop to deutonymphal stages of *E. scutalis*, when fed on untreated *P. gossypiella* eggs and *T. urticae*, respectively. It was longer (6.1 days and 7.4 days), when fed on *P. gossypiella* eggs, and increased to 7.2 and 8.8 days when provided with *T. urticae* spryied by *B. bassiana* and emamectin, respectively (Tables, 3 and 4). Sholla *et al*. (2017) reported that the total developmental period of immature stages of *E. scutalis* were 6.6 days /♀ and 5.03 days /♂ on *P. gossypiella* eggs, prolonged to 6.68 days/♀ and 5.92 days/♂ on *T. urticae*. Osman *et al.* (2013) stated that the larval stage of *E. scutalis* lasted (2.31 days), when fed on nymphs of *T. urticae*, the proto-nymphal period was (2.56 days), deuto-nymph lasted (2.31 days) and total immature stages (7.06 days), when fed on nymphs of *T. urticae*, respectively.

5.Percent mortality of predator when reared on *Pectinophora gossypiella* eggs and *Tetranychus urticae* treated:

Data recorded in Tables (3 and 4) indicated that high significant difference ($P < 0.05$) between the predator mortality rates when the predator reared on *P. gossypiella* eggs or *T. urticae* treated with *B. bassiana* and emamectin; it were (17 and 33%mortality), when *E. scutalis* was fed on PBW eggs treated with *B. bassiana* and emamectin, respectively, compared to (4%) in untreated (control). While the respective, rates increased (23 and 39%, mortality) when fed on *T. urticae*, compared to (5%) in the control (Table, 4).

Table (3): Developmental time of the predatory mite *Euseius scutalis* when fed on *Pectinophora gossypiella* eggs treated with LC_{50} values of *Beauveria bassiana* and emamectin benzoate under laboratory conditions.

Treatments		Egg stage	Immature stages (days ± SE)				Life span days ± SE	Increase in duration	Mortality%
		Incubation period	Larvae	Prto-Nymph	Deuto-nymph	Total immature stages			
P .gossypiella	*B. bassiana*	2.3 ±0.1	1.6 ±0.1	2.1 ±0.2	2.4 ±0.3	6.1 ±0.5	8.4 ±0.5	1.1	17
	E. benzoate		1.9 ±0.1	2.6 ±0.1	2.9 ±0.2	7.4 ±0.6	9.7 ±0.61	1.26	33
	Untreated		1.3 ±0.2	1.8 ±0.1	2.30 ±0.3	5.4 ±0.2	7.7 ±0.3	------	4
LSD			0.114	0.133	0.027	0.103	0.99	-	-
P			**	**	**	***	***	-	-

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

Table (4): Developmental time of the predatory mite *Euseius scutalis* when fed on *Tetranychus urticae* treated with LC_{50} values of *Beauveria bassiana* and emamectin benzoate under laboratory conditions

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Treatments		Egg stage	Immature stages (days ± SE)				Life span days ± SE	Increase in duration	Mortality%
		Incub-ation period	Larvae	Prto-Nymph	Deuto-nymph	Total immature stages			
T. urticae	*B. bassiana*	2.70 ±0.2	1.8 ±0.1	2.3 ±0.1	3.1 ±0.2	7.2 ±0.4	9.9 ±0.5	1.2	23
	E. benzoate		2.1 ±0.1	2.9±0.3	3.8 ±0.4	8.8 ±0.5	11.5 ±0.7	1.5	39
	Untreated		1.5 ±0.2	2.10 ±0.1	2.4 ±0.1	6.0 ±0.4	8.7 ±0.6	-----	5
	LSD	-	0.247	0.35	0.114	0.348	0.133	-	-
	P	-	**	**	***	**	**	-	-

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

The increase in mortality percent when *E. scutalis* was fed on *T. urticae* can be explained as a high susceptibility of the moving stages of the prey towards the two compounds than PBW eggs.

6.Effect of preys treated on food consumption of *Euseius scutalis* immature stages:

The data recorded in Tables (5 and 6) showed that there was a high significant difference (P < 0.05) between the all immature stages of *E. scutalis* consumption when fed on treated preys than the untreated; because the low consumption recorded when fed on treated preys. They consumed an average

of (18.0, 20.9 and 23.6) from PBW eggs; and (15.6, 18.3 and 20.0) from *T. urticae* in control for larvae, protonymph and deutonymphs of *E. scutalis*, respectively. On the other hand, it decreased to (14.3, 15.9 and 20.3), when fed on PBW eggs treated with *B. bassiana* and to (11.6, 13.3 and 18.6) prey/mite, respectively, when fed on PBW eggs treated with emamectin. These values gradually decreased to (8.8, 11.5 and 14.9 prey/ mite/ day) for larvae, protonymph and deutonymphs, respectively, when fed on *T. urticae* treated with emamectin and (9.0, 14.3 and 17.0 prey/mite), respectively, when they were consumed *T. urticae* treated with *B. bassiana* as tabulated in Table (6). The total food consumption of the predator was (69.5 preys) from untreated PBW eggs and (50.9 preys) from untreated *T. urticae*. At the same time, the total consumption of mite decreased to (43.5 and 50.5 preys) by fed on treated PBW eggs and to (35.2 and 40.3 preys) from *T. urticae* treated, respectively. The results agree with Sholla *et al*. (2017) who found that the total food consumption of the female and male predator were (66.43 and 54.33 preys) from PBW eggs, respectively, and (48.5 7 and 41.6 prey/mite)female and male, respectively, when fed on *T. urticae*.

7. Reduction in food consumption predator mite *Euseius scutalis*:

The effect of food source treatment on reduction of preys' *E. scutalis* consumption was presented in Tables (5 and 6). The highest reduction, ranged from (21.2 to 36.4%) and (25.5 to 43.6%) was found, when the predacious mite was fed on PBW or *T. urticae* treated with emamectin, while the lowest reduction recording (3.9 to 23.9 and 15 to 29.5 %), was recorded when *E. scutalis* was fed on PBW or *T. urticae* treated with *B. bassiana*. From the previous results, it can be concluded that the *T. urticae* was high susceptibility to the two compounds than PBW eggs and the treated PBW eggs or *T. urticae* by emamectin caused a high reduction in consumption of the predator than *B. bassiana* treated.

Table (5):Food consumptions of the predacious mite *Euseius scutalis* when fed on *Pectinophora gossypiella* eggs under laboratory conditions

Stages of predator\	Average numbers of prays consumption in a day/ predator ± SE						
	P. gossypiella treated with		*P. gossypiella* untreated			% Reduction in consumption due to fed on	
	E. benzoate	*B. bassiana*		LSD	P	E. benzoate	*B. bassiana*
Larvae	11.6±1.6	14.3±1.2	18.0±0.59	2.571	**	35.5	20.5
Prtonymphal	13.3±1.2	15.9±1.5	20.9±0.7	1.353	**	36.4	23.9
Deutonymphals	18.6±1.9	20.3±1.8	23.6±1.4	1.988	**	21.2	3.9
Total consumption	43.5±3.2	50.5±4.3	62.5±2.9	5.211	***	30.4	27.3

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

Table (6):Food consumptions of the predacious mite *Euseius scutalis* when fed on *T. urticae* under laboratory conditions.

Stages of predator\	Average numbers of prays consumption in a day/ predator ± SE					% Reduction in consumption due to fed on	
	T. urticae treated with		Untreated				
	E. benzoate	*B. bassiana*		LSD	P	E. benzoate	*B. bassiana*
Larvae	8.8±0.9	11.0±1.4	15.6±0.7	1.377	**	43.6	29.5
Prtonymphal	11.5±1.4	14.3±1.8	18.3±1.2	2.322	**	37.1	21.8
Deutonymphals	14.9±1.3	17.0±1.6	20.0±0.9	2.111	**	25.5	15
Total consumption	35.2±0.5a	40.3±3.3	50.9±0.9	6.217	***	30.8	20.8

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

From all the aforementioned results, we may concluded that can be used two bio chemicals' *B. bassiana* and Emamectin successfully in controlling the spider *T. urticae* because it was highly susceptibility to both compounds than PBW eggs.But; Emamectin caused a high reduction in consumption of the predator *E. scutalis* than that treated with *B. bassiana*. Biological control with *B. bassiana* is a promising alternative to bio-chemical control against PBW eggs or *T. urticae* that causes alittle damage to the predacious mite, *E. scutalis* with no damage to the environment. So it can be used *B. bassiana* products in the Integrated Pest Management Program of spider mites or PBW eggs with the predator, on cotton fields.

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Comparative biology and life table parameters of citrus brown mite *Eutetranychus orientalis* (Acari: Tetranychidae) on different grapevine cultivars

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Abstract:

The influence of the three grapevine cultivars king rubi, crimson seedless and thompson seedless on biology and life table parameters of the citrus brown mite *Eutetranychus orientalis* (Klein) (Acari: Acariformes: Tetranychidae) was studied under laboratory conditions of 25 and 30±1°C and 70±5% R.H. The biology of *E. orientalis* consisted of egg, larvae, protonymph, deutonymph and adult stages. The total duration of female from egg to adult was found to be the least in king rubi (16.4 and 8.3 days) followed by thompson seedless (10.02 and 9.53 days) and maximum in crimson seedless (19.18 and 10.18 days) at 25 and 30°C, respectively. The highest fecundity was 35.4 and 29.8 eggs/ female on king rubi and the lowest fecundity was 26.6 and 23.4eggs/ female on crimson seedless at 25 and 30°C, respectively. The higher values of net reproductive rate ($R_{0)}$, intrinsic rate of increase ($r_{m)}$, finite rate of increase (λ) the shortest mean generation time (T) was observed in king Rubi followed by thompson Seedless and crimson seedless. The results suggested that king rubi was the most suitable variety with higher survival rate of mites, shortest development time and higher fecundity while crimson seedless was the least suitable variety because of the lowest survival rate, longest development period and lower fecundity.

Introduction

The grapevine, *Vitis vinifera* L. is an important commercial fruit crop in Egypt. In Egypt, grape occupies the second ranked fruit after citrus and it has a great importance and plays an important role in the agricultural economy (Abido *et al.*, 2013). The total grapevine area in Egypt reached 778,950 hectares with an annual production of 1.703 million tons with an average of about 9.2 tons per feddan. In Gharbia Governorate, it occupies an area of about 10435 feddan

with annual production of 93722 tones according to the statistics of FAO (2017).

The citrus brown mite *Eutetranychus orientalis* (Klein) (Acari: Acariformes: Tetranychidae) is an important pest of citrus and is a persistent pest in Upper Egypt. It also infests a wide range of hosts including deciduous fruit trees, filed crops and ornamental plants. This tetranychid mite mostly feeds on the upper leaf surface, although feeding could extend to the lower leaf surface at high population levels. Damage in host plants is shown by a bronze tone on the leaves, which was shown to be associated with the rates of oviposition and female production (Jeppson *et al.* 1975; Zaher, 1984 and Elhalawany, 2019).

Despite the economic importance of grapevines in Egypt and the damage of phytophagous mites cause to them. Very little information is available on this pest though it is a polyphagous mite occurring on several cultivated plants all over Egypt. Thus, the aim of this study was to compare the effect of different grapevine cultivars and temperature on the biology and life table parameters of *E. orientalis*.

Materials and methods

The field experiment was carried out at Gemmeiza Station, Gharbia Governorate during season 2019.

1.Mite culture:

The first population of *E.orientalis* was collected from infested grape, *V. vinifera* . The stock culture was maintained on grapevine leaves in a rearing chamber at 25 ±2oc; 70 ± 5% RH.

2.Development and biology of *Eutetranychus orientalis* at different temperatures:

Experiments were conducted on king rubi, crimson seedless and thompson seedless varieties leaf discs at 25 and 30 ± 1 °C and 70 ± 5% RH. One leaflet from each variety was chosen and washed with running water to remove any possible residuals or mites. Leaf discs of about 2.5cm in diameter were made surrounded by tangle foot and placed lower surface down on of moisten cotton wool in Petri dishes of 15-cm diameter. Ten individuals of *E.orientalis* couple (male and female) were placed on each disc, for each variety. These petri dishes were kept at two temperatures, for 24 hours to allow mating, thereafter, males were removed, while female served as a source for known-age eggs, and larvae. About 50 hatching larvae were kept singly using a fine camel hair brush and released over the leaf discs and left to continue their life span, for each variety. The leaf discs were replaced at regular intervals before they dried out. Newly emerged females were copulated and left to deposit their eggs. Observation was conducted twice daily and essential records were noted. The observations on the survival and development of each life stage of mites were recorded. This method was proposed by (Elhalawany, 2019; and Elhalawany and Abdel-Wahed, 2013).

3. Life table parameters of the *Eutetranychus orientalis* :

The adult females emerged were collected and released over the leaf discs of the respective ciltivars for feeding and oviposition. The number of eggs laid, survival and fecundity by a female were recorded till the death of the last female. Life table parameters were estimated according to (Birch, 1948) using the Life 48, BASIC Computer program (Abou-Setta *et al.*, 1986).

4. Statistical analysis:

Data were statistically analyzed using one-way and two-way analysis of variance ANOVA and mean separation was conducted using Duncan's multiple range test ($P \leq 0.05$). These analyses

were conducted using SAS statistical software (SAS Institute, 2003).

Results and discussion

1. Developmental time and longevity at 25°C:

The life cycle of the citrus brown mite *E. orientalis* is completed and passed through four developmental stages with quiescence stages at the end of larval and nymphal stages. The duration of different developmental stages of *E. orientalis* on leaves of three grape cultivars: king rubi, thompson seedless and crimson seedless at 25˚C and 70% RH. is presented in Table (1). There is significant difference between the three varieties during egg incubation period of female and male. Female incubation period was 6.20, 6.72 and 6.90 days and male incubation period was 5.95, 6.70 and 6.60 days on king rubi, thompson seedless and crimson seedless, respectively. Statistical analysis indicated that significant differences were found between the three rearing varieties. The shortest female larva, protonymphal, deutonymphal stages, total immature stages, life cycle and generation period were 3.4, 3.5, 3.3, 10.2, 16.4 and 18.2 days at 25°C on king rubi variety; while the longest were 4.13, 3.95, 4.2, 12.28, 19.18 and 21.38 days on crimson seedless variety, respectively.

The longevity of adult female *E. orientalis* and the length of the oviposition, and post-oviposition periods differed significantly between the three grape varieties. The longest oviposition period was observed on king rubi was 13.0 days and the shortest period on crimson seedless was 9.7 days with significantly differences .The king rubi had the highest longevity 16.75 and 11.95 days, while the lowest longevity was recorded on crimson seedless 13.40 and10.20 days, for female and male, respectively at 25˚C. The highest mean number of eggs laid by female was 35.4 eggs/female with a daily rate of 2.78 eggs/♀/day on King Rubi while, the lowest fecundity was 26.60 eggs/♀ on crimson seedless with a daily rate of 2.74 eggs /♀/day at 25˚C. No significant differences between female and male life span on three hosts at 25˚C.

Table (1): Mean developmental times and longevity in days of *Eutetranychus orientalis* females and males reared on selected cultivars at 25˚C.

Biological aspects	Mean duration of female stages			L.S.D at 0.05	Mean duration of male stages			L.S.D at 0.05
	King Rubi	Thompson Seedless	Crimson Seedless		King Rubi	Thompson Seedless	Crimson Seedless	
Egg	6.20 b	6.72 a	6.90 a	0.37	5.95 b	6.70 a	6.60 a	0.62
Larva	3.40 b	3.70 b	4.13a	0.41	3.15 b	3.45 b	4.15 a	0.53
Protonymph	3.50 b	3.83 ab	3.95 a	0.39	3.40 a	3.65 a	3.80 a	0.53
Deutonymph	3.30 b	3.78 a	4.20 a	0.45	2.95 b	3.25 ab	3.60 a	0.59
Immature	10.20 c	11.30 b	12.28 a	0.62	9.50 b	10.35 b	11.55 a	0.98
life cycle	16.40 c	18.02 b	19.18 a	0.71	15.45 b	17.05 a	18.15 a	1.21
Generation	18.20 c	20.12 b	21.38 a	0.74	-	-	-	-
Preoviposition	1.80 a	2.10 a	2.20 a	0.39	-	-	-	-
Oviposition	13.00 a	10.85 b	9.70 c	0.87	-	-	-	-
Postoviposition	1.95 a	1.70 ab	1.50 b	0.26	-	-	-	-
Longevity	16.75 a	14.65 b	13.40 c	1.04	11.95 a	10.75 b	10.20 b	1.15
Fecundity	35.40 a	31.00 b	26.60 c	2.93	-	-	-	-
Daily rate	2.78 a	2.89 a	2.74 a	0.32	-	-	-	-
Life span	33.15 a	32.67 a	32.58 a	1.16	27.40 a	27.80 a	28.35 a	1.76

The means with the same letters at the same row are not significantly different at 0.05% level.

2.Developmental time and longevity at 30°C:

Data as shown in (Table, 2) indicated that significant differences between the three grape cultivars at 30°C. All female and male immature stages developed faster on king rubi than on thompson seedless and crimson seedless. Significant difference was observed for the duration of incubation period, larva, protonymph, deutonymph, immature stages and life cycle, the shortest period were recorded on king rubi and the longest periods were found on crimson seedless at 30°C. The shortest generation time of the *E. orientalis* fed on king rubi was (9.30 days) and significantly shorter than of those fed on crimson seedless was (12.0 days) at 30°C.

The female longevity was longest on king rubi (14.25 days), followed by thompson seedless (13.43 days), and was shortest on crimson seedless (12.33 days) with significant differences. A similar trend was observed for males the longest on king rubi (10.40 days), followed by thompson seedless (10.3 days), and was shortest on crimson Seedless (9.0 days).

Significant differences were found in fecundity the highest being in those fed on, king rubi 29.8 eggs/♀, followed by those fed on thompson seedless 26.4 eggs/♀, while the lowest fecundity was 23.4 eggs/♀ fed on crimson seedless at 30°C (Table, 2).

Table (2): Mean developmental times and longevity in days of *Eutetranychus orientalis* females and males reared on selected cultivars at 30°C.

Biological aspects	Mean duration of female stages			L.S.D at 0.05	Mean duration of male stages			L.S.D at 0.05
	King Rubi	Thompson Seedless	Crimson Seedless		King Rubi	Thompson Seedless	Crimson Seedless	
Egg	4.15 b	4.25 b	4.60 a	0.30	3.80 a	3.90 a	3.80 a	0.33
larva	1.50 b	1.80 a	1.98 a	0.18	1.15 b	1.60 a	1.50 ab	0.37
Protonymph	1.38 b	1.75 a	1.85 a	0.23	1.25 a	1.40 a	1.60 a	0.39
Deutonymph	1.28 b	1.73 a	1.75 a	0.21	1.25 a	1.30 a	1.50 a	0.36
Immature	4.15 b	5.28 a	5.58 a	0.32	3.65 b	4.30 ab	4.60 a	0.70
life cycle	8.30 c	9.53 b	10.18 a	0.40	7.45 b	8.20 a	8.40 a	0.73
Generation	9.30 b	11.63 a	12.00 a	0.47	-	-	-	-
Preoviposition	1.00 b	2.10 a	1.83 a	0.34	-	-	-	-
Oviposition	11.80 a	10.00 b	9.40 b	0.82	-	-	-	-
Postoviposition	1.45 a	1.33 ab	1.10 b	0.32	-	-	-	-
longevity	14.25 a	13.43 ab	12.33 b	1.10	10.40 a	10.30 a	9.00 b	1.25
Fecundity	29.80 a	26.40 b	23.40 c	2.22	-	-	-	-
Daily rate	2.55 a	2.65 a	2.49 a	0.18	-	-	-	-
Life span	22.55 a	22.95 a	22.50 a	1.13	17.85 a	18.50 a	17.40 a	1.66

The means with the same letters at the same row are not significantly different at 0.05% level.

These results are agreement with finding by Atwa *et al.* (1987) who indicated that at 30°C, egg incubation period and immature stage development period and adult longevity were 3.57, 10.0 and 7.36 days, respectively. The egg to adult developmental time of the female was 12.43 days at 30°C. The longevity of the female ranges from 16.57 days at 20°C to 7.50 days at 30°C. The fecundity ranges from 14.56 to 16.33 eggs per female, and the sex ratio is 75–80% female. Al-Gboory (1991) found that developmental time of *E. orientalis* was 11.85 days on mandarin at 28 oc. The mean longevity of females was 8.75 days, whereas the female produced only 5 eggs during oviposition period. Assari (2001) reported that *E. orientalis* life span at 28 oc and 20 % relative humidity was 5 days for males and 8 days for females. These results are lower than those recorded by Elhalawany and Abdel-Wahed (2013) indicated that the reproduction, survival, and life table parameters of *T. urticae* on kostata and hachiya persimmon cultivars leaves were studied under laboratory conditions of 15, 20, 25 and 30°C, 70% RH. The shortest period of incubation, immature stages and female longevity were 3.27, 8.92 and 12.98 days, while these periods on males were 3.35, 7.8 and 11.8 days at 30°C on kostata persimmon variety, respectively. Elhalawany (2019) who studied the biology of *E. orientalis* on leaves of six host plants. The highest life cycle was 19.95 and 9.38 and 19.31and 9.8 days for male and female on date palm at 25 and 30°C, while, the lowest value of this period was obtained on castor bean were 16.45 and 16.75 days for male and female at 25°C and 6.31 and 8.43 days for male and female at 30°C on Indian laburnum, respectively. Mean longevity of female *E. orientalis* ranged from 13.53 to 15.13 days at 25°C and from 9.9 to 16.9 days at 30°C. The highest mean total fecundity was 19.45 and 14.1 eggs/ female on Indian laburnum and castor bean at 25 and 30°C, respectively.

3.Life table parameters of *Eutetranychus orientalis* on grape cultivars:

Results presented in Table (3) showed that, the shortest mean generation time (Tc) was observed on king rubi was 21.63 and 13.12 days, while the longest were 23.51 and 14.39 days recorded on crimson seedless at 25 and 30˚C, respectively. Whereas, the shortest time for population density doubling (DT) was 4.81 and 3.22 days at 25 and 30˚C on king rubi variety while the longest period was 6.03 and 4.03 days at 25 and 30˚C on crimson seedless cultivar.

The maximum net reproductive rate (R_o) occurred at 25˚C on king rubi recorded 22.56 individuals/ generation, followed by on thompson seedless was 18.46 individuals/ generation, while the lowest value on crimson seedless was 14.39 individuals/generation at 30˚C.

The maximum intrinsic rate of natural increase (r_m) the difference between birth rate and death rate was obtained at temperature of 30°C whereas, the lowest values were recorded at 25˚C. These values were 0.144 and 0.215; 0.127 and 0.193 and 0.115 and 0.172 individuals/♀/day at 25 and 30˚C on king rubi, thompson seedless and crimson seedless, respectively.

The finite rate of increase (λ) ranged from 1.12 offspring/ individual/day at 25°C on crimson seedless to 1.24 offspring/ individual/day at 30°C on king rubi cultivar. Gross reproduction rate (GRR) recorded the highest value at 25°C on king rubi was 28.3 eggs/ individual and the lowest value 17.9 eggs/ individual on crimson

seedless. The sex ratio ranged from 0.7 to 0.75 female/ total not affected by temperature and host plant Table (3).

Table (3): Life table parameters of *Eutetranychus orientalis* under different temperatures.

Parameter	King Rubi		Thompson Seedless		Crimson Seedless	
	25°C	30°C	25°C	30°C	25°C	30°C
Mean generation time (T_c) [a]	21.63	13.12	22.84	13.95	23.51	14.39
Doubling time (DT) [a]	4.81	3.22	5.45	3.59	6.03	4.03
Net reproductive rate (R_o) [b]	22.56	16.81	18.46	14.79	15.24	14.39
Intrinsic rate of increase (r_m) [c]	0.144	0.215	0.127	0.193	0.115	0.172
Finite rate of increase (λ)[c]	1.15	1.24	1.13	1.21	1.12	1.18
Gross reproduction rate (GRR)[b]	28.3	24.2	25.2	22.9	20.2	17.9
Survival rate %	0.85	0.77	0.8	0.75	0.77	0.73
Sex ratio (♀/total)	0.75	0.70	0.72	0.72	0.70	0.70

[a] Days [b] per generation [c] Individuals/female/ day

Age specific survivorship (l_x) and fecundity (m_x) curves for *E. orientalis* are shown in Figure (1). The daily age-specific survival rate was highest at 25oc and decreased as the temperature increased on three host plants. The maximum number of eggs produced on thompson seedless was at 25oc (day 22: 2.92 egg/♀/day), the lowest value was obtained at 30oc (day 13: 2.38 egg/♀/day) on crimson seedless. The highest survival rate of females was 0.85 % on king rubi at 25°C, while lowest value was 0.73% on crimson seedless at 30°C.

These results agree with that of Imani *et al.* (2009) found that the mean generation time (T) is 22.83 days, the net reproductive rate (R_0) is 154.08 and the intrinsic rate of increase (r_m) is 0.221. The intrinsic rate of increase (r_m) ranges from 0.144 at 30°C to 0.094 individuals per female daily at 20°C. The population doubles in 4.79 days at 30°C and in 7.33 days at 20°C. Sangeetha *et al.* (2013) reared *E. orientalis* on neem leaf discs at 35°C, development times from egg to adult stage were 9.48 days, oviposition period averaged 7.7 days and Fecundity averaged 30.1 eggs/ female. Elhalawany (2019) indicated that the highest intrinsic rate of increase (r_m) was 0.143 on Indian laburnum at 25°C and 0.138 (individuals/ female/ day) on castor bean at 30 oc. The individuals had the ability to double with the shortest time at 30oc (4.81 days) on Indian laburnum and the longest time at 25 °C (12.38 days) on date palm. The mean generation time (T) and generation doubling time (DT) values decreased with temperature increase.

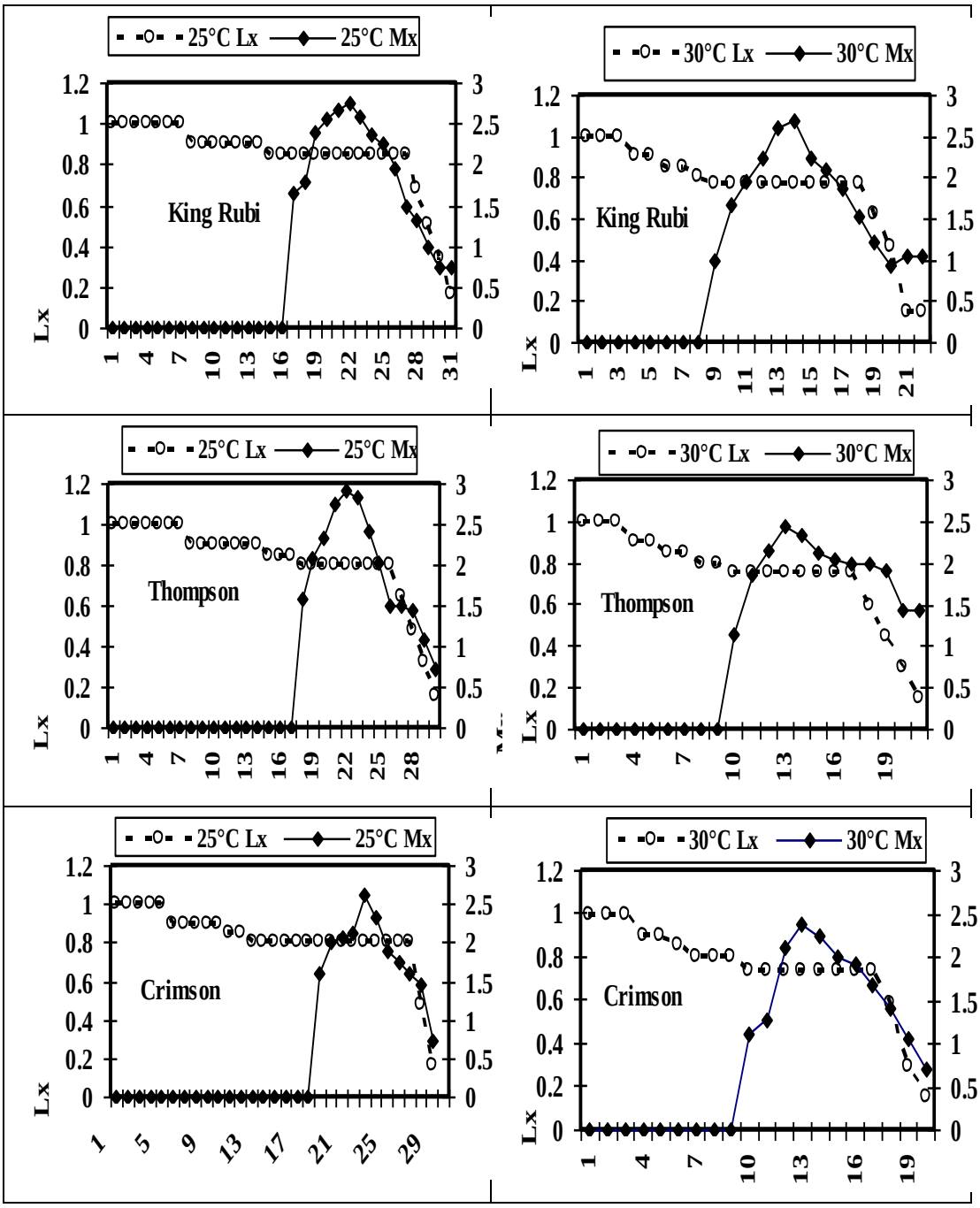

Figure (1): Age specific survivorship (Lx) and age specific fecundity (M_x) for *Eutetranychus orientalis* on grape verities and two different temperatures.

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Interaction of agricultural drainage water cations with insecticide potency

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Abstract:

The insecticides potency for controlling target insects in field may affected by many cations which present in water used in insecticides preparation in the field. The toxicity of three types of insecticides lufenuron, lambda-cyhalothrin and tetramthrin and dimethoate. The LC_{50} values for each compound against the 4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) 72 hours was 0.001, 0.21 and 0.046 ppm. Six different types of cobalt, sodium, potassium, calcium, magnesium and manganese all in a chloride salt to study their toxicity in presence and absence of LC_{50} of tested insecticides to examine their interaction and interference with insecticides toxicity. Sodium and magnesium chloride had categorically decreased the toxicity of all tested insecticides at concentration 100, 0.01 ppm mortality decreased to 10, 40 and 36 % and 30, 30, 50 %, respectively. Inhibition percentage of total ATPase and AChE were investigated. Na^+ counteracts effects of all tested insecticides with significant decrease in the levels of ATPase inhibition. Conversely, Mg^{2+} decrease in the levels of AChE inhibition. The toxicity and biochemical data had shown a very interrupted effect due to the presence of these cations due to their interference with the site of action of these pesticides.

Introduction

The cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most devastating agricultural lepidopterous pests. It can attack abundant economic field and vegetable crops over the year in Egypt (Kandil *et al.*, 2003). The efficacy of the pesticides to cause its toxic effect depends on surrounding condition in which the effect occurs (Pietrock and Marcogliese, 2003). Several abiotic characteristics of water and soil, such as temperature (Rotich *et al.*, 2004), pH (Boss and Mott, 1980), dissolved oxygen content (Panigrahi *et al.*, 2014), hardness (Persoone *et al.*, 1989), salinity (Huang

and Brattsten. 2007), cations (Lo and Lee, 1989) and heavy metals (Broerse and van Gestel, 2010) may affect the toxicity of pesticides on organisms.

As a result of the increase in population and with increased demand for water. In Egypt, the increasing scarcity water with the expansion of the cultivation of new land in Sinai and Western Sahara. The reuse of agricultural drainage water provides an integral supplement to the water supply during the coming years. The recycling of this water influences the quality of water in flowing through Egypt's irrigation network. Therefore, the Government works to improve the quality of agricultural drainage water. Exploit it to the water irrigation deficit (Barnes, 2014). Major cations including Ca^{2+}, K^{+}, Mg^{2+}, Na^{+} were listed in drains which may be attributed to high fertilizers, pesticides, other contaminants (Ezzat and Reham, 2012 and Nasr and Zahran, 2015).

ATPases plays an important role in the ionic transfer across the membrane that is a target of the neurotoxic effect of OPs and pyrethroid compounds (Nozdrenko *et al*., 2016 and Kakko *et al*., 2003). Cations facilitate neuromuscular excitability, enzymatic reactions and retention of membrane permeability. Cations counteracted effects of insecticides by promoting normal ATPase activities (Kiss and Fazekas, 1983). Wherefore cations affect the sensitivity of ATPases and AChE inhibition by pesticides. Moreover, it may interfere with the pesticides efficacy on the target and non-target organisms (Takano *et al.*, 1983; Shaker *et al*., 1987; Sarma *et al*., 2013; El-Alfy *et al.*, 2001 and Senger *et al*., 2011).

The aim of this work is to study the problem of contamination soil and agricultural drainage water with cations and know relationship of the pesticides efficacy and its toxicity to the pest studied.

Materials and methods

1. Insect rearing:

A laboratory strain (Lab) of cotton leafworm *S. littoralis* was obtained from Central Lab. of Pesticides, Agricultural Research Center (ARC) Cairo, Egypt that was reared under laboratory conditions for several years without exposure to insecticides. The colony was kept at a temperature of 27±2 ºC and 65±5 RH. (El-Defrawi *et al*., 1964). Larvae were reared on castor oil leaves (*Ricinus communis* L.), the 4th larvae selection for bioassays and biochemical assessments.

2. Insecticides and chemicals:

One insect growth regulators lufenuron (Wormatin, 5% EC) from Bayer Crop Science Jordan, one pyrethroid lambda-cyhalothrin 2.5% and tetramthrin 2.5% (Lambada plus 5 % EC) provided from El-Helb Pesticides and Chemicals Dumyat Al Jadidah, Dumyat, Egypt and one organophosphorus insecticides dimethoate (Belthethoate, 40% EC) provided from BR Agrotech Ltp India. Six different salts in chloride form (Cobalt and manganese) was obtained from Sigma Aldrich Co. and (Sodium, potassium, calcium and magnesium) chloride was obtained EL Nasr pharmaceutical chemicals Co., Egypt.

3. Toxicity of tested insecticides against 4th larvae of *Spodoptera littoralis*:

The leaf-dipping bioassay method was used to determine (LC_{50}) values of tested insecticides against 4th instar larvae

of *S. littoralis.* Castor leaves were cut into discs (9 cm). Each disc was dipped in different concentrations of insecticides that prepared in distilled water for 10s. Treated and control discs were held vertically to allow excess dilution to drip off, and were air-dried for 2 hrs. Disc offered to ten larvae in each treatment with three replicates and kept under laboratory conditions (27 ±2 ºC and 65-70% RH.). Mortality counts were recorded after 24, 48 and 72 hr. of treatment.

4. Toxicity of tested insecticides in the presence of different cations concentrations:

For analysis of the effects of synergists or antagonist effect on toxicity of lufenuron lambda-cyhalothrin and tetramthrin and dimethoat in present of different salt solution of ($Cocl_2$, NaCl, KCl, $CaCl_2$, $MgCl_2$ and $MnCl_2$). Castor leaves discs treated with LC_{50} concentration of tested insecticides that prepared in distilled water containing 0.01,1,100 ppm of each salt solution. Number of dead larvae per each replicate was counted after 72 hr. of treatment. Mortality percentage was calculated and correcting for natural death according to Abbott equation (Abbott, 1925).

5. Enzyme preparation and AChE activity:

Fourth instar larvae of *S. littoralis* treated with LC_{50} of tested insecticides alone and with 100 ppm salt solutions of NaCl or $MgCl_2$, after 72hr. of bioassay test. One gm from treated and untreated larvae were homogenized in ice cold 40 mM Tris-HCl (pH 7.4) for 50 sec. then the homogenates were centrifuged at 5000 rpm for 15min at 4 ºC. The resulting supernatants were filtered and recentrifuged at 10,000 rpm for 30 min. The resulting supernatants were stored at (-20 ºC) for used as enzyme source. AChE activity determined according to method reported by (Ellman *et al.*, 1961), in total volume of three ml, 100µl of .01 M 5,5 dithio bis-(2-dinitrobenzoic acid) (DTNB) dissolved in 0.1 M phosphate buffer pH 7.4 , 30µl of 0.075 M acetylthiochoholine iodide (ATChI) and 50 µl of enzyme. The reactions were incubating at 37 ºC for 15 min. enzyme activity is measured spectrophotometrically as λ 412nm. The enzyme specific activity was computed as (ΔO.D. λ_{412} /mg protein/min). Inhibition percentage (I %) of AChE activity was calculated as follows:

Inhibition % = $[1- SA_T/SA_C] \times 100$, where SA_C is specific activity of the enzyme in the control and SA_T is specific activity of the enzyme in the treatment.

6. Enzyme preparation and ATPase activity:

One gram of treated and untreated of 4^{th} instar larvae homogenized in 10 ml in solution (40 mM Tris–HCl, 320 mM sucrose, 1 mM EDTA, buffer pH 7.4). the homogenates were centrifuged at 5,000 rpm for 10 min at -4ºC. Supernatant was then recentrifuged at 17,000 rpm for 30 min at 4ºC. The formed pellets were resuspended in the buffer and stored at (-20 ºC) for use. Total ATPases activity was determined according to Koch *et al*. (1969), with slight modification by (Morshedy,1980) using Tris–HCl buffer instead of imidazole buffer. the enzyme source (100 µl) was mixed with a reaction mixture 850 µl contained 40 mM Tris–HCl pH 7.4, 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 5 mM ATP. The mixture was incubated for at 37 ºC 15 min. the reaction was stopped by adding

150 µl of TCA (30 % w/v). The hydrolysis Pi was measured according to (Taussky and Shorr, 1953) by adding 4 mL of fresh coloring reagent (5 g $FeSO_4$ in10 % Amm. Molybdate in 10 N H_2SO_4). The absorbance was measured at 750nm against blank using spectrophotometer. The enzyme activity was represented as inorganic (Pi µmole/mg protein/ h). Inhibition percentage of ATPases activity was calculated. The standard curve of Pi was made using KH_2PO_4 (concentrations from 10 to 100 µmol/ml). 4 ml of the coloring reagent was added to 1 ml of each concentration. The color was measured at 750 nm.

The protein content in prepared homogenates of larvae of *S. littoralis* was assayed spectrophotometrically by methods of (Lowry *et al*., 1951) using bovine serum albumin (BSA) as a standard protein.

7. Statistical analysis:

Data were subjected to one-way analysis of variance (ANOVA) using the IBM SPSS statistics version 25.0 software package. Mean separations were performed by Tukey-Kramer honestly significant differences (HDS) and the results were considered statistically significant when $P < 0.05$. The LC_{50}, their 95% confidence limits, slopes and (Chi) were calculated according to (Finney, 1971) using computerize Ldp-line program.

Results and discussion

1. Toxicity of tested insecticides against 4th larvae of *Spodoptera littoralis*:

Toxicity results of tested insecticides expressed as LC_{50} values are given in (Table, 1). Lambda was the highest toxic compound against fourth instar larvae of *S. littoralis* followed by lufenuron and dimethoate after 24hr. exposure period LC_{50} values was 0.041, 0.05 and 0.78%. The LC_{50} values after 48hr. was 0.0 19, 0.033 and 0.053% for lufenuron, lambda-cyhalothrin and tetramthrin and dimethoate. After 72hr. lufenuron was the highest effective insecticide followed by lambda-cyhalothrin and tetramthrin but dimethoate was the least effective compound LC_{50} values was 0.001, 0.021 and 0.046%, respectively. Furthermore, the confidence limits of LC_{50}, s after 72 hr. were not overlapped. Slopes of Ldp lines were greater for all insecticides except from lufenuron, which show a low slope in its Ldp lines1.8, 1.0, 0.9 after 24, 48,72hr. exposure period these low slopes probably reflect the heterogeneity of response to the lufenuron in these population. These finding agree with Maqsood *et al*. (2016) reported, lufenuron proved the most effective insecticide against *S. lituraiis* followed by chloropyrifos, spinethylin, acrinathrin, gamma cyhalothrin, emamectin benzoate, thiodicarb and flubendiamide. Also, Bakr *et al.* (2013) found *S. littoralis* was most susceptible to IGRs, chitin synthesis inhibitors lufenuron than molting hormone agonist tebufenozide. Lufenuron was the highest effective insecticides against the 2nd instar larvae of *S. littoral is* followed by methomyle and diple 2x (*Bacillus thuringiensis*) (Abdel-Aal and El- Shikh, 2012).

Table (1): Toxicity of tested insecticides against 4th instar larvae of *Spodoptera littoralis*.

Insecticides	Time exposure (hrs.)	LC_{50} (%) [a]	Confidence limits (%) 95%		Slope [b] b ± S.E	χ^{2} [c]
			lower	Upper		
Lufenuron	24	0.050	0.041	0.063	1.80±0.21	**0.01**
	48	0.019	0.012	0.028	1.00±0.18	**2.44**
	72	0.001	0.0009	0.002	0.91±0.11	**5.20**
lambada-cyhalothrin and tetramthrin	24	0.041	0.039	0.043	6.72±1.43	**2.14**
	48	0.033	0.028	0.035	8.66±1.76	**5.10**
	72	0.021	0.019	0.023	3.50±0.25	**1.83**
Dimethoate	24	0.078	0.066	0.107	2.79±0.55	**0.62**
	48	0.053	0.049	0.058	4.28±0.55	**2.26**
	72	0.046	0.043	0.049	5.23±0.56	**0.04**

a The concentration that causes 50% mortality.

b Slope of the concentration–inhibition regression line ± standard error

2.Toxicity of tested insecticides in the presence of different cation concentrations:

All concentrations of cations have no effect on fourth instars larvae of *S. littoralis* after 72h exposure period. Sodium, Potassium and Cobalt decrease the toxicity of lufenuron to *S. littoralis*. Sodium chloride at 100 ppm significantly decreases lufenuron toxicity, the mortality decreases from 50% to 10% ($p < 0.05$). Calcium and manganese chloride did not significantly change the lufenuron toxicity. Magnesium chloride decreases the lufenuron toxicity at concentration 0.01 ppm, the mortality decreased to 30% (Figure, 1a). Sodium chloride reduces lambda-cyhalothrin and tetramthrin toxicity in significant at all tested concentrations. Cobalt, potassium and manganese chloride had no significant. Conversely, 0.01, 1 ppm magnesium and calcium chloride led to significantly decreased of lambda-cyhalothrin and tetramthrin toxicity (Figure, 1b). Cobalt and manganese chloride did not affect of dimethoate toxicity. Sodium and potassium chloride at 1, 100 ppm achieved significantly lessens on dimethoate toxicity. While, magnesium and calcium chloride are lessening dimethoate toxicity at 0.01ppm. This suggests that sodium chloride had categorically decrease toxicity of all tested insecticides at concentrations 1 and 100 ppm and magnesium chloride decreased insecticides toxicity at 0.01 ppm (Figure, 1c). The present results agreement with which found before that salinity decreased toxicity of beta-cypermethrin, acephate, temephos and atrazine (Wang *et al*., 2013; Huang and Brattsten, 2007 and Hall *et al*., 1994). Under isosmotic conditions, less mortality was observed in compared with hyperosmotic conditions (Song and Brown. 1998). While increases in the salinity led to a significant increase in the toxicity of insecticides toxicity (El-Alfy *et al*., 2001). Magnesium divalent cation have been found to reduce the toxicity of OP or pyrethroides and reduced mortality. It therefore gives ca-antidote to treat pesticide-poisoning (Pajoumand *et al*., 2004; Singh *et al*., 1998 and Ajilore *et al*., 2018). Existence calcium reduced the toxicity of Deltamethrin. Ca^{+2} is known to be the antagonist of the nervous excitation or the phosphorylation inhibition or modification of the receptivity of the sodium channels which is the main known target of pyrethroids (Ghillebaert *et al*., 1996 and Matsumura, 1987). Therefore, cations levels can interfere with insecticides and increase or decrease its toxicities.

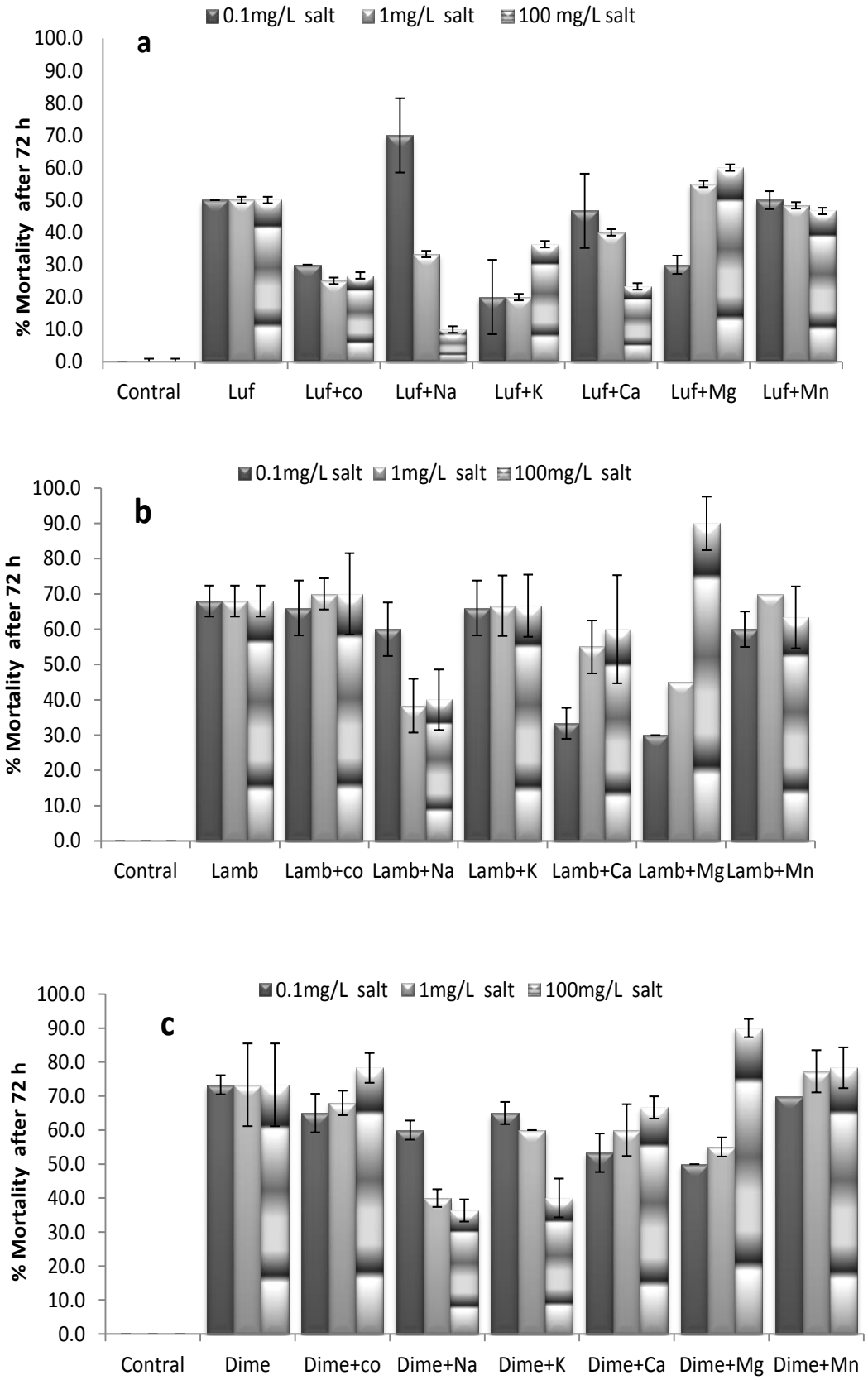

Figure (1): Effect of three concentrations of different cations on the potency of lufenuron lambda-cyhalothrin and tetramthrin and dimethoat against 4th instar larvae of *Spodoptera littoralis.*

3. Biochemical studies:

The specific activity and inhibition percentage of both total ATPase and AChE in case of insecticides in present and absent of sodium and magnesium chloride summarized in (Table, 2). The presence of sodium and magnesium ions with the insecticides reduces the inhibition of ATPase caused by those insecticides. Sodium and magnesium chloride decrease lufenuron inhibition from 67.55% to 20.20 and 50.78%. As well as, ATPase inhibition achieved highly significant between lambda-cyhalothrin and tetramthrin in absent or present both cations. Sodium and magnesium decrease enzyme inhibition from 64.06% to 29.14, 42.22%. Applied dimethoate with sodium or magnesium cations decreases ATPase inhibition approximately half less than dimethoate alone. Sodium and magnesium cations show a decrease of ATPase inhibition, which due to the acting mechanism of cations as a cofactor or as a ligand or effect proteins through a variety of mechanisms (Clark, 1958). This finding is like the earlier reports, Magnesium reactivates the membrane Na^{+}-K^{+}-ATPase and antagonist the direct toxic inhibitory effect of organophosphates on Na^{+}\ K^{+}-ATPase (Kiss and Fazekas, 1983 and Ajilore *et al*., 2018). Sodium, potassium, calcium, cobalt, magnesium, manganese showed a decreased of ATPase inhibition (Shaker *et al*., 1987). The presence of some salts such as Sodium and Magnesium chloride may be equivalent to the shortage of those ions. Which, effect on membranes permeability due to exposure to insecticides and reduced their toxicity to the target pests.

Table (2): In vitro inhibition of acetylcholinesterase and total adenosine triphosphatase isolated from 4th instar larvae of *Spodoptera littoralis* by selected two cations on potency some insecticides.

Insecticide LC_{50} (%)	Total ATPase		AchE	
	Specific activity ± S.E*	Inhibation ±S.E(%)	Specific activity ±S.E**	Inhibation ± S.E(%)
Control	14.80±3.80 a	00.00±0.00 b	$9.76\times10^{-3}\pm2.07\times10^{-3}$ a	00.00±0.00 c
Sodium chloride	7.37±0.56 b	50.18±3.81 a	$5.77\times10\text{-}^{3}\pm5.40\times10^{-4}$ ab	40.87±5.52 a
Magnesium chloride	7.70±0.52 b	47.96±3.54 a	$6.77\times10\text{-}^{3}\pm3.50\times10^{-4}$ b	30.60±3.57 b
Lufenuron	4.80±0.13 b	67.55±0.87 a	$3.10\times10^{-3}\pm3.32\times10^{-4}$ c	68.21±6.47 a
lufenuron+ sodium chloride	11.81±1.61 a	20.20±10.84 b	$8.34\times10^{-3}\pm1.03\times10^{-3}$ a	14.53±10.59 c
lufenuron+magnesium chloride	7.29±0.99 b	50.78±6.69 a	$6.22\times10^{-3}\pm3.86\times10^{-6}$ b	36.22±0.04 b
Lambada	5.32±0.36 c	64.06±2.40 a	$3.81\times10^{-3}\pm8.89\times10^{-4}$ b	60.99±9.10 a
lambada+ sodium chloride	10.49±0.56 a	29.14±3.79 c	$5.99\times10^{-3}\pm2.60\times10^{-4}$ a	46.95±6.82 ab
lambada+magnesium chloride	8.55±0.27 b	42.22±0.71 b	$5.92\times10^{-3}\pm2.61\times10^{-4}$ a	39.30±2.67 b
Dimethoate	4.88±0.19 b	63.70±4.51 a	$3.86\times10^{-3}\pm6.17\times10^{-5}$ c	60.42±0.63 a
Dimethoate + sodium chloride	9.73±1.69 a	34.22±1.71 b	$4.62\times10^{-3}\pm3.31\times10^{-}$ b	56.03±0.00 b
Dimethoate +magnesium chloride	9.27±3.10 a	37.35±11.41 b	$7.10\times10^{-3}\pm1.98\times10^{-3}$ a	27.61±1.16 c

*** Specific activity (Pi µmole/mg protein/ hr). ** Specific activity (ΔOD/mg protein/min).**
Means followed by the same letter are not significantly different (Tukey test, $p < 0.05$).

The highest AChE inhibition 68.21% was found with lufenuron. However, sodium and magnesium chloride decreased this inhibition to 14.53, 36.22%, respectively. Magnesium was the most cation decreases inhibition of lambda-cyhalothrin and tetramthrin from 60.99% to 46.95% followed by sodium 46.95%. It can be noted that highest enzyme inhibition with

dimethoate was 60.42%. The enzyme inhibition decreased to 27.61 % when magnesium chloride applied with dimethoate, sodium decreased enzyme inhibition unremarkable. Magnesium was most effective than sodium in reducing enzyme inhibition achieved by tested insecticides (Table, 2). These results are agreement with (Shaker *et al*., 1987) AChE Inhibition may be explained by its ability to inhibit acetylcholine and to antagonize the effects of insecticide (Pajoumand *et al*., 2004). OPs insecticides react with AChE by nucleophilic reaction of the serine hydroxyl functional group. Magnesium divalent cation is combined with serine hydroxyl group reduces the pKa of serine gives higher basicity to serine that in turn results in decreased reactivity and nucleophilicity of the serinic hydroxyl group for nucleophilic reaction with OPs and hence favors the reaction of serine with OP, resulting in a more polar extractable reaction product (Shetab-Boushehri *et al*., 2012). Also, Smissaert (1981) explanted that, activation of Acetylcholinesterase by Monovalent Na^+ by associated specific binding of the Na ions with the anionic subsite of the catalytic center reduces the reactivity of the (AChE). Low ionic strength, monovalent (Na^+, K^+) and divalent ($Ca2^{+,}$ $Mg^{2+)}$ metal ions enhanced the AChE enzymatic activity (Hofer *et al*., 1984). The activity of AChE was improved by monovalent and divalent cations whereby the activation caused by second group is much greater than caused by first group (Nachmansohn, 1940). This explains why magnesium chloride reduces AchE inhibition caused by both lambda-cyhalothrin and tetramthrin, dimethoate greater than reduced caused by sodium chloride.

In conclusion, this study results indicated that several actions play an essential role in the biochemical characteristic of living organisms. Cations interfere with enzymes activity i.e., acetylcholinesterase and adenosine triphosphatase activity. This may indicate that most cations are present in the cell body in a certain balance concentration and change of these balance equilibrium causes a toxic effect to the cell. Cations interfere with pesticide actions by effect their conjugations manner occur more harmful effect to non-target or decrease their effect by causing insensitivity to the pesticide targets at a certain concentration. It is clear from the above using agriculture contaminated water in preparing and dilution of pesticides will affect its potency in controlling different pests in the field applications. Subsequently, Government constructs more recycling drainage water stations before directing it back into the Egypt's irrigation network for reuse in agriculture.

It is concluded that Tthe toxicity of lufenuron, lambda-cyhalothrin, tetramethrin, and dimethoate against cotton leafworm *S. littoralis* has been seriously affected by presence of cation in water that used in insecticides dilution. Sodium and magnesium chloride had categorically decreased the toxicity of lufenuron, lambda-cyhalothrin and tetramthrin, and dimethoate. Sodium counteracts effects insecticides with significant decrease in the levels of ATPase inhibition. Magnesium decreases the levels of AChE inhibition. Therefore, cations showed a very discontinuous effect due to their interference with the site of action of these pesticides.

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Ecobiological studies on two land snail species at Sharkia Governorate

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Abstract:

The present experiment was aimed to through light on some environmental parameters on two land snails, the glassy clover snail *Monacha cartusiana* (Müller) (Gastropoda: Hygromiidae) and the amber snail *Succinea putris* L. (Gastropoda: Succineidae). The laboratory experiments showed that daily food consumption of different shell height of *S. putris* snail arranged as follow: 13.20 mg by snails with shell height (8-10mm) < 13.33mg (6-8mm) < 15.34 mg (12-14mm) < 15.45mg (10-12mm) < 16.35mg (14-16mm). The favorite places of aestivation in four different directions site for *M. cartusiana* during year were: Western (231.8) snail > Southern (197.5) > Northern (134) > Eastern (131.8). While, the favorite places of rest for *S. putris* could be arranged as follows: South (207) snails > East (152.2) > North (136.2) > West (100.9) and it was not entered in aestivation. On the other hand, the size frequency of *S. putris* snails with shell height of 5-6, 7-8 and 9-10 mm and with shell width 3-4 and 4-5 mm were detected during all months from January to August 2016, while the size frequency of *M. cartusiana* snails with shell height 6-7 and 7-8 mm and with shell width 11-12 and 13-14mm were observed during February to August 2016.

Introduction

Terrestrial gastropods are most serious pests attacking agricultural crops around the world. They cause damage to field, vegetable crops and fruit trees, and ornamental plants (Godan, 1983). Succineidae are distributed almost everywhere in the world (Kerney and Cameron, 1979). In Egypt, the glassy clover snail *Monacha cartusiana* (Müller) (Gastropoda: Hygromiidae) and the amber snail *Succinea putris* L. (Gastropoda: Succineidae) are considered, the most abundant mollusks infesting and causing damage to the Egyptian clover fields and some filed crops especially in Sharkia and Ismailia Governorates (Ismail, 1997 and Lokma, 2013). Abdel-Aal (2001) showed that the one adult snail of *M. cartusiana* ate from

9.8 to 47.85 mg for 24 hours depending on the host plant.

Snails aestivate during the hot summer and hibernate during the cold winter (Kassab and Daoud, 1964). Block (1971) indicated that in the dry summer monthly many snails enter a period of suspended activity called aestivation, they remain firmly attached by hardened mucus to the bark of trees, to leaves, twigs and branches, often 2 to 4 m above the ground with the body with drawn into the shell. The succineidae snails were able to persist through dry periods in an aestivating state (Patterson, 1973). The degrees of temperature and the percentages of relative humidity are the factors inducing aestivation in *Achatina fulica* (Férussac) (Gastropoda: Achatinidae) of course, just at the onset of aestivation (Saydeedur Rahman and Raut, 2010). *Helicella vestalis* (Pfiffer) (Gastropoda: Helicidae) and *M. cartusiana* were observed aestivate in lower portion in the trunk of navel orange trees, under weeds on bits of irrigation canals, on weeds in orchards, on lower portion in border of the orchard (Mahrous *et al*., 2002).The terrestrial gastropods do not inhabit and cool environments but also habitat in which hot and dry conditions prevail. Snail species that can cope with such climatic conditions are thus expected to have developed multi-faceted strategies and mechanisms to ensure their survival and reproduction under heat and drought stress (Schweizer *et al*., 2019).

The aim of this study was to determining daily food consumption of *S. putris* under laboratory conditions and estimated some environmental parameters (the favorite direction to aestivation and size frequency) for *M. cartusiana* and *S. putris* snails under field condition.

Materials and methods

1. Laboratory experiment:

Laboratory experiment was carried out to estimate daily food consumption by *S. putris* snails.

1.1. Daily food consumption of different shell height for *Succinea putris*:

S. putris snails with different shell height were collected from highly infested field cultivated with Egyptian clover, located on El- Qurana village, Abo-Hammad district, Sharkia Governorate during April, 2016. Snails were transferred to laboratory in cloth bags contained Egyptian clover leaves. Once on laboratory, snails were put on rearing box 50×30×30 cm containing moister clay soil up to 7cm depth and supplied daily with cabbage leaves till 15 days for acclimatization. Snails were divided into five groups according to shell height (6-8), (8-10), (10-12), (12-14) and (14-16) mm. Five individuals from each group were put on plastic box without soil and covered with muslin clothes, each group were replicated four times. All snails group were starved for 24 hours prior to testing then two cabbage leaves discs were introduced to each box. The discs were weighted using digital balance with accuracy 0.001 g before and after testing and compared with control treatment without snails. Food consumption for each snail with different shell heights was calculated daily to a period of five days (Baur, 1993).

2. Field experiments:

2.1. Study area:

The experiments were carried out at El- Qurana village (31.71°N, 30.51°E), Abo-Hammad district, Sharkia Governorate. Study area occupies about 2 feddan cultivated with Egyptian clover during winter season and rice during summer season (Figure, 1).

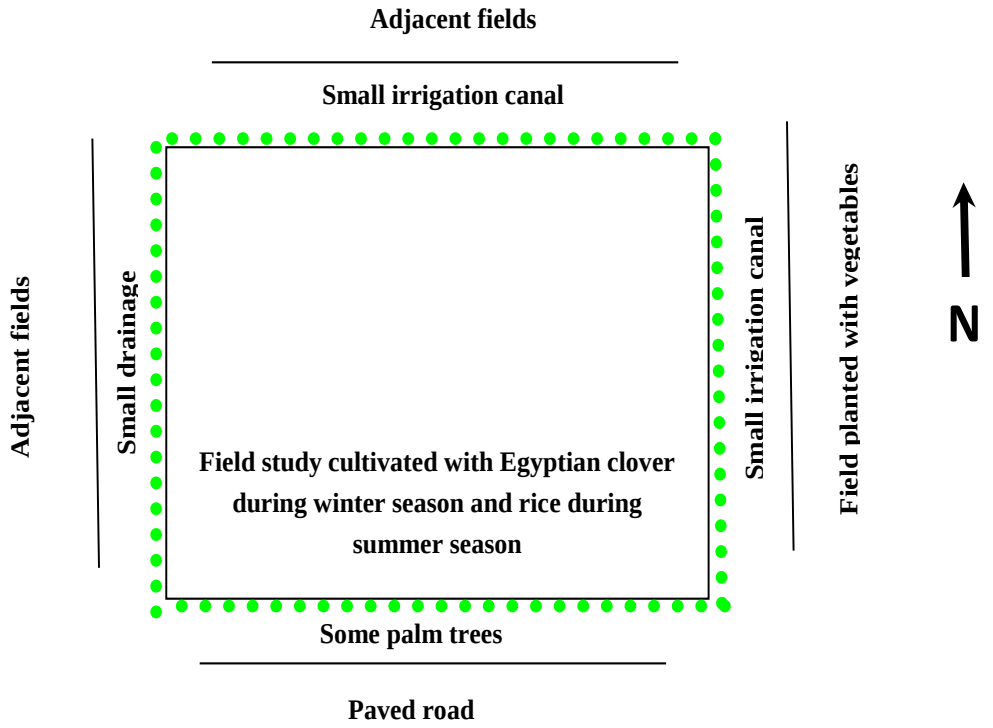


Figure (1): The field study and its borders.

2.2. Observation of aestivation for *Monacha cartusiana* and *Succinea putris* snails:

This trial were carried out in field cultivated with Egyptian clover in the winter and rice in the summer and infested with glassy clover snail, *M. cartusiana* and the amber snail, *S. putris* snail at El-Qurana village at Abou-Hammad district, Sharkia Governorate, during January to December 2016. Five replicates (50×50 cm) were chosen to each four direction North, South, East and West at different directions in the active and aestivation months. Selected of bermuda grass (*Cynodon daclylon* L.) grow on the inner belt of the irrigation canal. Number of all aestivated snails (active and epiphragmed) in each quadrate sample was counted biweekly intervals. Each sample was marked placing sticky label in the border of each quadrant. Moreover, epiphragmed snails were detected in aestivated places in each sample. *M. cartusiana* snail were counted as adult and Juvenile stages and number (different size) of large, medium and small size of *S. putris* (Lokma, 2013).

2.3. Size frequency distribution for *Monacha cartusiana* and *Succinea putris*:

This experiment carried out to estimate different shell growth of *M. cartusiana* and *S. putris* snails in the active and aestivation months. Snails were collected from the above-mentioned study field. Five quadrates replicates (50 X 50 cm) were chosen at four different directions (North, South, East and West) in aestivation location at the edge of field. Snails which found in the quadrates were collected monthly and 25 individuals were taken randomly in the early morning. The shell width and height of

each snail was quantified using Vernier caliper with accurate to 0.02 mm during the period from January to December 2016 (Staikou and Lazaridou-Dimitriadou, 1990).

Results and discussion

1. Daily food consumption of different shell height for *Succinea putris* under laboratory conditions:

Manifestation caused by *S. putris* snail in field on some vegetable and field crops are illustrated in (Figure, 2). For this reason, the average daily food consumption of this snail was estimated. Daily food consumption by *S. putris* snails which have different shell height was measured under laboratory conditions (Table, 1). The highest value of daily food consumption by *S. putris* was 18.99 mg/snail recorded by the largest shell height (14-16 mm) at the 5^{th} days, while the lowest value was 9.45 mg/snail recorded by snails which have shell height (12-14mm) at 3^{rd} days. General mean of consumed leaves by different shell height were arranged as follow: 13.20 mg by snails (8-10 mm) < 13.33mg (6-8mm) < 15.34 mg (12-14 mm) < 15.45mg (10-12 mm) < 16.35mg (14-16mm). Lokma (1998) indicated that the average daily consumption values for. *M. cartusiana* on alfalfa leaves, date palm, pindans and hibiscus were 24.0, 7.8, 6.8 and 6.2 mg/individual, respectively. Snail did not approach leaves of washingtonia palm. Abdel-Aal (2001) reported that one adult snail of *M. cartusiana* ate from 9.8 to 47.85 mg for 24 hours depending on the host plant. The highest values were found with lettuce (47.85 mg) followed by guava (40.6 mg), while the lowest values were determined with mango, wheat and Egyptian clover with means of hours depending on the host plant. The highest values were found with lettuce (47.85 mg) followed by guava (40.6 mg), while the lowest values were determined with mango, wheat and Egyptian clover with means of 9.8, 10, and 10.3 mg, respectively. However, cabbage and broad bean showed intermediate values of 36.95 to 21.55 mg, respectively. Lokma (2013) studies the food consumption of snail, *Monacha cartusiana* on certain vegetable crops under laboratory conditions. The tested material can be arranged descending according to their suitability as follows: kidney bean 103.66 mg < watermelon 80.88 mg < strawberry succulent fruit 74.06 mg < tomato 60.60 mg < strawberry leaves 51.85 mg < strawberry green dead fruit 38.20 mg. Maduabuchi and Bede (2019) cleared that tested leafy vegetables can be successfully utilized as diets for rearing of *Archachatina marginata* (Swainson) (Gastropoda: Achatinidae) for farmers to achieve better result, the inclusion of fluted pumpkin leaf *Carica papya* and pawpaw leaves. *Vernonia amygdalina* in the diets of *A. marginata* is highly recommended in snail rearing businesses.

Rice (*Oryza sativa* L.)

Egyptian clover (*Trifolium alexandrium* L.)

Tomato (*Solanum lycopersicum* L.)

Cabbage (*Brassica oleracea* Liver. capitata)

Figure (2): Manifestation caused by the amber snail *Succinea putris* on some field and vegetable crops under field conditions.

Table (1): Daily food consumption (mg) on cabbage leaves per one snail of different shell height for *Succinea putris* under laboratory conditions.

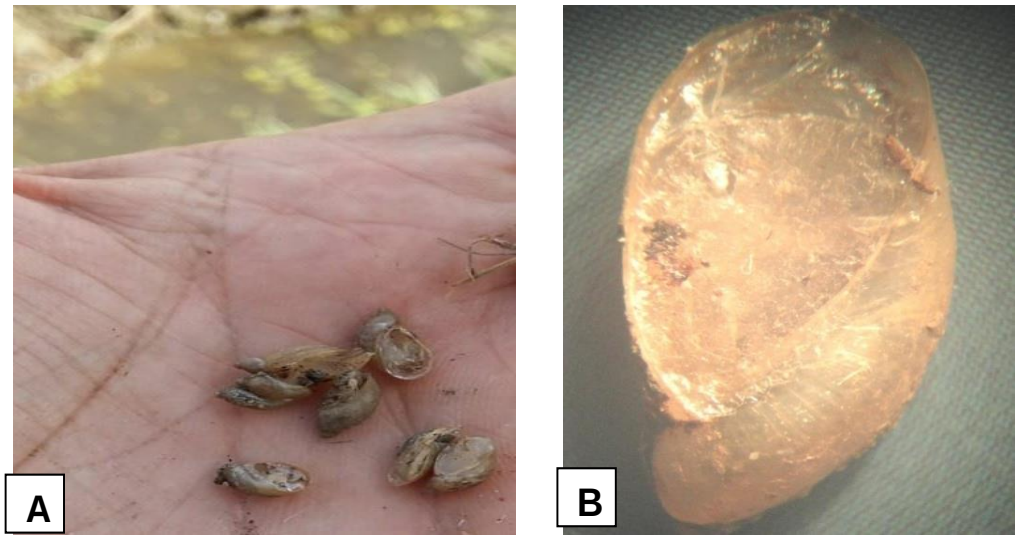
Days	Different shell height (mm)					Mean
	(6-8)	(8-10)	(10-12)	(12-14)	(14-16)	
1 day	10.80	15.78	17.03	17.55	18.12	15.86
2 day	15.37	12.60	14.39	16.58	18.17	15.42
3 day	10.74	10.71	11.77	9.45	12.20	10.98
4 day	14.25	12.68	15.68	17.35	14.25	14.84
5 day	15.49	14.25	18.37	15.75	18.99	16.57
Mean	13.33	13.20	15.45	15.34	16.35	

2. Determining the favorable direction location to aestivation for *Succinea putris* and *Monacha cartusiana* in Egyptian clover field:

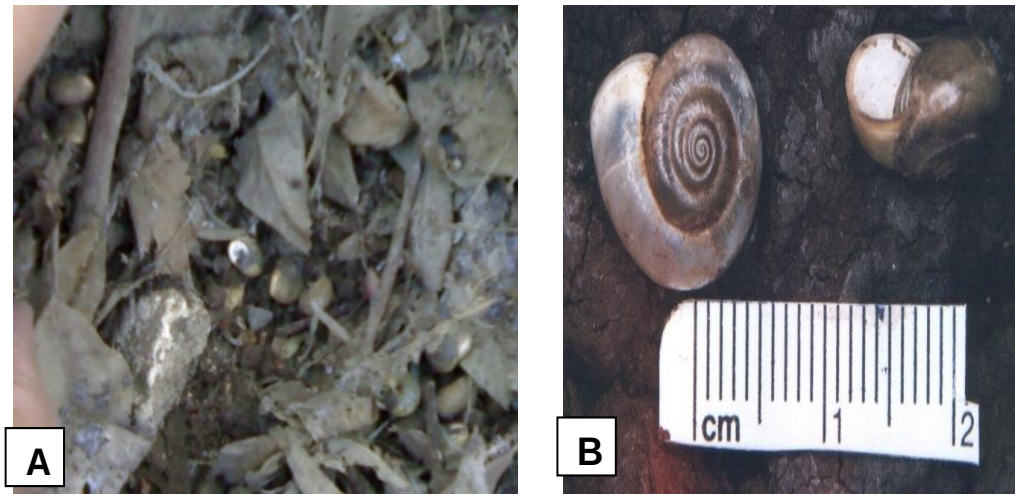
This study was placed in Egyptian clover field heavy infesting with *S. putris* and *M. cartasiana* in Abou-Hammad district, Sharkia Governorate. Snails were observed aestivating under damp habitats either under the Bermuda grasses in edge of the irrigation canals and in soil cracks (Figure, 3).

2.1. Observation on the favorite direction location to rest for *Succinea putris*:

Results in (Table, 2) showed that the Southern direction in edge of the irrigation canals of Egyptian clover field were highly number of individuals snail *S. putris* in the beginning of January with value 5.8 snails in 50×50 cm. While, in February in Northern direction of aestivation place were highly with numbers of snails 7.2 snails compared by other directions with values 6.4, 4.7 and 1.8 snails to South, North and West direction of aestivation place, respectively. Number of snails were increasing in April reached to highly number in Southern direction was 81.2 while in different direction with values 76.4, 61.6 and 43.8 snails at 50×50cm for Eastern, Northern and Western, respectively. However, in summer and autumn months number of snails was decreasing gradually until to November month, where number of snails were 1.8, 0.4 and 8.8 snails four different direction of resting places North, South and West, respectively. Grand total of *S. putris* snails in different directions places could be arranged as follows: South (207) snails 50×50 cm > East (152.2) >North (136.2) > West (100.9). It was noticed that in April and May only that amber snail *S. putris* resting by closing their shell by a thin transparent epiphragm, with low number of snails with value (3) snails in west direction and it was not entering in aestivation. Its importance to mentioned that in summer months when the rice grown some few of individuals of *S. putris* snails were seen active in rice plant in the beginning and middle of June with values 2.5 & 2.8 snail, mean of 5 replicates in 50×50 cm (Figure, 1) and the temperature degree were highly in this period. Also, in the half of September *S. putris* snails were mating in resting sites and the new hatching were appeared in the beginning of December.



Shell aperture of *Succinea putris* sealed with transparent layer of epiphragm, A: In field, B: In laboratory.



Shell aperture of *Monacha cartusiana* sealed with white layer of epiphragm, A: In field, B: In laboratory.

Figure (3) : Aestivation shell shape of land snail *Succinea putris* and *Monacha cartusiana*.

Table (2): Number of *Succinea putris* snails with different size and directions in border of Egyptian clover field.

Date	Snail size	North		South		East		West	
		Mean	Total	Mean	Total	Mean	Total	Mean	Total
Jan.	L	-	2.4	-	5.8	0.2	0.5	0.2	1.2
	M	0.8		1.2		0.2		-	
	S	1.6		4.6		0.1		1.8	
Feb.	L	3	7.2	0.4	6.4	-	1.8	1.8	4.7
	M	2.6		1.8		1.6		1	
	S	1.4		4.2		0.2		1.6	
Mar.	L	5.4	16	1.2	13.2	0.8	8.4	2.6	18
	M	2.6		1.8		3.2		12.8	
	S	8		10.2		4.4		2.6	
Apr.	L	15	61.6	9	81.2	10.8	76.4	11.8	43.8
	M	25.2		7.6		25.4		16.4	
	S	21.4		64.6		40.2		15.6	
May	L	8.2	24	3.2	60	3.8	40.6	4	14.6
	M	8.4		22.2		28.6		5	
	S	7.4		3.8		8.2		5.6	
Jun.	L	-	12.4	9	31.2	3.5	19.7	0.4	5.4
	M	5.8		15.2		8.8		2.6	
	S	6.6		7		7.4		2.4	
Jul.	L	0.8	5.4	1.2	5.4	-	-	0.2	3
	M	2.4		3		-		2.6	
	S	2.2		1.2		-		0.2	
Aug.	L	-	1.4	-	-	-	3.8	-	-
	M	0.2		-		0.2		-	
	S	1.2		-		3.6		-	
Sep.	L	-	0.8	-	1.6	-	-	-	-
	M	0.6		-		-		-	
	S	0.2		1.6		-		-	
Oct.	L	-	1.6	-	1.2	-	-	-	-
	M	-		1.2		-		-	
	S	1.6		-		-		-	
Nov.	L	-	1.8	-	0.4	-	-	5.4	8.8
	M	0.4		0.2		-		3	
	S	1.4		0.2		-		0.4	
Dec.	L	0.2	1.6	-	0.6	0.2	1	0.2	1.4
	M	1.4		0.6		0.6		-	
	S	-		-		0.2		1.2	
Grand total			136.2		207		152.2		100.9

L: Large; M: Medium; S: Small

2.2. Observation on the favorite direction location to aestivation for *Monacha cartusiana*:

Data in (Table, 3) showed that *M. cartusiana* take different direction of selected aestivation sites during the period from January to December 2016, where a relatively low numbers in January without epiphragmed layers gradually to April month, number of snails in different direction in January were 1.4, 5, 0.8 and 0.6 snail/50×50 cm in Northern, Southern, East and West directions, respectively. In the beginning of April number of aestivated snails with epiphargmed was appeared and gradually increasing in all directions with values 3.6 and 10.8 snails in Northern and East direction only, aestivated snails were in adult stages. In May month the maximum numbers of aestivated snails were counted, and the west was most favorable direction with values 77.6 epiphargmed snails in 50×50 cm followed by Southern, East and Northern direction with values 57.4, 33 and 0.8 epiphargmed snails, respectively. Also, non-individual of Juvenile stages was seen aestivated in

four different directions in all examined months. Grand total of aestivated snails for *M. cartusiana* in four different directions during year were: western (231.8) snail > southern (197.5) > northern (134) > eastern (131.8). Kassab and Daoud (1964) showed that the openings of snails (shell aperture) remain close with a white liquid secreted from the mantle from the end of November to the end of February, this liquid forms a mucus sheet, which soon hardens to the epiphragm. Block (1971) indicated that in the dry summer monthly many snails enter a period of suspended activity called aestivation, they remain firmly attached by hardened mucus to the bark of trees, to leaves, twigs and branches, often 2 to 4 m above the ground, with the body and drawn into the shell. In the cool of the evening, when dew falls, the snails reemerge and feed. In long period of continuous dryness, this resting stage is uninterrupted, in this case the case the mouth of the shell is closed by several layers of dried mucus and each separated from the next by an air-filled space. Pollard (1975) observed seasonal migratory patterns in *H. pomatia*, indicating that they return to traditional hibernating sites. Most Stylommatophran snails can aestivate over periods of unfavorable conditions, with the animal into the shell and the shell aperture sealed with one or more epiphragms (Riddle, 1983). On the other hand, Mahrous *et al.* (2002) and Mortada (2002) they reported that land snails aestivate during summer months where temperature and relative humidity are not suitable for their growth and development.

Table (3): Number of *Monacha cartusiana* snails aestivated and un aestivated with different stages and direction in borders of Egyptian clover field

Date	Snails	North			South			East			West		
		Epi	Non epi	Total	Epi	Non epi	Total	Epi	Non epi	Total	Epi	Non epi	Total
Jan.	A	-	-	1.4	-	-	5	-	-	0.8	-	-	0.6
	J	-	1.4		-	5		-	0.8		-	0.6	
Feb.	A	-	1.4	8.4	-	0.3	5.9	-	0.6	5	-	0.4	7.2
	J	-	7		-	5.6		-	4.4		-	6.8	
Mar.	A	-	19	33.4	-	5.2	8.8	-	3.4	8.4	-	6.6	12
	J	-	14.4		-	3.6		-	5		-	5.4	
Apr.	A	3.6	22.6	46.6	-	15.4	23.2	10.8	11.8	25	-	2.8	2.8
	J	-	20.4		-	7.8		-	2.4		-	-	
May.	A	0.8	3.8	5.0	57.4	39	96.6	33	9.2	43	77.6	99.2	176.8
	J	-	0.4		-	0.2		-	0.8		-	-	
Jun.	A	15	12.6	27.6	31.8	18	49.8	16.6	2	18.6	18.8	3	21.8
	J	-	-		-	-		-	-		-	-	
Jul.	A	7.4	3.6	11.0	3.6	2.2	5.8	-	-	-	5.4	4.2	9.6
	J	-	-		-	-		-	-		-	-	
Aug.	A	0.4	-	0.4	2.4	-	2.4	18.4	12.4	30.8	-	-	-
	J	-	-		-	-		-	-		-	-	
Sep.	A	-	-	-	-	-	-	-	-	-	-	-	-
	J	-	-		-	-		-	-		-	-	
Oct.	A	-	-	-	-	-	-	-	-	0.2	-	-	-
	J		-		-	-		0.2	-		-	-	
Nov.	A	-	-	-	-	-	-	-	-	-	-	0.6	0.6
	J	-	-		-	-		-	-		-	-	
Dec.	A	-	0.2	0.2	-	-	-	-	-	-	-	-	-
	J	-	-		-	-		-	-		-	-	
Grand total				134			197.5			131.8			231.8

A: Adult; J: Juvenile; Epi: Epiphargmed snails; Non epi: Non epiphargmed snails

Lokma (2007) studies the aestivation of *M. cartusiana* in Egyptian clover fields, snails aestivate, during summer month under plants grown in the irrigation canals. Sugar cane was the most preferable one followed by elephant grass, while Bermuda grass was the least one in this respect, general means of aestivated snails in 50×50 cm² under the plants were 207.57, 168.62 and 70.67 snails, respectively. The terrestrial gastropods do not inhabit cool environments but also habitat in which hot and dry conditions prevail. Snail species that can cope with such climatic conditions are thus expected to have developed multi-faceted strategies and mechanisms to ensure their survival and reproduction under heat and desiccation stress (Schweizer *et al.*, 2019).

2. Size frequency distribution for *Monacha cartusiana* and *Succinea putris*:

2.1. Size frequency of *Succinea putris*:

The size frequency distribution for *S. putris* was conducted at monthly intervals during the growing season of Egyptian clover at El-Qurana village, Abo-Hammad district, Sharkia Governorate. Data in (Figure, 4) declare that the newly hatched juveniles of shell height less than 5 mm were found during January and February 2016 only. However, snails with shell height of 5-6, 7-8 and 9-10mm were detected in all months from January to August 2016. While, snails with shell height 14-16 mm recorded during February to May. It was noticed that the highest number of individuals during all months from January to August 2016 were with shell height 7-8 mm except in March and May was with shell height 9-10 mm. Data in (Figure, 5) reveal that the shell width of *S. putris* 2-3, 3-4, 4-5, 5-6 and 6-7mm found in January were 5, 9, 5, 4 and 2 snail/sample, respectively, while the snails with shell width of 2-3, 3-4, 4-5, 5-6, 6-7, 7-8 and 8-9 mm were 4, 5, 5, 1, 5, 4 and 1snail/sample, respectively during February 2016. It was noticed that during March the snail with shell width of 3-4, 4-5, 5-6, 6-7 and 7-8 mm were 6, 8, 6, 3 and snail/sample, respectively, but the snail with shell width of 2-3, 3-4, 4-5, 5-6, 6-7and 7-8 mm were 2, 4, 6, 4, 3 and 6 snail/sample, respectively during April 2016. It is worthy to indicate that the snails with shell width of 2-3, 3-4, 4-5 and 5-6 mm were 1 and 3, 11 and 6, 11 and 10 and 2 and 6 snail/sample during May and June, respectively. While, the snails with shell width of 2-3, 3-4 and 4-5 recorded in July and August 2016 only.

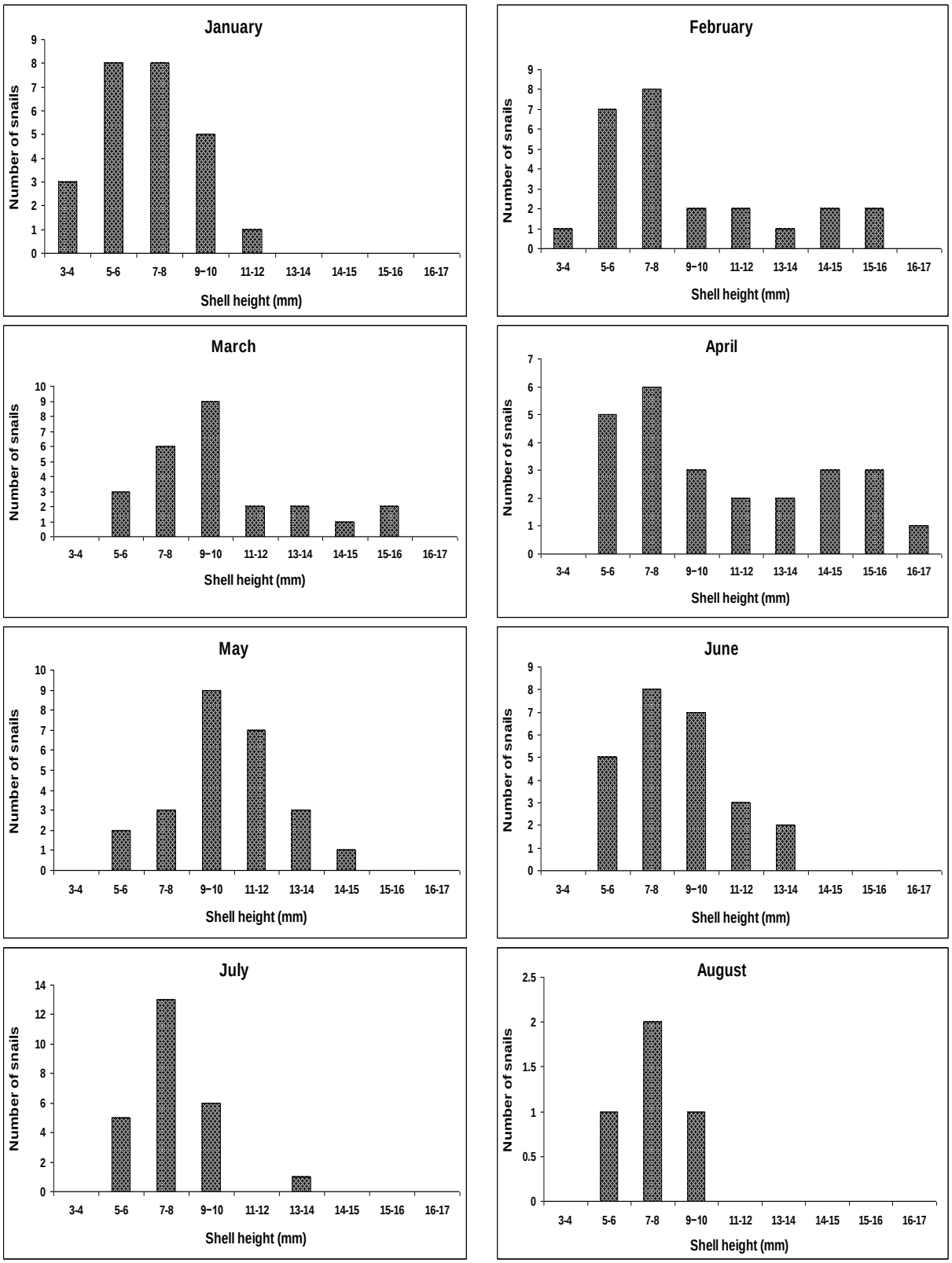


Figure (4): Size frequency histogram (shell height) of *Succinea putris* on Egyptian clover at El-Qurana village, Abo-Hammad district, Sharkia Governorate during the period from January to August 2016.

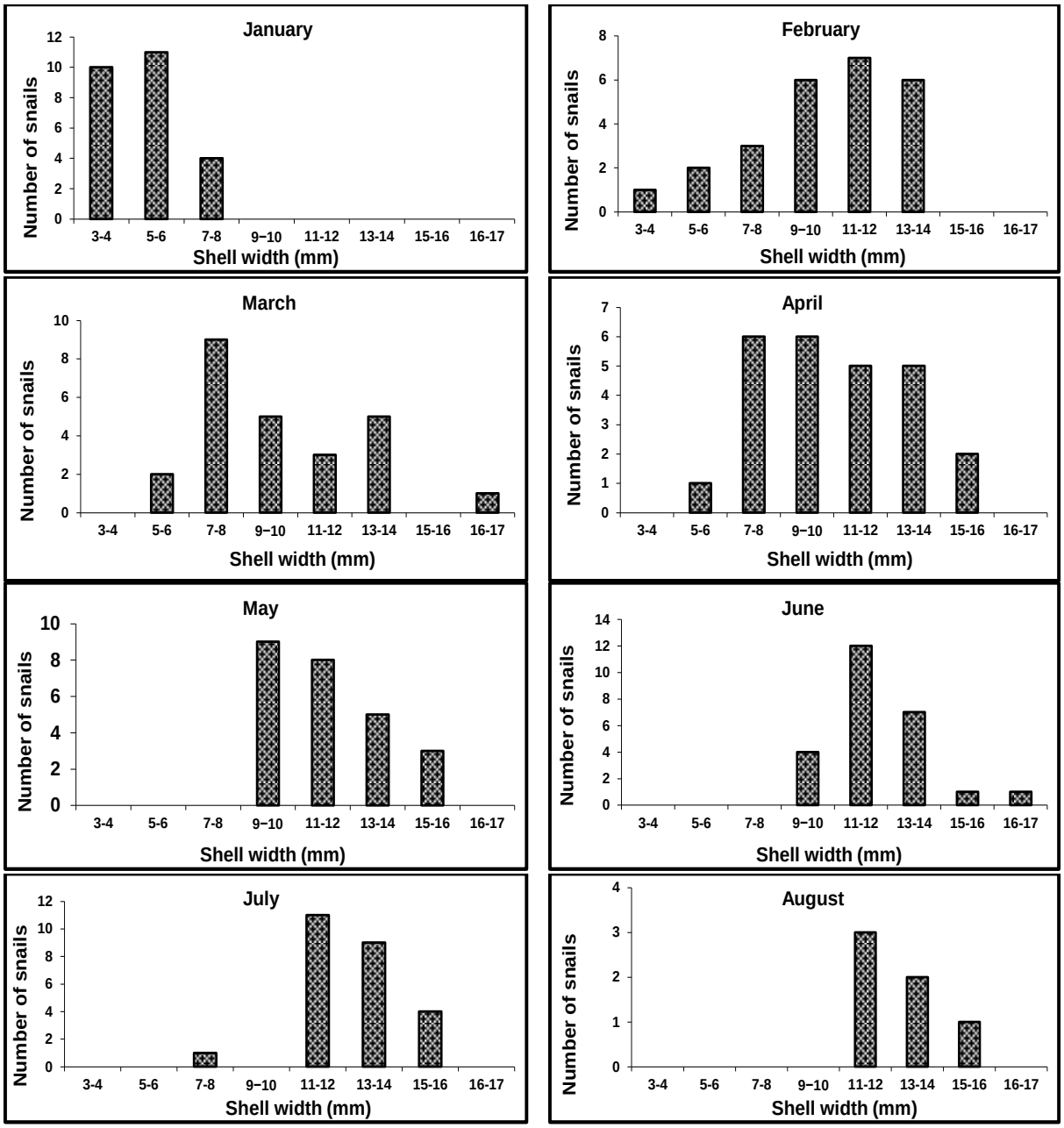


Figure (5): Size frequency histogram (shell width) of *Succinea putris* on Egyptian clover at El-Qurana village, Abo-Hammad district, Sharkia Governorate during the period from January to August 2016.

2.2. Size frequency of *Monacha cartusiana*:

Data in (Figures, 6 and 7) illustrate the size frequency distribution for *M. cartusiana* was conducted at monthly intervals on Egyptian clover at El-Qurana village, Abo-Hammad district, Sharkia Governorate. It is clear from the data in (Figure, 6) that the newly hatched juveniles of shell height less than 4 mm were found during January and February 2016 only. However, snails with shell height of 4-5 mm were recorded in three months from January to March, while, snails with shell height 5-6 mm were found in all months from February to June 2016. It was noticed that snails with shell height 6-7 and 7-8 were detected during all months from February to August, but snails with shell height 8-9 were found from April to August 2016. Data in (Figure, 7) indicated that the shell width of *M. cartusiana* 3-4 mm found in January and February were 10 and 1

snail/sample, respectively, while the snails with shell width of 5-6 and 7-8 mm were detected during January to April 2016. It is worthy to indicate that snails with shell width of 9-10 mm were found during February to June, also, snails with shell width 11-12 and 13-14 were observed during February to August. However, snails with shell width 15-16 were recorded during April to August; also, snails with shell width 16-17 were detected in Mach 2016 only.

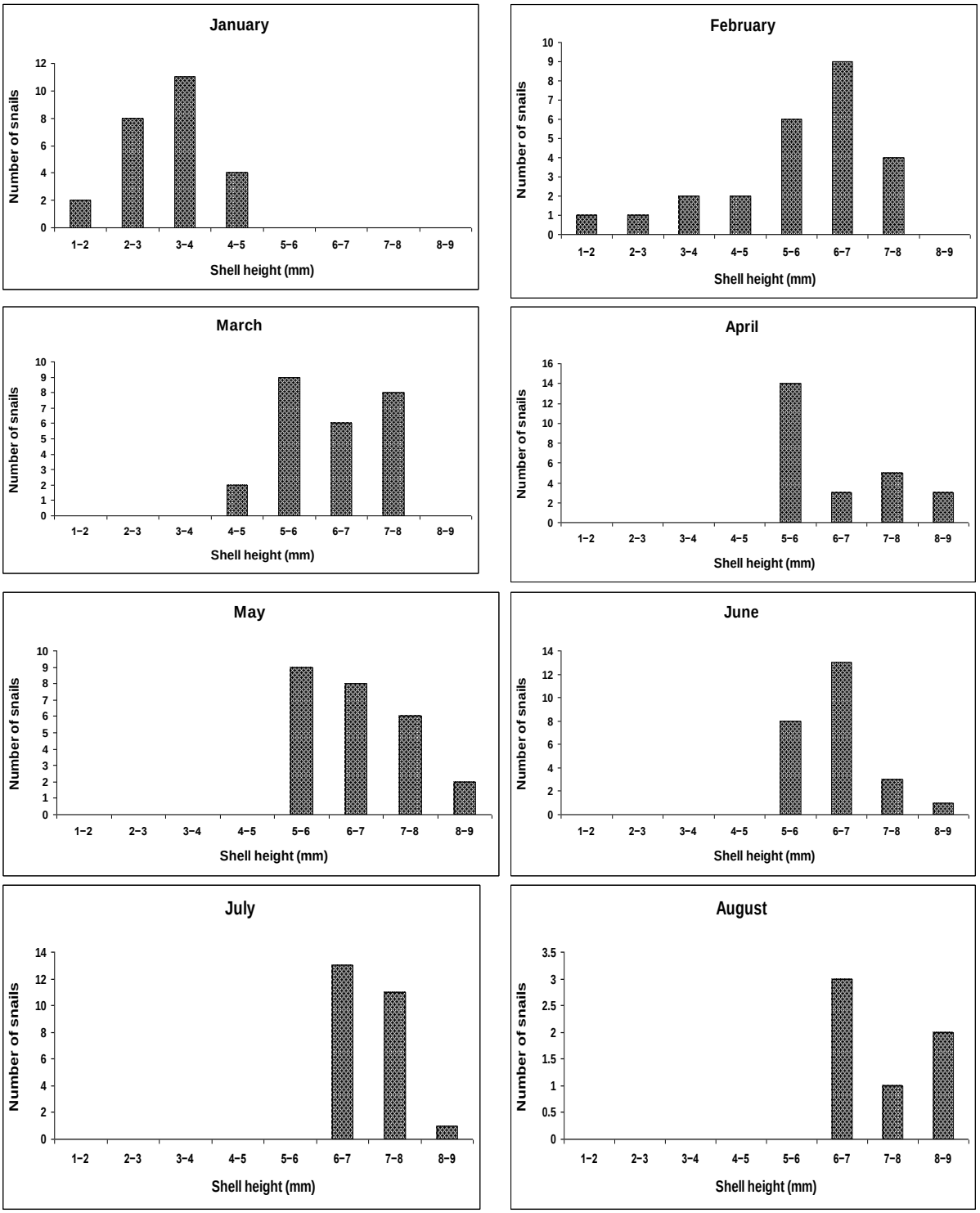


Figure (6): Size frequency histogram (shell height) of *Monacha cartusiana*on Egyptian clover at El-Qurana village, Abo-Hammad district, Sharkia Governorate during the period from January to August 2016.

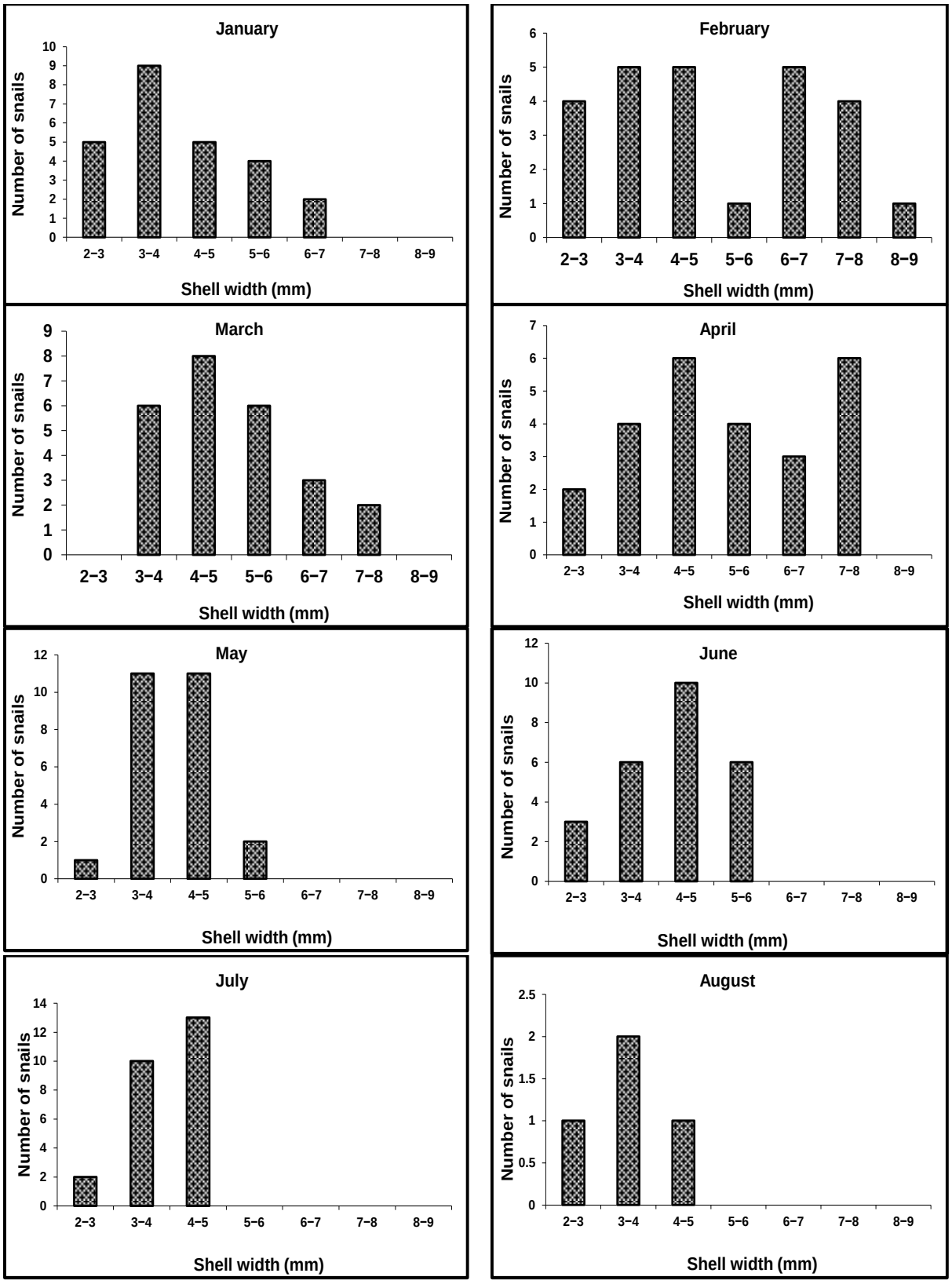


Figure (7): Size frequency histogram (shell width) of *Monacha cartusiana*on Egyptian clover at El-Qurana village, Abo-Hammad district, Sharkia Governorate during the period from January to August 2016.

In Greece, Staikou and Lazaridou-Dimitridou (1990) reported that *M. cartusiana* snails reached maturity within one year at a size of 8-10 mm. They could be lay eggs immediately upon maturation, died soon afterwards, while most of a population reached maturity and laid eggs two years after hatching. Adult snails were died after the productive period. Villalobos *et al*. (1997) showed that the neotropical terrestrial snail *succinea contarieana* had become a quarantenary pest in ornamental plants *(Dracaena marginata*, Dracaenaceae), they reached a density of 282900 individuals/ha. In field, reproduction it contained (as is rain fall) and eggs, young and copulation pairs are found mainly under moist litter. Ismail (1997) mentioned that feeding *Monacha cartusiana* on lettuce and cabbage leaves gave the highest growth in shell diameter after six months of the feeding on lettuce leaves and shell diameter were 8.8 and 8.6 mm for lettuce and cabbage, respectively. Carlos and Julian (2004) studied the yearly body size distribution of *Succinea costaricana* von Martens (Gastropoda: Succineidae) on an ornamental plant. Body size distribution (measured in the shells) indicated a capacity to produce year- round with a peak when pluviosity decrease in December. At this time of year, the population was dominated by snail under 4mm in shell length (longest individual: 12.06 mm). However, the yearly vain fall pattern does not correlate with shell, length width or width/length ratio than remain that rainfall alone is not the most important factor affecting population dynamics. Abed (2011) studied the relation between shell diameter and number of eggs of *M. cartusiana* during the breeding season. Result revealed that the clutch size of *M. cartusiana* snail as influenced by shell diameter of the three tested snail shell diameters descending as follows: 12mm ×10mm (22.9) and 10×10mm (16.9) eggs/ one pair snail, respectively. Lokma (2013) noticed that during April the number of *S. putris* snail in Egyptian clover filed was 5& 11 snail/sample of size frequency 2-3 and 3-4mm respectively. No snails with shell diameter less than 3-4mm were detected, while the snails with shell diameter of 3-4, 5-6 and 6-7mm were 8.8 and 10 and 5 snails/sample during May & June 2008, respectively. Moreover, the amber snail *S. putris* aestivated during summer months, closing their shell aperture by a thin, transparent epipharagm, snails were observed aestivating under damp habitats either under the grasses or edging of the irrigation canals and in soil cracks under masses, leaves in upper layers of soil.

It is concluded that *S. putris* consumed different amount of cabbage leaves, which increasing gradually with increasing shell highest under laboratory conditions. This snail was favorite the southern direction in the field to resting and appeared as active in different locations in all months during year and it was not enter in aestivation, while *M. cartusiana* snails were aestivated as adult stage with sealed white layer of epiphragm in the beginning of April until November and western direction was the favorite place to aestivation. The most size frequency of *S. putris* was ranged between 5-10 mm shell highest, but *M. cartusiana* was ranged between 11-14 mm shell width.

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Role of faba bean planted around and within sugar beet fields on insect infestations

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Abstract:

Faba bean is a good source of pollen and nectar for attracting natural enemies of insects, also it is a major source of protein for human and animals feeding in Egypt. Therefore, the present study was conducted at Tayfa village, Kafr El-Sheikh Governorate during 2017/2018 and 2018/2019 for investigating the role of faba bean planted around and within sugar beet fields (not intercropping) on insect infestations, natural enemies and farmer income. Obtained results demonstrated that mean numbers of infested plants (10 plants each replicate) with beet fly *Pegomyia mixta* Vill. (Deptera: Anthomyiidae), beet moth *Scrobipalpa ocellatella* (Boyd.) (Lepidoptera: Gelechiidae), tortoise beetle *Cassida vittata* Vill. (Coleoptera: Chrysomelidae) and aphid (*Aphis* spp.) in a sugar beet + faba bean field were recorded 3.00, 2.67, 2.33 and 4.50 in the first season, while, in the second season it recorded 3.67, 2.00, 4.00 and 5.00, respectively, while, in a sole sugar beet field the mean numbers of infested plants were 5.44, 6.56, 6.11 and 6.56 in the first season and 6.33, 6.16, 8.06 and 7.94 in the second season, respectively. Data also cleared that field mixed beet and faba infestation by beet fly, beet moth and tortoise beetle were beginning about 2 months (on February 25) later than sole beet, while, aphis infestation was beginning in the same time in both treatments. Statistical analysis proved significant differences between both fields during the both seasons have been detected. Further, total population of natural enemies, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), *Syrphus corollae* Fabricius (Diptera: Syrphidae), *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae), *Scymnus* sp. (Coccinellidae: Coleoptera) as predators and *Opius nitidulator* Nees (Hymenoptera : Braconidae) , *Monorthochaeta nigra* Blood (Hymenoptera : Trichogrammatidae) , *Agathis* sp. (Hymenoptera:Braconidae) and *Diadegma oranginator* Aubert (Hymenoptera:Ichneumonidae) as parasitoids in a sugar beet + faba bean plant were recorded 30.22 and 42.33 during 2017/2018 and 2018/2019, respectively. Whereas, in a sole sugar beet plant were recorded 7.06 and 7.39 in both seasons, respectively. Meantime, the additional return beside price the main crop was 1690 and 1470 L.E (Egyptian pound) to a sugar beet + faba bean in comparison with a sole sugar beet field.

Introduction

Nowadays, sugar beet in Egypt ranked the first in sugar production followed by sugar cane, where the total sugar production recorded 2.5 million ton in harvesting season of 2018-2019. Where sugar beet cultivation was extended to reach about 621000 feddan (about 261000 hectar) (Sugar Crops Council, 2018) in Delta (Northern Egypt).

One of the main problems associated with the Egyptian agriculture system is the low area of cultivated land per farmer. In average, 43% of the farmers own or work in fields of area one feddan or less. This led to an increase need to maximize land usage to enhance farmer's income (Ahmed *et al.* 2009). Farghaly *et al.* (2003) reported that the highest values of land equivalent ratio were found when sugar beet was intercropped with onion or faba bean. Some Egyptian Farmers used to grow faba bean in sugar beet fields (Hamdany and El-Assar, 2017).

From the insect control point of view, Risch (1984) and Baliddawa (1985) reported that population of several insect pests have been reduced under conditions of plant species diversity, indicating that intercropping could be used for the control of some insect pests. Further, the multiple cropping could be a powerful component of cultural pest control, as well as it satisfies the socio-economic objectives of the growers (Perrin, 1977). Omar *et al.* (1994) reported that reductions were recorded in cotton infestations with major insects when intercropped with cowpea, as compared with infestations in sole cotton. Wnuk and Wojciechowicz-Zytko (2007) pointed out that intercropping of two crop plants which are not shared hosts for insects is a method for insect control without usage insecticides.

Modern agriculture has often caused the simplification of biological and environmental structures in the agro-ecosystem mainly through intensive cropping practices. One of the methods of enhancing the population of natural enemies is enriching the field neighborhood with flowering plants. Wnuk and Wojciechowicz-Zytko (2007) showed that *Phacelia tanacetifolia* Benth is a good source of pollen and nectar for beneficial insects (Predators + parasitoids). They added that *P. tanacetifolia* was intercropped with Faba bean, the population of *Aphis faba*e Scop. was reduced because of the synergistic effect of *P. tanacetifolia* pollens and nectars to the predatory Syrphids that feed upon aphids. The rate of infestations by *Pegomyia mixta* Vill. (Deptera: Anthomyiidae) and *Cassida vittata* Vill. (Coleoptera: Chrysomelidae) were less in sugar beet plants intercropped with faba bean as compared with their numbers in sole sugar beet (El- Fakharany *et al.*, 2012). In addition, higher population densities of the insect predators, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), *Paederus alfierii* Koch (Coleoptera: Staphylinidae) and *Scymnus* spp. (Coccinellidae: Coleoptera) were recorded in intercropped fields. Badawi and Shalaby (2015) indicated that in plant protection programs, it has become necessary to use non-chemical methods for controlling insect pests. In such concern, intercropping of two crops which do not act as hosts for the same pest can contribute in reducing insect pest populations. Thus, adoption of intercropping is to create more favorable conditions for beneficial insect species and inhibit pest infestations.

The current investigation aimed to study the effect of faba bean planting within (on canal and detachers) and around (on borders) sugar beet fields on insect infestations, natural enemies and the net farmers income.

Materials and methods

The current investigation was carried out at Tayfa village, Kafr El-Sheikh Governorate, during 2017/2018 and 2018/2019 growing seasons. This study aimed at the role of faba bean planted around and within sugar beet fields (not intercropping) on insect infestations, natural enemies and farmer income. The experimental area was about one feddan divided into two halves, the first half was planted with sugar beet only (Karam variety). The second half was planted with the same sugar beet variety + faba bean (Sakha variety) sowing within and around the second half. Distance as border between the halves about 200 meters left without sowing.

Every half divided into three equal area plots acted as three replicates es. The experimental design was Randomized Complete Block (RCBD). Sugar beet was cultivated on 20th October, whereas, faba bean was cultivated on 15th November during the two seasons. The study was carried out by:

1. **Recording infestation by four insects *i.e. Pegomyia mixta, Scrobipalpa ocellatella, Cassida vittata* and *Aphis* spp.:**

Numbers of infested plants were counted by visual examined monthly using randomly 10 plants from each replicate, from 30th December till 10th May during two seasons.

2. **Recording insect predators and parasitoids:**

Numbers of insect predators (*C. carnea* larvae + adult and *Syrphus corollae* Fabricius (Diptera: Syrphidae) adult, *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) larvae + adult, *Scymnus* spp. and adult parasitoids such as *Opius nitidulator* Nees (Hymenoptera : Braconidae), Monorthochaeta nigra Blood (Hymenoptera : Trichogrammatidae) , *Agathis* sp. (Hymenoptera:Braconidae) and *Diadegma oranginator* Aubert (Hymenoptera:Ichneumonidae) were taken by sweep net method (50 double strikes per examination). After sweeping, the catch was put into paper pages, after that transferred to the laboratory and it put into refrigerator for 30 minutes to anethetizate the catch. Finally, the catches were put into petri dishes containing 70% ethyl alcohol for identifying by a stereoscope (4.8 – 56.0 x magnification).

3. **Statistical analysis:**

Mean numbers of infested plants and natural enemies population during 2017/2018 and 2018/2019 seasons in sugar beet + faba bean and a sole sugar beet were statistically analyzed according to the method described by Gomez and Gomez (1984). Means of the treatments were compared using the least significant difference (LSD) at 5 % level of probability.

Results and discussion

1. **Effect of faba bean planting around and within sugar beet on infestations with major insect pests and their associated natural enemies:**

Data presented in Tables (1, 2, 3 and 4) showed that the effect of f. bean planting around and within sugar beet fields (not intercropping) on infestations with certain insect pests during 2017/2018 and 2018/2019 seasons in comparison with a sugar beet field alone without faba bean. Mean numbers of

infested plants per 10 plants with beet fly (*P. mixta*), beet moth (*S. ocellatella*), tortoise beetle (*C. vittata*) and aphid species (*Aphis* spp.) in a sugar beet + faba bean field were 3.00, 2.67, 2.33 and 4.50 in the first season, respectively, while, in the second season were recorded 3.67, 2.00, 4.00 and 5.00, respectively. In a sole sugar beet field, the mean numbers of infested plants with the same insects were recorded 5.44, 6.56, 6.11 and 6.56 in the first season, whereas, in the second season number of infested plants with the four insects were 6.33, 6.16, 8.06 and 7.94, respectively. Statistical analysis showed that faba bean planted within (on canal and detachers), and around (on borders) sugar beet fields have reduced significantly the rate of sugar beet pest infestations as compared with their infestations in sole sugar beet during the two seasons.

Table (1): Mean of infested sugar beet plants with *Pegomyia mixta* / 10 plants each examination during 2017/2018 and 2018/2019 seasons.

Examination Date	2017/2018		2018/2019	
	Sugar beet + Faba bean	Sugar beet	Sugar beet + Faba bean	Sugar beet
30/12	0.00	1.00	0.00	1.67
26/1	0.00	2.33	0.00	4.33
25/2	3.33	5.00	4.33	6.33
30/3	5.00	9.67	6.33	9.67
24/4	5.00	8.33	6.00	8.33
10/5	4.67	6.33	5.33	7.67
Mean	3.00	5.44	3.67	6.33
Significant Status	**L.S.D Value at 0.05 = 2.389**		**L.S.D Value at 0.05 = 2.499**	

Table (2): Mean of infested sugar beet plants with *Scrobipalpa ocellatella* / 10 plants each examination during 2017/2018 and 2018/2019 seasons.

Examination Date	2017/2018		2018/2019	
	Sugar beet + Faba bean	Sugar beet	Sugar beet + Faba bean	Sugar beet
30/12	0.00	3.00	0.00	3.33
26/1	0.00	3.00	2.00	5.00
25/2	0.00	6.33	0.00	5.67
30/3	3.67	8.33	3.00	7.00
24/4	6.33	9.67	3.33	7.67
10/5	6.00	9.00	3.67	8.33
Mean	2.67	6.56	2.00	6.16
Significant status	**L.S.D Value at 0.05 = 3.031**		**L.S.D Value at 0.05 = 3.623**	

Table (3): Mean of infested sugar beet plants with *Cassida vittata* / 10 plants each examination during 2017/2018 and 2018/2019 seasons.

Examination Date	2017/2018		2018/2019	
	Sugar beet + Faba bean	Sugar beet	Sugar beet + Faba bean	Sugar beet
30/12	0.00	0.00	0.00	5.67
26/1	0.00	3.67	0.00	7.33
25/2	0.00	5.33	5.00	8.67
30/3	3.00	8.67	7.33	10.00
24/4	6.67	9.67	6.33	9.33
10/5	4.33	9.33	5.33	7.33
Mean	2.33	6.11	4.00	8.06
Significant status	**L.S.D Value at 0.05 = 3.576**		**L.S.D Value at 0.05 = 3.446**	

Table (4): Mean of infested sugar beet plant with aphid species / 10 plants each examination during 2017/2018 and 2018/2019 seasons.

Examination Date	2017/2018		2018/2019	
	Sugar beet + Faba bean	Sugar beet	Sugar beet + Faba bean	Sugar beet
30/12	1.33	4.67	1.33	6.00
26/1	2.00	3.33	2.67	5.33
25/2	4.67	6.33	5.33	7.67
30/3	6.33	8.33	6.00	9.33
24/4	7.00	9.00	7.00	10.00
10/5	5.33	7.67	7.67	9.33
Mean	4.50	6.56	5.00	7.94
Significant status	**L.S.D Value at 0.05 = 1.953**		**L.S.D Value at 0.05 = 2.111**	

Worth to mention that the infestation by beet fly, beet moth and tortoise beetle were began about 2 months (on February, 25) later in sugar beet+ faba bean as compared by sole sugar beet where the infestation began in the end of Dec. (Tables, 1, 2 and 3), meantime, aphis infestation was began in the same time in both beet + faba and sole beet (Table, 4). Such effect give evidence that delayed plant infestation has a vital role in lesser the damage caused by these insects in beet crop. .

Concerning the natural enemies, data in Table (5) showed that mean population of natural enemies in a sugar beet + faba bean field were 30.22 and 42.33 during 2017/2018 and 2018/2019, respectively, while, the mean population in a sole sugar beet field were 7.06 and 7.39 during both seasons, respectively. Statistical analysis showed that faba bean planted within (on canal and detachers), and around (on borders) sugar beet fields have increased significantly number of natural enemies as compared with sole sugar beet during two seasons. These results indicated that the reduction of sugar beet insect infestations in sugar beet + faba bean field may be due to the high populations of various natural enemies in this field in comparison with sole sugar beet ones.

Table (5): Mean of natural enemies in sole sugar beet and sugar beet + faba bean by sweep net (50 double strikes) each examination during 2017/2018 and 2018/2019 seasons.

Examination Date	2017/2018		2018/2019	
	Sugar beet + Faba bean	Sugar beet	Sugar beet + Faba bean	Sugar beet
30/12	17.33	3.33	23.67	3.67
26/1	20.33	6.00	29.00	6.33
25/2	22.00	7.33	33.00	6.33
30/3	29.67	7.00	41.00	8.00
24/4	41.67	10.00	57.33	9.33
10/5	50.33	8.67	70.00	10.67
Mean	30.22	7.06	42.33	7.39
Significant status	**L.S.D Value at 0.05 = 3.778**		**L.S.D Value at 0.05 = 3.881**	

The obtained results are in agreement with those of Baliddawa (1985), Perrin (1987), Omar *et al.* (1994), Farghaly *et al.* (2003), Wnuk and Wojciechowicz-Zytko (2007), El-Fakharany *et al.* (2012) and Badawy and Shalaby (2015) who demonstrated that the rate of infestations by sugar beet insects were less in sugar beet plants intercropped with faba bean as compared with in sole sugar beet. Moreover, higher populations of natural enemies were recorded in intercropped fields. Sengonca and Frings (1988) referred a reduction in *Aphis faba*e Scopoli (Hemiptera: Aphididae) population on sugar beet crop when *Phacelia* sp. was sown in beet field.

In this connection, Ruppert and Mollhan (1991) indicated that one of the methods of enhancing the population of natural enemies is enriching the field neighborhood with flowering plants. Altieria (1999) demonstrated that modern agriculture has often caused the simplification of biological and environmental structures in the agro – ecosystem mainly through intensive cropping practices. Morris and Li (2000) stated that coriander attracts hover flies and reduce pest infestation. Rizk (2005) found that *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) population were significantly diminished on different tomato strains intercropped with coriander as compared with control treatment. Risk (2011) also added that intercropping faba bean crop with *Coriandrum sativum* is a highly recommended method in pest control programs, it is a cheap, effective and safe method to minimize *Aphis craccivora* Koch (Hemiptera: Aphididae) population, to attract more predators as well to conserve biodiversity. Finally, Al-Beltagy (2015) suggested that intercropping systems create more favorable conditions for natural enemies and reduce insect infestations.

Concerning the aphid species, Hokkanen (1991) reported that trap crops are plant stands that are grown to attract insects to protect target crops from insect attack.

2. Economic benefits of faba bean planting around and within sugar beet:

Data presented in Table (6) showed that the importance of faba bean planted around and within sugar beet to farmer's income. Data cleared that sugar beet + faba bean have not spraying with insecticides, at the same time the farmer income increases due to the selling faba bean seeds after harvest. Therefore, the total sum income of sugar beet + faba

bean was 1690 and 1470 L.E (Egyptian pound) at the two seasons, respectively comparison with sole sugar beet. This profit considered as additional return beside price of the main crop.

Table (6): Effect of faba bean planting around and within sugar beet on farmers income during 2017/2018 and 2018/2019 seasons.

Seasons	2017/2018		2018/2019	
Crops	**Sugar beet + faba bean**	**Sugar beet**	**Sugar beet + faba bean**	**Sugar beet**
Insecticides spraying (L.E)	—	500	—	480
Price of faba bean seeds (L.E)	1190 (119 kg × 10 L.E)	—	990 (99 kg× 10 L.E)	—
Total return (L.E)	1690		1470	

The obtained results agree with those of Badawy and Shalaby (2015), El-Shamy *et al.* (2016) and Hamdany and El-Assar (2017).

It is concluded that the importance of faba bean for attracting and enhancing natural enemies, subsequently reducing the insect infestations. Further, additional increase in farmer income due to faba bean crop and to some extend to saving the cost of insecticides.

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Toxicological and biochemical parameters of microbial preparations on the cotton leafworm *Spodopter alittoralis* (Lepidoptera :Noctuidae)

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Abstract:

Laboratory experiments were conducted to evaluate the efficacy by sequential treatments of the two entomopathoginc nematode isolates, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* and the entomopathoginc fungus, *Beauveria bassiana* (Biopower1.4%WP) as well as the combined effect of them against the 3rd instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Data revealed that the nematode, *H. bacteriophora* was more potent than *S. carpocapsae* where their Lc_{50} values after 72h of treatment recorded 53.3 and 81.41 Jv/ml, respectively. On the other hand, the LC_{50} value of the entomopathoginc fungi, *B. bassiana* scored 20.08 gm/l. The combination between the three tested bio-agents using sequential method (*B. bassiana*+ *H. bacteriophora*) and (*B. bassiana* + *S. carpocapsae*) against *S. littoralis* 3rd instar larvae indicated a potantiation effect .The highest larval mortality percentage (93.75%) was recorded by treatment with the combination of the two bioagents. The effect of these pathogens on certain biochemical and physiological aspects of the treated larvae showed that the total protein content and the activity of transaminases were decreased post-infection with each tested bio-agent individually and this reduction was higher when they were used in combination. In contrast, there were an increase in the activity of acid and alkaline phosphatases. The increased activities were higher in treatment with the combined bio-agents than in treatment with each bio-agent only.

Introduction

The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered one of the most destructive phytophagous insect pests in Egypt, not only to cotton plants, but also to other field crops and vegetables (Kandil *et al*., 2003). Intensive use of chemical insecticides for

controlling this pest usually leads to adverse effects on non-target organisms and development of high levels of resistance to organophosphates, carbamates and pyretheroids (Alford, 2000). Therefore, there is always a need for finding out new material having specific modes of action to replace the conventional insecticides. Among the most suitable biological control agents for controlling the cotton leafworm are the entomopathoginc nematodes of the families Steinernematidae and Heterorhabditidae.Those were considered good biocontrol agents because they cause rapid mortality of the insect host without side effects on non-target organisms(Poinar, 1986).The third-stage juvenile of these nematodes is theinfective stage. Those are capable of long-term survival without feeding. These juveniles carry symbiotic bacteria (*Xenorhabdus sp*.) in their intestine to be released into the host`s haemocoel leading to septicemia followed by death of the host insect then the nematodes reproduce within the cadaver (Molyneux *et al*., 1983). Entomopathoginc fungi are similar to most fungal pathogens where they infect their hosts through the external cuticle. The infective units are the conidia which born on conidiophores. The sporulation and germination require high humidity. Fungi gain access to the insect directly through the insect's integument. After germination of the conidia on the insect's cuticle, the fungus penetrates the integument and proliferates throughout the host, ultimately resulting in mortality of the infected host. Host specificity of entomopathoginc fungi varies considerably; some species have a broad host range and others are more restricted (Mudroncekova *et al*., 2013).The present investigation was planned to study the efficacy of the two nematode species, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* as well as the entomopathogenic fungus *Beauveria bassiana* only or in combination using sequential method on toxicological and some biochemical parameters of the cotton leaf worm *S. littoralis* under laboratory conditions.

Materials and methods

1.Tested insect:

A laboratory strain of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was obtained from Plant Protection Research Institute and it was reared on castor bean leaves under laboratory conditions at 25±2 ^{0}C using the method described by El-Defrawi *et al.* (1964). The 3rd instar larvae were used in all laboratory experiments.

2.Microbial agents:

2.1. Entomopathogenic nematodes:

Two strains *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* Poinar (Rhabditida: Steinernematidae : Heterorhabditidae) were obtained from Pest Physiology Department, Plant Protection Research Institute.

2.2. Entomopathogenic fungus:

Beauveria bassiana as the commercial product Biopower (1.4%WP), produced by/S.T. stares company limit-India**.**

3.Toxicological studies:

Pathogenicity of the nematodes was performed against the freshly moulted 3rdinstar larvae of *S. littoralis*. The inoculums of IJ from *H. bacteriophora* and *S. carrbocabsia* were applied by placing every ten larvae in petri-dishe lined with filter papers. The filter papers were contaminated with 40,80,160 and 200 IJs of each nematode strain. Each concentration was replicated four times. Non-infected larvae were used

as control. Mortality percentages were recorded after three days and corrected using Abbott's formula (Abbott, 1925) whenever necessary.

Median lethal concentration (LC_{50}) of the fungi, Biopower as determined as follow: series of concentrations (4,6,8 and10 gm/100 l.) were prepared by diluting the formulated compound with distilled water. The3rdinstar larvae of *S. littoralis* were placed into plastic cups lined with filter paper and offered to treated leaves (using leaf dipping technique). Each concentration was replicated four times, ten larvae per each replicate. Mortality percentage was recorded after treatment and corrected using Abbott's formula (Abbott, 1925). The data were statistically analyzed using Ldp line to find out the LC_{25} and LC_{50} value.

3.1. Joint action and sequential treatments of fungi, Biopower on larvae infected with nematode, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*:

To examine the interaction between Biopower and the entomopathoginc nematodes, *H. bacteriophora* and *S. carpocapsae* the $LC_{25}$of each nematode (18.15-18.34 Jv/ml) was applied firstly to ten 3rd instar larvae of *S.littoralis*, then after 24h the fungus was applied at the LC_{25} level(6.95gm/l.). In addition, another ten larvae were infected with LC_{25} of the fungus then LC_{25} of each nematode was applied. The experiments were incubated at 25 ± 2^0Cand replicated four times. Ten non-infected larvae were fed on untreated leaves as control. The mortality percentage was recorded and corrected after 72h of infection. The co-toxicity factor was calculated according to (Mansour *et al*., 1966) equation to differentiate the final effect of the combinations were categorized as synergism, antagonism or additive effect. A positive factor of 20 or more was considered potentiation, a negative factor of -20 or less mean antagonism, while a value between -20 and +20 was the additive effect.

3.2. Biochemical studies:

Total body samples were collected from the 3rdinstarlarvae treated with the tested microbial agents, separately or in combination after 72 hours post treatment. Total protein content was measured according to the method described by (Bradford, 1976), acidic and alkaline phosphatases activity was determined according to the method of (Laufer and Schon, 1971). Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) also were determined as given by (Reitman and Frankel, 1957).

4. Statistical analysis:

Median lethal concentration values (LC_{50}, s) and the regression lines were statistically measured according to Finney (1971) using a software computer program (Ldp-line). Analysis of variance (ANOVA) conducted on all data using SPSS computer program software. And significance between treatment were compared by Duncan's multiple range test (Duncan, 1955).

Results and discussion

Toxicological studies:

1. Toxicity response of *Spodopter alittoralis* 3rd instar larvae to each *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *Beauveria bassiana* (Biopower) separately after 72h post treatment:

The LC_{25} values of *H. bacteriophora*, *S. carpocapsae* and *B. bassiana* were 18.15Jv/ml,18.34Jv/mland 6.95g/l, respectively (Table,1). Also, the LC_{50} values of the three pathogens were

53.3Jv/ml,81,4Jv/ml and20.08 g/l, respectively. The current results agree with those mentioned by Reyad (2001) who showed that the tested inoculums's level of *S. carpocapsae* and *H. bacteriophora*was effective against the 3rdlarval instar of *S. littoralis*. Moreover, the level 40 infective juveniles/ml distilled water caused 100% mortality of the host. On the other hand, Anand and Tiwary (2009) observed a high larval morality percentage against the 2nd instar larvae of *S. litura*at the higher fungal spores concentration. Mortality caused by EPF was low at lowerspores' concentrations. It increased with increase of spores' concentration. The (LC_{50}) of *B. bassiana*.value for 3rd instar larvae was (20.08g/l), showing that B. *bassiana* was the least effective agent. This might be attributed to defense mechanisms of target insect. It is well documented that older instars of the cotton leaf worm can tolerate toxic effect of this fungus. EPF species that infect insects have received considerable attention by scientists for their potential use in biological pests control. Some pathogenic fungi have restricted host ranges while others have a wide host range, *e.g., B. bassiana*. Many researchers have focused on the selection of virulent strains for target pests and their development as biological control agents (Godonou *et al*., 2009). Similarly, Anand and Tiwary (2009) observed highest morality rates against 2nd instar larvae of *S. litura*at the highest spores' concentration of fungal isolates. The growth of mycelium was indicated by white spores of *B. bassiana* on the dead *Agrotis ipsilon* larvae treated with the LC_{50} (2 x 10^8spores/ml) after 7, 10, 13 and 16 days. The mycelium started to grow after 7 days from death of infected larvae, and then the insect cadaver was covered by mycelium after 10 days later, the formation and discharge of spores were detected after 13 and 16 days, respectively (Gabarty *et al*., 2014).

Table (1): Toxicity response of *Spodopter alittoralis* 3rdinstar larvae to the tested entomopathogenic nematode and fungi at 72h post treatment.

Treatments	LC25	95% Fiducial limit		LC50	95%Fiducial limit		Slope ±SE
		Lower	Upper		Lower	Upper	
H. bacteriophora	18.15(Jv/ml)	11.70	24.4	53.3(Jv/ml)	43.00	64.29	1.44±0.16
S. carpocapsae	18.34(Jv/ml)	9.46	27.1	81.4(Jv/ml)	63.12	106.79	1.04±0.15
B. bassiana	6.95(g/l.)	5.68	9.0	20.08(g/l.)	13.67	46.24	1.46±0.29

2. Joint action and sequential treatment of the entomopathogenic fungi, *Beauveria bassiana* (Biopower) on larvae infected with each of (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*):

Data in Table (2) indicated that the combined effect of *B. bassiana* and each entomopathogenic nematode (*H. bacteriophora* and *S.carpocapsae*) as sequential treatment differed from the infection with each pathogen only. In the present study, all combinations showed an increase in the host mortality and gave potentiation effect. The highest effect was observed with the combination of *B. bassiana+H. bacteriophora* (+87.70) followed by *H. bacteriophora+B. bassiana* (+79.16) and *B. bassiana+S.carpocapsae(*+73.90) mixtures, then *S. carpocapsae* + *B. bassiana* (+58.33) mixture. These results agree with those mentioned by Shaira and Noah (2014) who observed that the combination of *B. bassiana*and the nematode*H. Bacteriophora* showed high

larval mortality among *S. littoralis* 3rd instar, which increased with increasing concentrations of fungal spores and/or nematode juveniles the interaction between fungi and nematodes may allow reducing chemical application rates. Additionally, the nematodes may become established and begin to offer a long-term reduction in the larval populations (Klein and Georgis, 1992). This study gives additional support to the importance of combinations between the entomopathogenic fungi and the nematodes for increasing the potentiality control insect pests.

Table (2): Co-toxicity factor and final effect of binary mixtures of entomopathogenic agents against 3rd instar larvae of *Spodoptera littoralis* by using sequential method.

Treatments	Concentration	Observed mortality%	Expected mortality%	Co-toxicity factor	Type of synergistic action
H. bacteriophora+B. bassiana	18.15Jv/ml+6.95g/l.	89.58	50	+79.16	Potentiative
S. carpocapsae+ B. bassiana	18.34Jv/ml+6.95g/l.	79.10	50	+58.33	Potentiative
B. bassiana+H. bacteriophora	6.95g/l.+18.15Jv/ml	93.75	50	+87.70	Potentiative
B. bassiana+S.carpocapsae	6.95g/l.+18.34Jv/ml	86.97	50	+73.90	Potentiative

3. Biochemical influences of *Heterorhabditis bacteriophora, Steinernema carpocapsae a*nd *Beauveria bassiana* on *Spodoptera littoralis* larvae:

3.1. Total protein content:

3.1.1. Effect of treatment with LC_{50} of each tested bio-agent alone on total protein content:

The obtained results in Table (3) showed a significant reduction in total protein content of *S. littoralis* 3rd instar larvae 72h post-infection with each bio-agent separately compared to control. The highest decrease was recorded in case of infection by *H. bacteriophora*, followed by *S. carpocapsae* and *B. bassiana* with percentages of change - 53.70, - 40.73 and - 30.12%, respectively. The results agreed with Ahmed *et al*. (2014) who found a reduction in total protein of the host larvae post-infection with the nematodes *S. riobrave* and *H. bacteriophora.* This toxic effect of the entomopathogenic nematodes is related to the symbiotic multiply bacteria, rapidly when released into the haemocoel causing a lethal septicemia to the insect host (Dutly, 1959 and Nickle and Welch, 1984). Thus, biochemical changes in the hemolymph composition were expected, since the hemolymph is the main site of action. On the other hand, the present results are in consistence with those obtained by Mazet and Boucias (1995).They found that during the vegetative development of *B.bassiana* in the haemocoel of the beet armyworm,*S.eixuga*; the mycelia tissue invasion phase inhibited host protein synthesis and produced a range of exocellular peptides.

Table (3): Effect of each LC_{50} value of *Heterorhabditis bacteriophora, Steinernema carpocapsae a*nd *Beauveria bassiana* on the total protein content at 72h post-infection of *Spodoptera littoralis* 3rd instar larvae.

Treatments	Total protein (mg/g.b. wt)		
	Mean±SE	Change%	Activity ratio
H.bacteriophora	8.07±0.11e	-53.70	0.462
S.carpocapsae	10.33±0.29c	-40.73	0.592
B. bassiana	12.18±0.17b	-30.12	0.698
Control	17.43±0.20a	---------	---------

***means with the same letters are not significantly different at$P \leq 0.05$**

3.1.2. Effect of treatment with combination between the tested bio-agents on total protein content:

Data in Table (4) indicated a significant reduction in the total protein content of *S. littoralis* larvae treated with binary mixtures of the tested bio-agents compared with the control with percentages of change (-91.4, -91.2, -90.1 and -90.9%) for treatment with the binary mixtures of (*H. bacteriophora+B. bassiana*), (*S.carpocapsae+B. bassiana*), (*B. bassiana+H. bacteriophora*) and (*B. bassiana + S. carpocapsae*), respectively. According to lee and Atkinson (1976), this high reduction in protein content could be referred that many nematodes secrete chemicals that facilitate penetration and migration through host tissues, feeding and avoidance of host immunity responses. These chemicals include toxins and digestive enzymes. Such as, proteases which are digestive enzymes that catalyze the cleavage of peptide bonds in proteins. Moreover, some animal parasitic nematodes secrete proteases to assist in skin and tissue penetration (Von Brando, 1973). On the other hand, the cyclic peptide metabolite beauverolide l, cyclosporine a and cyclic were produced by several genera of entomopathogenic fungi. When those were injected into last-instar of *Galleria mellonella*, it activated humoral response and induced a significant release of isozyme and cecopin-like activity into the haemolymph, suggesting stimulatory activity on humoral immune responses. These finding may explain the reduction of protein synthesis after treatment with the entomopathogenic fungi (Vilcinskas *et al.*, 1999).

Table (4): **Total protein content of 3rd instar larvae of *Spodoptera littoralis* treated with of the tested binary combinations of bioagents.**

Treatments	Total protein (mg/g.b. wt)		
	Mean ±SE	Change%	Activity ratio
H. bacteriophora+B. bassiana	2.15±0.08c	-91.4	0.08
S. carpocapsae + B. bassiana	2.20±0.05c	-91.2	0.09
B. bassiana+H. bacteriophora	2.48±0.09b	-90.1	0.09
B. bassiana+Scarpocapsae	2.26±0.11b	-90.9	0.09
Control	25.11±0.07a	--------	--------

***means with the same letters are not significantly different at$P \leq 0.05$**

3.2. Transaminase activities (Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase**):**

3.2.1. Effect of treatment with LC_{50} of each tested bio-agent only:

Data in Table (5) indicated that infection by *H.bacteriophora*, *S.carpocapsae*and *B. bassiana* significantly decreased the activity of GOT by percentages of change (-36.89, -29.60 and -14.37%) and GPT (-50.98, -44.48 and -32.60%), respectively as compared to the control. The current data agree with Ahmed *et al.* (2014) who found that the activities of GOT and GPT were highly decreased with infection by *H. bacteriophora* and *S. riobrave* juveniles. In the present study, the significant decline of GOT in *S. carpocapsae* larvae after 72 hr. post-infection by *H. bacteriochlorin* and *B. bassiana*, as compared to control treatment, may be attributed to the significant decline in free amino acids content, as has been pointed out by Kaur *et al.* (1985). Soliman (2002) reported that GOT and GPT activities decreased in *Ceratitis. Capitata* last instar larva infected with *S. riobrave* and *Heterorhabditis* sp. Agreeing with that of the present study. They added that the quantum of free amino acids directly influenced the activity of transaminase at the time of protein synthesis.

Table (5): Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities of *Spodoptera littoralis* 3rd instar larvae treated with the LC_{50} of each bio-agent only.

Treatments	Glutamate-oxaloacetate transaminase (GOT)			Glutamate-pyruvate transaminase (GPT)		
	Mean ±SE	Change %	Activity ratio	Mean ±SE	Chang%	Activity ratio
H. bacteriophora	16.37±0.35d	-36.89	0.631	18.10±0.11d	-50.98	0.490
S. carpocapsae	18.26±0.21c	-29.60	0.703	20.50±0.32c	-44.48	0.555
B. bassiana	22.21±0.24b	-14.37	0.856	24.89±0.28b	-32.60	0.673
Control	25.94±0.78a	---------	----------	36.93±0.24a	---------	----------

*Vertical means with the same letters are not significantly different at P≤ 0.05.

3.2.2. Effect of treatment with the combination between the tested bio-agents on Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities:

As shown in Table (6), there were a significant reductions in GOT and GPT activities of *S. littoralis* 3rd instar larvae treated with binary mixtures of (*H. bacteriophora+B. bassiana*), (*S.carpocapsae+B.bassiana*), (*B. bassiana+H. bacteriophora*) and (*B. bassiana+S.carpocapsae)* compared with the control by percentages of change (-39.04, -15.25, -28.73 and -34.18%) and (-35.43, -25.51, -39.96 and -33.12), respectively. This significant decline in GOT activity that occurred 72h post-infection by *H. bacteriophora* and *B. bassiana*may be attributed to the reduction in free aminoacids content as had been pointed out by Kaur *et al.* (1985).In addition, the current result agreed with that of Soliman (2002) who reported that GOT and GPT activities decreased in *C. capitata* larvae infected with *S. riobrave* and *H.bacteriophora.* They added that the quantum of free amino acids directly influenced the activity of transaminase at the time of protein synthesis.

Table (6): Glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase activities of the 3rd instar larvae of *Spodoptera littoralis* treated with the LC_{50}values of bio-agents in combination.

Treatments	GOT			GPT		
	Mean±SE	Change %	Activity ratio	Mean±SE	Change %	Activity ratio
H. bacteriophora + B. bassiana	11.41±0.16c	-39.04	0.60	25.20±0.03c	-35.43	0.64
S. carpocapsae+ B. bassiana	14.34±0.10b	-15.25	0.76	29.07±0.05a	-25.51	0.74
B. bassiana + H. bacteriophora	13.34±0.05a	-28.73	0.71	23.43±0.09d	-39.96	0.60
B. bassiana + S.carpocapsae	12.32±0.09 d	-34.18	0.65	26.10±0.3b	-33.12	0.66
Control	18.72±0.03b	---------	----------	39.03±0.04a	----------	----------

***Vertical means with the same letters are not significantly different at $P \leq 0.05$.**

3.3. Acidic and alkaline phosphatase activities:

3.3.1. Effect of treatment with LC_{50} of each tested bio-agent only:

From data in Table (7), the activity of alkaline and acid phosphatases, significantly, increased in the treated larvae with *H.bacteriophora*, *S.carpocapsae* and *B. bassiana* by percentages of change (188.7, 303.9 and 29.3%) and (81.33, 140.80 and 39.41%), respectively as compared to the control. The present results are in accordance with Xia *et al*. (2000) who suggested that acid phosphatase as a lysosomal enzyme, might have a role in autophagy and cell turnover as well as defense. Therefore, it appeared that the enhancement of acid phosphatase activity in *S. littoralis* larvae infected with *S. riobrave*, *H. bacteriophora* and *B. bassiana* was an attempt by the insect to defend itself against the invasion of the three pathogens. The same authors also added that phagocytosis is known to stimulate the production of lysosomal enzymes of which acid phosphatase is a key component. In addition, acid phosphatase had been found in insect haemocytes and shown to be released into the plasma (Lai-Fook, 1973 and Rowley and Rakcliffe, 1979). Moreover, Cheng (1983) reported that there was a hypersynthesis of acid phosphatase by haemocytes of the mollusk, *Biomphalaria glabrata* during phagocytoses. On the other hand, alkaline phosphatase of secreting products across cell boundaries. The present results agree with Ahmed *et al.* (2014) who reported that the activities of acid and alkaline phosphatase increased because of infection by *Steinernema riobrave* and *H. bacteriophora*.

Table (7): Alkaline and acid phosphatase activities in *Spodoptera littoralis* 3rd instar larvae treated with the LC_{50}value of each bio-agent alone.

Treatments	Alkaline phosphatase			Acid phosphatase		
	Mean±SE	Change%	Activity ratio	Mean±SE	Change%	Activity ratio
H. bacteriophora	5.11±0.073b	188.7	2.88	13.02±0.06b	81.33	1.81
S. carpocapsae	7.15±0.19a	303.9	4.03	17.29±0.12a	140.80	2.40
B. bassiana	2.29±0.16c	29.3	1.29	10.01±0.16c	39.41	1.39
Control	1.77±0.11d	----------	----------	7.18±0.03d	----------	----------

***Vertical means with the same letters are not significantly different at $P \leq 0.05$.**

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.3.2. Effect of treatment with the combination between the tested bio-agents on alkaline and acid phosphatase activities:

Results in Table (8) showed that there was a significant increase in alkaline and acid phosphatases' activity of *S. littoralis* 3rd instar larvae treated with binary mixtures of (*H. bacteriophora+B. bassiana*), (*S.carpocapsae+B. bassiana*), (*B. bassiana+H. bacteriophora*) and (*B. bassiana+S.carpocapsae*) compared with the control by percentages of change (-39.04, -15.25, -28.73 and -34.18%) and (-35.43, -25.51, -39.96 and -33.12), respectively. The present results agree with those obtained by Soliman (2002) who found that acid and alkaline phosphatases activity increased in the larvae of *C. capitata* infected with *S. riobrave* and *H. bacteriophora.*

Table (8): Alkaline and acid phosphatase activities of *Spodoptera littoralis* 3rd instar larvae treated with the LC_{50} value of bio-agents in combination.

Treatments	Alkaline phosphatase			Acid phosphatase		
	Mean±SE	Change%	Activity ratio	Mean±SE	Change%	Activity ratio
H. bacteriophora+B. bassiana	15.14±0.08c	62.97	1.62	14.31±0.1a	72.20	1.72
S. carpocapsae +B. bassiana	10.31±0.10e	10.97	1.10	9.12±0.1d	9.74	1.09
B. bassiana+H. bacteriophora	16.04±0.041a	72.65	1.72	13.07±0.07b	57.28	1.57
B. bassiana+S.carpocapsae	15.65±0.12b	68.46	1.68	12.56±0.04c	51.14	1.51
Control	9.29±0.09d	---------	---------	8.31±0.1e	--------	---------

***Vertical means with the same letters are not significantly different at $P \leq 0.05$.**

In conclusion' using the tested entomopathogenic nematodes and fungi in separate treatments or in combination through using sequential treatment to *S. littoralis* larvae affected some biochemical aspects in the treated larvae. This effect was more potent in treatment with the bio-agents in combination than treatment with each bio-agent alone. Therefore, the current study gives additional support to the importance of combination between the entomopathogenic fungi and nematodes for controlling the cotton leaf worm, *S. littoralis*.

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Taxonomy of genus *Brachymeria* species (Hymenoptera: Chalcididae) in Egyptian fauna

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Abstract:

Brachymeria Westwood (Hymenoptera: Chalcididae) is widely distributed and it considered the most common genus of chalcid parasites of many pests of agricultural importance in Egypt. The valid species of *Brachymeria* which are studied : *B. aegyptiaca* Masi, *B. albicrus* Klug , *B. ancilla* Masi, *B. brevicornis* Klug, *B. excarinata* Gahan, *B. femorata* Panzer , *B. fonscolombei* Dufour , *B. kassalensis* Kirby, *B. libyca* Masi as the first record in Egypt , *B. minuta* Linnaeus, *B. somalica* Masi , *B. vicina* Walker , and recorded from Egypt. This study is including description which support by illustration photography; distribution data and key of 12 *Brachymeria* species. The hosts of some species in Egypt are showed .DNA sequences of *B. femorata* obtained.

Introduction

Chalcidids comprise a very important beneficial group of parasitoids .Many species of the family are important parasitoids that have been used successfully for the biological control of many insect pest species. The genus *Brachymeria* Westwood , belongs to the subfamily Chalcidinae. Apparently, there are almost 300 species of *Brachymeria* in the world (Noyes, 2011). *Brachymeria* parasitize of the mature larvae and pupae with wide range species of various orders. They play significant role in the ecosystem of various economic important crops. In Egypt *Brachymeria* includes the most common and wide taxa distributed of the family Chalcididae .Many species of this genus are primary endoparasitoids of families Lepidoptera; Diptera and Coloeoptera. On the other hand , sometime hyperparasitic species are found on parasitize on Diptera (Tachinidae) and Hymenoptera (ichneumonid). Therefore the identification of the species concerned are highly important of many host-parasite which study for biological control involving this genus (Joseph *et al.*, 1973). Accurate techniques to detect and identify parasitoids are a prerequisite for understanding and managing host–parasitoid needed to interactions: for example, they are measure and monitor parasitism rates (Agusti *et al.*, 2005).

Today parasitoids have been detected within hosts of Diptera, Lepidoptera, Heteroptera, and Coloeoptera by DNA-based methods. These studies, utilizing the Polymerase Chain Reaction (PCR) ,have shown that parasitoid detection is possible at high specificity and sensitivity level (Greenstone, 2006). Studies on the taxonomy, ecology and genetic of parasitoids can be supply the basic information which necessary for biological control and for its efficient operations as strategy point undertaking integrated control plan in Egypt.

Materials and methods

1. Morphological methods:

The taxonomic study of family Chalcididae in Egypt depending on specimens which collected from the field survey and as well as the materials which kept in the main reference insect collections in Egypt . The Egyptian insect collection included, Ministry of Agriculture, Ain Shams University , Cairo University , and Al-Azhar University. The identifications or compare of specimens and terms were, mostly, carried out using Bouček (1952, 1956 and 1988); Habu (1960); Masi (1929 and 1936); Nikol'skaya (1952); Joseph et al. (1973) and Narendran and Achterberg (2016). Descriptions of all specimens were based mainly upon external morphological characters of the adult males and females whatever available . Some parts of insects measured by the gradual lens, then compare them. Using the Sony lens 20.1 Mega pixels .The different body orientation of the insects were photography as well as the parts in the description object. All examination, descriptions, distinction, measurement process and Photographer operations for specimens were made by use of a stereoscopic binocular microscope.

2.Genetic method:

Due to the relative numerical abundance of B. femorata parasitoid in the field and their accessibility in some areas were used in the genetic experiment as fallow:

2.1.Collected the parasitoids :

Pieris rapae (L.) (Lepidoptera : Pieridae) pupae collected from the fields of cabbage , (1/2 Fadden) , located in Qaha , Qalyubia during September and october, pupa stages of cabbage worm were collected in cloth bags, closed with rubber band and transferred to laboratory. The collected parasitoid pupae were confined individually in test tube (1.5× 1.5 cm.), covered by muslin cloth and tighted with a rubber band . A droplets of pure bee honey were put inside the glass tube wall for feeding by emerged parasitoids and kept under laboratory condition . Six live individuals from the parasitoid were used for the experiment. All the follow steps are specific to protocols of each of GeneJET Genomic DNA Purification Kit #K0721, #K0722. (Zilinskiene, 2012) and PCR Purification Spin Protocol (QIAquick Spin Handbook03/2008).

2.2. Primer28s :

F(GACCCGTCTTGAAACACGGA3')R (5' TGCGAAGGAACCAGCTACTA 3')

2.3. Machines used:

The PCR machine used is "Veriti 96 Well Thermal Cycler" from Applied Biosystems. The sequencer details is "3500 Genetic Analyzer" Applied Biosystems. Gel documentation (G:BOX) (SYNGENE model 680XHR) Made in UK. Species and related species were identified by the GeneBank.

Results and discussion

1. Key of *Brachymeria* species in Egypt:

1- Preorbital carina and postorbital carina present……..…3

- Preorbital carina and postorbital carina absent…… *B. albicrus* Klug.

2-Preorbital carina present and postorbital carina absent……...….7

- Preorbital carina absent and postorbital carina present……...…8

3-Sixth abdominal tergite weakly pitted and sparsely bristled…….....4

- Sixth abdominal tergite with coarse dense punctures and covered with dense bristles……6

4- Hind femora length more than or at least 1.80-2.00 times of width, with apical patch; hind tibia red, with sub basal and apical patches. apical patch of hind femora ;sub basal and apical patches of the hind tibia whitish.……*B. fonscolombei* Dufour.

- Hind femora length equal or less 1.80 times of width, mostly black, apical yellow; hind tibia mostly black. Apical and subbasal part yellow or brownish-yellow. ……..…….5

5-scape, mostly light color, hind femur with expanded yellow spot apically, and dark parts of tibiae reddish …….*B. brevicorinis* Klug.

-scape dorsally dark color and tibiae dark parts brownish black ……..*B. minuta* L.

6- Hind femur less elongate ,shiny, covered with pubescence, with one big reddish patch, and apical with yellowish ring ; provided with 12 black teeth ……...*B. vicina* Walker

-Hind femur more elongate, weakly shining , outer part pubescence, brownish red and yellow; provided with 11 dark brown teeth ……...*B.ancilla* Masi

7-Hind tibia black with basal slightly reddish and clear yellowish patches subbasally and apically , scrobe extended to front ocellus.…….. *B. excarinata* Gahan

- Hind tibia yellowish, scorbe slightly distant from front ocellus…....*B.somalica* Masi

8 -Hind coxae dentate below. *B. kassalensis* Kirby.

- Hind coxa not dentate below……9

9- Antenna, mostly black…..…… 10

-Antenna completely orange - reddish ……*B. libyca* Masi

10- Hind femur black in median dorsal ……..……..*B. femorata* Panzer

- Hind femur black, opaque with small yellow mark apically....*B. aegyptiaca* Masi

2. Description:

2.1. *Brachymeria aegyptiacae* Masi, 1931 (Figure ,1) :

Body : Length 4.0 mm, black, with short erected silver hairs.

Head: Flat dorsally ,provided with three bright yellow ocelli, compound eyes yellow, dim , ovate and protruding ; face with minute sculpture ; scrobe area deep dark; epistoma tubercle obliterate, clypeus margin motivate ; postorbital carina thin and perpendicular. occiput little oblique behind eyes and narrow; ocelli small ,circular and scattered, compound eyes small developed and into circle; width of ocellar area equal three fourth of inter-ocular space width at level of hind ocelli; scrobe cavity narrow; interorbital space high equal wide ; antenna black yellowish ;radical strong, small and yellow , scape brown ;pedicel bright brown, ball shaped and elongate; flagellum dark brown and 9 flagellomeres, 1st and 2nd flagellomere elongate, other seven flagellomeres long equal width ; club rounded end.

Thorax: Long equal one and half times of width with dense shallow punctured ; pronotum black, basal ridge, interrupted in middle with angle apically and rounded in median third ; parapsidal furrows deep ; scutellum convex , flatten, with rounded apical; scutellum with complete apex ; scutum and scutellum contiguous and symmetrically; propodeum plated; thorax sloping gradually behind scutellum ; tegula yellowish and triangular shaped with silver short hairs distally; forewing with marginal vein length equal one-half of sub –marginal vein , three times of post marginal vein , and equal four times of stigma vein length ; veins brown and submarginal base yellow ;leg yellowish and brown, covered with soft short hairs ; femur apex yellow with short spot; anterior tibia black, proximal and distal

thirds yellow with black median or proximal half yellow and distal half black brownish; tarsi yellow, rufous with five segmented , ended with two black thinly claws; hind legs enlarged; hind coxa fusiform, hairy and blackish; trochanter brownish, rounded and covered with hairs ; hind fumer strong , black ,dorso-venteral bright ,covered with small silver soft hairs, distal yellowish, external margin with dense , punctuate and provided10 teeth perfectly clear; teeth of distal margin small and convergent, last two teeth invisible; hind tibia yellowish brown, equal femur in length, curved shaped ,proximal and distal yellowish , large with reddish black ring in ventral median, apex truncated, arolium brownish and short.

Abdomen: Glazy ,spindle, conical and with pointed apical consisted 7 segments , first 4 segments separate and rest of conjunctivitis; 1st tergite smooth and dorsal bright black ; 1st and 2nd segments glazy dorso-ventrally;3rd and 4thtergites covered with one row of bristled ; from 5thtergite to last segment covered with silver hairs, 5th segment with punctures and bristles ; tergites 2-5 with finely reticulate and covered with scales; 6thtergite small , last two segmented formed genital capsule.

Specimens examined:1♀,Alexendria,5.9.2013; 1♀,Faiyum, 10.2016 ; 1♀ Giza 30-11-2014; 1♀ ,Giza 23.11.2014; 1♂, Mitghamer , 16.11.2014; 1♀ ,Wadi Al-Arish 9.2014; 2♀, Wadi El Natrun, 10.2015 and 11.2017 on Olive , Pomegranate from *Virachola livia* and *Palpita unionalis* pupae. **Geographical zone:** Costal stripes, Lower Egypt and Sinai.

Distribution: Cyprus **(Bouček, 1956)**, Iraq **(Al-Maliky and Al-Izzi, 1986)** and Palestine.

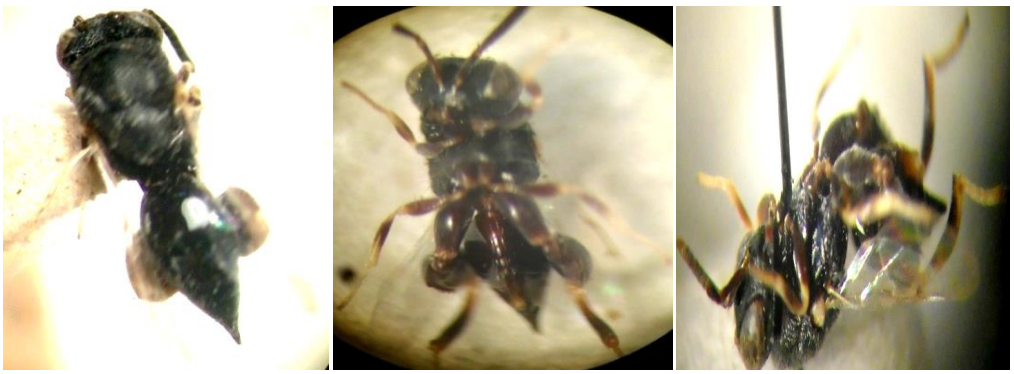


Figure (1): ***Brachymeria aegyptiacae*** **Masi (1931) ♀ .**
a. orsal **b. Ventral** **c . Lateral**

2.2.*Brachymeria albicrus* Klug, 1834 (*Pseudochalcis indica* Mani, 1935) (Figure ,2):

Body: Female length 5 - 5.5 mm ; black with reddish; fatness; covered with small silver and pubescence .

Head: Flat ; punctuate; vertex with interspaces between pits rugose; occiput with strong sloping; ocelli brownish ; distance between median and lateral ocellus equal 0.4 times of interoceller distance; face covered with dense velvet short silver hairs; height of compound eyes equal 2.3 times of width; scorbe length 1.5 times of width; inter antennal projection triangular ; malar space height equal 0.22 times of eye height; frons without preorbital carina , postorbital

carina absent; antennae stout; scape not exceeding to front ocellus; club biarticulate.

Thorax: Punctuate, width equal two thirds of length, pronotum plano concave; mesoscutum less half of length thorax; scutellum rounded ,bilobate raised proxmally, wide equal long , perpendicularly declined posteriorly; metathorax scabrous; propodeum with acute lateral tooth ; tegula triangular shape and light yellowish. Wings limpid; veins brownish .First and second legs light yellow, third legs reddish. Hind coxae hairy , ventral with densely minute punctured; hind femora red, enlarged, strong, equal 1.75 times of wide , with minutely punctured, distal margin with a row of 13 black teeth not equal in size ; hind tibia curved , equal femur in length; tarsi yellowish , 5th tarsomeres claws dark brownish .

Abdomen: Reddish, ovate .smooth 6th covered with dense tuft hairs; hypopygium smooth; ovipositor short.

Specimens examined: 2 ♀, Aswan, 21.6.2013; 1♀, Wadi El Natrun, 9.2014 on pomegranate by *Danais chrysippus* pupae.

Geographical zone: Upper Egypt and Western Desert.

Distribution: Ethiopia **(Azerefegne, 1999)**, India **(Gowri *et al.*, 2016)**, Iran **(Lotfalizadeh *et al.*, 2012)**, Nepal **(Walker, 1846)**, Pakistan (**Fry, 1989)** Papua New Guinea **(Narendran and Joseph, 1975)** and Somal.

Figure (2): ***Brachymeria albicrus*** **Klug (1834)** ♀.

a. Dorsal **b. Ventral** **c. Lateral**

2.3. *Brachymeria anecilla* Masi, 1951a (Figure , 3):

Body: Length 4 mm width 0.8 mm ,black; covered with short white hairs and some fuzz.

Head: Blackish; equal thorax width with dense punctures; dorsal pitted, ocelli small , circular and dark brown; distance between lateral ocelli equal three times of distance between compound eye and lateral ocellus; scrobe cavity touching madian ocellus; eyes leather texture, color black brown and convex shaped; malar space trapezoid form; preorbital carinae, genal carinae , clypeus and inter-antennal projection distinct; right mandible with two blunt teeth; antennae brownish red and stout ; torulus circular; scape slightly yellowish and short; pedicel length equal width; ring segment narrow and transverse; flagellum coarse, thickened towards apex, first basal segments long and narrow , segments 3-7 transverse; club semi spire.

Thorax: Curved; densely punctured, hairy; pronotum long laterally and short medially ; scutellum nearly rounded, moderately convex, divided in two lobes; propodeum provided laterally with small triangular tooth; tegulae yellowish and triangular shaped, wings hyaline; veins dark brown, forewing covered abdominal

segmented; marginal vein of hind wing yellowish and equal two times of post-marginal vein or equal one half of sub-marginal vein or equal one and half stigma vein ; 1st coxa blackish; trochanter black, fumer brown yellowish; tibia yellowish with spurs; tarsus yellowish with 5 tarsomeres, 1st tarsomere length large; arolium and claws brown; second leg brownish and yellowish; third legs enlarged and robust ; hind coxa spindle shaped , black with simple yellow ring distally, trochanter oblong, with circular bit, and dark brownish; hind fumer enlarged, elongate, weakly shining , outer side with pubescence, brownish red with yellow; lower margin with small dark brown with 11 teeth; hind tibia yellow with middle brownish red; hind tarsi yellowish red.

Abdomen: Conical shaped , shiny black and brown on ventral side; female end pointed, as small funnel, nearly as long as thorax; with dorsal white prominent specks, 1sttergite shiny and enlarged followed by second tergite; 2nd tergite equal one- quarter of 1st and covered with long bristles; each of 3rd ,4th and 5thtargites equal quarter of second tergite; 6th tergite hairy with densely punctured; segments 7-8 compressed laterally; ovipositor sheath blackish ,more visible in dorsal and ventral .

Specimens examined: ♀, Giza, 15.10.2013, Sweeping

Geographical Zone: Lower Egypt

Figure (3): ***Brachymeria anecilla*** **Masi (1951) ♀.**

a.Dorsal **b. Ventral** **c. Lateral**

2.4. ***Brachymeria brevicornis*** **Klug, 1834 (*Chalcis brevicornis* Klug, 1834) (Figure ,4):**

Body: Female length 3 - 6 mm, male length 3-4 mm . body black covered with silver hair.

Head: Wide equal 2.5 times of long; distance between lateral ocellus and compound eye equal 0.88 times of median ocellus diameter; mandible elongate, brown and red ,with two black teeth; clypeus conspicuously transverse, with piliferous points; postorbital carina present; gena with sparse puncturation, frons with preorbital carina; eyes small ;scape not reaching to median ocellus, long 3.6 times of wide ,pedicel transverse; flagellum dark brown to black , fusiform ;funiculars transverse and decreasing progressively in length; club

red yellowish ; tapering from base to apex.

Thorax: Long equal 1.37 times of broad; mesonotum with golden setae and punctures; scutellum long 0.88 times of broad; propodeum steeply sloped. Fore wing long equal 2.8 of broad; marginal vein long equal 2.38 times of postmarginal vein; hindcoxa moderately slender; hindfemur long 1.8 times of broad ; ventral margin with 12 teeth progressively closer to each other; tarsi yellowish. *Abdomen* oval shape, equal 1.79 times of broad; punctured anterior laterally. 1st tergite smooth with concave posterior margin; 2nd tergite with piliferous points basally and setae laterally; tergites 3–5 with setae subapically; 6th tergites covered with setae and piliferous points; ovipositor sheath short.

Specimens examined: ♂**,** Cairo, 11-10-2016; ♀, Cairo, 27.10. 2016; ♀, Giza, 8.2016 ; ♀, Qaha, 9.2015; ♀, Nag Hamadi, 20.9.2017 on Cotton from *Earias insulana* pupae

Geographical zone: Lower Egypt and Upper Egypt.

Figure (4): ***Brachymeria brevicornis*** **Klug (1834)** ♀**.**

a. Dorsal **b. Ventral** **c. Lateral**

2.5. ***Brachymeria excarinata*** **Gahan, 1925 (*B. apantelesi* Risbec, 1956) (Figure, 5):**

Body: Length 3 mm, shiny; black with yellowish, reddish or brownish spots; covered with silver pubescence and bristles.

Head: Width less thorax width ;dorsal with weakly pitted; vertex umbilicate punctate; width of ocellar area equal 0.75 of interocular space at level of hind ocelli; interocellar distance equal 2.33 times as wide as hind ocelli major axis; eyes brown and convex , width of eye equal 0.75 of height; dorsal frons irregularly carinate and ventral faintly carinate; preorbital carinae distinct ;scrobe polished , reaching to front ocellus and deep; inter – antennal projection narrow; malar space height less half of compound eye height; postorbital carina absent; fronto-genal suture complete; front and hind genal angle rounded; clypeus shining with few shallow punctures; right mandible with three pointed tooth ;antenna blackish , not stout; scape elongate ,brown , smooth ,equal combined segments 4 to 7 in length and apical one-third contracted; pedicel black; flagellum with same thickness; segments 4 - 10 equal in length; club brownish red equal two times of segment 10 .

Thorax: Pronotum with apical rounded, lateral carinate; parapsidal furrows as shallow grooved ;scutellum apical narrow explanate and reflexed , outer margin covered with long silver hairs, posterior margin slightly declined, not bilobed; wide equal 1.2 times of long ;propodeum

powerful declined ; tegulae yellow with basal reddish brown ; wings hyaline; veins dark reddish brown , length of fore wing equal 1.6 times of width; postmarginal vein less one half of marginal vein; legs black with yellow ; hind tibiae curved, black with reddish basal and subbasally, apex with long yellow patch ,with brown outer side ,inner side with patch becoming brownish and attaining both ventro-lateral carina and apical margin , equal hind femur length; hind coxae with distinct dense punctures, pubescence and distinct microsculpture ventrally; hind femur enlarged , width equal 0.4 of length ; outer side with dense pubescent, distinct reticulate and minute punctures ; inner side with less pubescent, and distinct punctures ;outer ventral margin with 12 teeth; basal with one large tooth; tarsi 5 segments and yellowish ; claws and arolium dark reddish brown.

Abdomen: Black, shiny with partially brownish, short, covered with silver hairs; width less length; pointed posteriorly; 1st tergite smooth and long; 2nd tergite finely dense punctate and bristles dorsally, lateral with distinct microsculpture ; 6th tergite very rough with shallow bristled pits and distinct microscupture; epipygium fairly compressed form sides, with middle carinate; genitalia elongate**.**

Specimens examined: 2♀**,** Helwan 27.9. 2014, Sugercane

Geographical zone: Lower Egypt

Distribution : Cameroon **(Narendran and Achterberg , 2016)** , China, India , Iran, Japan, Papua New Guinea, Philippines **(Herting, 1975)**, Taiwan **(Chien *et al.* ,1984)** and Vietnam.

Figure (5): ***Brachymeria excarinata*** **Gahan 1925 ♀.**
a.Dorsal **b. Ventral** **c. Lateral**

2.6. *Brachymeria femorata* Panzer, 1801 (*Chalcis ornatipes* Cameron, 1906) (Figure,6):

Body: Length 4.2 - 6.5 mm, black ; with yellow brown, batches and covered with silver pubescence hairs.

Head: Black , half-shiny; triangular shaped ; width of head converges with thorax width; vertex flat ; scrobe deep and smooth faintly rugose ; ocelli rounded and dark brownish ; width of ocellar area equal 0.75 of interocular space at level of hind ocelli ; compound eyes glabrous, black, with yellow or dim brown and convex; preorbital carinae not existing; postorbital carina distinctly; antenna black and stout , funicle with trichoid sensillae on ventral side (male) ; club long equal 2 times of segment 10th with slight red end.

Thorax: Thick structure, dorsal with pits, inter spaces between pits narrow and carinate; pronotum irregular shaped; scutum width approximates the length; scutellum fairly high laterally, semi-circular and less scutum, strongly, with

declined towards apex , apical widely explanate and reflexed and bilobate ; Propodeum with one blunt protuberance behind spiracle. Tegula bright yellow with basal dark reddish-brown , triangular and smooth ; wings semi-transparent and hyaline, veins dark brown; legs yellowish black and brown; hind femora enlarged , bright yellowish with black spot in middle; equal 1.7 times of wide , outer side with densely punctuate and pubescent, inner side with punctuate; outer ventral margin with eleven teeth .

Abdomen: Short ; pearly shaped, 1st tergite smooth,; 2nd tergite large , with bristled punctures, except ventro – lateral and basal areas; tergites 3 - 5 with densely pitted and bristled except ventro-lateral and basal area; tergite 6th covered with densely pitted ; ovipositor sheath small not visible from above and punctuated.

Specimens examined: ♀, Damanhor , 11.2017 ; 2♂ and 6♀ ,Giza , 3. 2013 ; ♀, Kafr Alaym ,8.2013; ♀, Kom Halin ,11.2016; ♀,Kotor 10.2015 ; ♀,Mansoura,10.2016; ♀,Qaha , 9.2015 ♀; ♀,Quesna ,10.2014; ♀, Shibin El Kom, 9.2013; ♂, Tokh Tanabsha ,7.2013; on Cabbage and Cauliflower by sweeping and *Pieris rapae* pupae.

Geographical zone: Lower Egypt and Costal stripes.

Distribution: Bosnia Hercegovina **(Bouček, 1977)**, Caucasus **(Nikol'skaya, 1978)**, China **(Baltazar, 1966)**, Europe , India **(Bhat and Bhagat, 2009)**, Indonsia, Iran, Iraq, Kazakhstan, Mongolia, Pakistan, Palestine, Russia and Turkey .

Figure (6): ***Brachymeria femorata*** **Panzer (1801) .**
a. Dorsal **b. Ventral** **c. Lateral**

2.7. ***Brachymeria fonscolombei*** **Dufour, 1841 (*B. podagrica* Fabricius, 1787) (Figure,7):**

Body: Length 6 mm, black with short silver brownish hairs and pubescence, half –shiny and shiny.

Head: With flat vertex, pitted; scrobe deep, smooth, width equal more 1.5 times of interocular space wide and reaching to front ocellus; ocelli round, brownish; width of ocellar area equal 0.6 times of interocular space of hind ocelli; compound eyes glabrous, convex; postorbital carina distinct , front genal angle and hind angle rectangular; mandibles dark brown, right mandible with two blunt teeth; antennae black and brown , stout; radical small and brownish; scape yellowish red ; length equal segments from 4 to 7 combined; pedicel dark brownish ,club length more two times of 10thsegment and brownish .

Thorax: Width less length by 16,67%; pronotum narrow, smooth, microsculpture , interspaces pits carinata; scutum almost as wide as long ;

scutellum declined with long silver hair posteriorly, apical part widely and reflexed , apex emarginated and bilobed; propodeum slope, coarsely sculptured with one sharp protuberance; tegulae white yellowish and blackish base; wings hyaline with small black particles and beginnings orange color; fore wing length equal 2.7 times of wide ; veins yellowish and dark red brown , margin vein equal 0.55 of submarginal , post margin equal 0.25 of marginal; legs shiny, black brown and yellowish; hind legs strong, hind coxa spindle shape, reddish brown with basal black; hind femur enlarged ,reddish brown with yellowish white spot distally ;length equal 1.9 times of width ,ventro-distally with one small protuberance; ventral margin saws shaped with ten brownish red and black large and acute teeth; hind tibia reddish brown with yellow spots ,inner margin dark brown ; tarsus brownish and 9 segmented .

Abdomen: Semi-conical, smooth and shiny, pointed posteriorly, less thorax length, equal thorax width; 1st tergite smooth; 2nd tergite with sparse minute punctures on dorsal base except middle, 6th tergite with weakly pitted and distinct microsculptures. Genital copsoul covered with long hairs laterally and 2 yellow line; hypopygium copper; epipygim sides compressed; ovipositor sheath visible above.

Specimen examined: ♀., Cairo, 27.8.2014 Grasses.

Geographical zone: Lower Egypt.

Distribution: Afrotropical, Australasian, Bangladesh **(Chowdhury and Howlader,1978)**, Brazil **(Marchiori *et al.*, 2003),** China, Europe, India , Indonesia **(Heller and Günther, 1936)**, Indopacific, Iran, Jamaica **(De Santis, 1979)**, Japan **(Pujade, 1994)**, Malaysia, Mexico, Mongolia **(Bouček, 1952),** North Africa, Palestine ,Philippines, Somalia **(Masi, 1938)**, South Africa **(Cameron ,1911)**, Thailand , Vietnam and Zambia.

Figure (7): ***Brachymeria fonscolombei*** **Dufour (1841)♀.**
a. Dorsal b. Ventral c. Lateral

2.8. *Brachymeria kassalensis* Kirby, 1886 (*B. bengalensis pulchellae* Joseph, Narendran and Joy, 1972) (Figure,8):

Body: Length 5- 6 mm. dark black with silver hairs, some parts half shiny and other shiny with finely and densely puncture.

Head: Black and brownish distally, width slightly more thorax width; occiput sloping behind compound eyes; width of interocular space equal 2.5 times of interocellar distance, latter equal 3.2 times of ocellocuar distance ; compound eyes large, convex , brown yellowish,

height equal 2.5 times of width; scrobe deep , smooth and reach to front ocellus, length equal 1.78 times of width ; frons without preorbital carina; postorbital carina reaching to genotemporal margin ; genal carina bifurcate; malar space equal one-third of compound eye height; mandible black with brownish end; antenna geniculate , brave, elongate; scape black; radical brownish and black, scape not exceeding to front ocellus , longe equal one to three combined flagellomeres; pedicel small , length equal approximately width, semi rounded; funicle black ; 1st flagellomere less thicker; 1stto 3rdflagellomeres with long more wide, 4th to 6th flagellomeres roughly square, 7th flagellomere transversed ; club divided in two segments and with reddish round end.

Thorax: With high level of head and abdomen, width equal abdomen width and more long; rounded reticulate; with densely umbilicate and close pits; pits interspaces curly shaped; pronotum plano concave; parapsidal furrows shallow;scutellum convex,width equal approximately three - quarters of length; propodeum coarsely sculputured; tegulae triangular shape, with blackish base; fore wing length equal 2.7 of width; base of submarginal veins yellowish ; marginal veins length equal 0.6 of submarginal veins length, postmarginal veins length slightly more three- fifth of marginal veins length, stigma vein equal one-quarter of postmarginal veins ; hind coxae obclavate shape , hairy, black, minute dense pitted ; hind femora thickened with minute pitted, denticulated (12 black teeth), reddish-brown, tip with large yellow spot, length equal 1.75 times of width; hind tibiae light yellow, with reddish-brown ring basely and long ventral carina, equal with fumer length,curved and thickness; tarsi yellowish , 5th segments , with claws black.

Abdomen: Ovate; length less pronotum ; scutum and scutellum combined, width less three- fifth of length, middle high , venteral side black brownish ; 1st tergite shagreen dorsally ; 2nd tergite middle with small sparsely minut punctures, base and proximal part glazy , lateral sides with dense pitted and bristls; 3rd tergite completely pitted ; tergite 4–5 punctured at proximal half; the 6th tergite with dense punctured and bristled. Ovipositor sheath clearly visible from dorsal side.

Specimen examined: ♀ , Halayeb 22.1.2014 and ♂, Halayeb,4.2.2016.

Geographical zone: Upper Egypt

Distribution: Ethiopia **(Masi, 1951b),** India **(Narendran, 1986)**, Senegal , South Africa, **(Prinsloo, 1980)**, Sudan **(Kirby, 1886)** and West Africa.

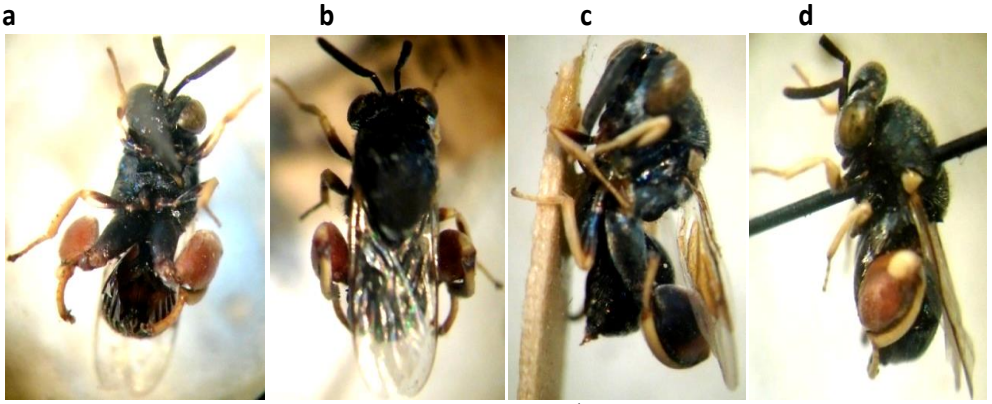


Figure (8): ***Brachymeria kassalensis*** **Kirby (1886) ♂ and♀.**
a Dorsal♀ b Ventral♀ c Lateral ♂ d Lateral ♀

2.9. *Brachymeria libyca* Masi , 1926 (*Chalcis libyca* Masi, 1926) (Figure,9):

Body: Length 5 mm ,black, some parts covered with whitish hairs and pubescence.

Head: Densely punctured, smooth and some parts rough; length slightly less width; sloping straightly behind eyes in front, upper as two lobes, one high and other reduced; vertex pitted , middle part slightly thin ; ocelli oval shape, bright brownish and oblique ; width of interocular space at level of hind ocelli equal one time and two- thirds of ocellar area width ; compound eyes black yellowish; eye height slightly less double eye width , width equal of height malar space; postorbitals carinae not clear , preorbital carinae not present; scrobe obovata ,deep and smooth, extended to front ocellus ;inter antennal projection elongated triangle ; fronto genal suture distinct ,gena immensity and extend below orbit ; clypeal sulcus deep ; mandible bidentate; antennae completely orange – reddish ,thick and short; scape smooth, less one- half of flagellum length and unequal thickness; pedicel semi-rounded ; anellus small ,flagellum increased in width parts and covered with small yellow hairs ; funicle 8 segments , 1st flagellomerewidth equal length, each rest segments width slightly more length ,club 2 segments and large of other funicle segments.

Thorax: Strong, glazy with silver short hairs and densely punctured, width equal one-sixth of length and elevated with curve; pronotum reduced and planer concave ; parapsidal furrows shallow ; scutum bell shaped ; scutellum with two lobes and convex laterally; propodeum sculptured with laterally into acute teeth ; tegula whitish yellow and triangular shaped ;wings hyaline ,veins yellowish orange forewing length equal two times of hind wing length and width equal 3 times of hind wing width ; submarginal vein equal three times of marginal, marginal vein equal two times of post marginal. legs some parts whitish yellow , orange and others copper color ;third legs strong and large , hind femur enlarged , length slightly less two times of width , with few fine hairy, punctures , first two-thirds orange and last third yellowish-white cream , ventral orange with teeth , inner margin with small tooth and external with nine black teeth, equal in size , first three closed together; hind tibia curved , strong , equal hind femur length, color orange, with pale yellow stripes, margin lower black and more wide near the tarsus, tarsus margins uneven ; tarsi yellowish with pubescence; 5 segments, and not equal in size , spur with two orange and short seta; claws dark brownish.

Abdomen: Oval shaped , approximately conical reddish black , shiny and smooth ; with small white protuberances and whitish bristle ,tip blunt; equal thorax width and height; dorsal reddish black; last four segments depression ; ventral copper blackish; 1st tergite large ,smooth; 2nd tergite with punctures on laterally; tergites 3-6 with row bristles and punctures on laterally; 6th tergite coarsely and gently pressed with dense punctures .

Specimens examined: ♀, Cairo, 3.6.2014.

Geographical zone: Lower Egypt.

Distribution: Libya and Syria.

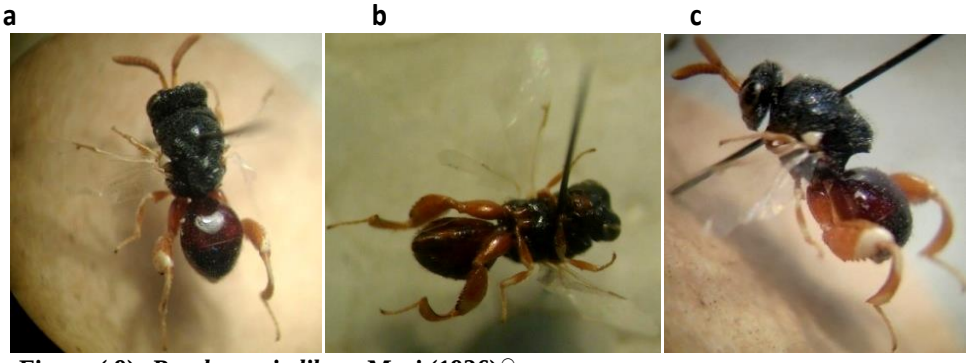


Figure (9): *Brachymeria libyca* Masi (1926)♀.
a.Dorsal b. Ventral c . Lateral

2.10. *Brachymeria minuta* Linnaeus, 1767 (*Brachymeria putturensis* Joseph, Narendran and Joy, 1971) (Figure,10):

Body: Length 3.5 - 6 mm, black, streamlined shape, shiny and half-shiny, covered with grayish white pubescence.

Head: Pitted with carinate ,except scrobe; ocelli rounded , yellowish ,width of ocellar area equal 0.67 wide of inter-ocular space at level of hind ocelli; compound eyes enlarged , glabrous ; convex; black light brownish; compound eyes high equal one-third of width; preorbital and postorbital carinae distinct; right mandible with two blunt teeth; antennae black, brave, club long and equal two segments of flagellum , slightly reddened; antennae of male narrow apically .

Thorax: Inflected , with long silver erect hairs , with shallow pitted dorsally and interspaces pits carinate; scutum wide equal 1.2 times of long; Parapsidal furrows distinct; scutellum apically extending outward in flat form and fold back, rough and consisting of two lobes; propodeum distinctly incline downward posterior. with one hurt indistinct tooth behind spiracles at sides. Forewing length equal 2.5 times of wide; veins brownish; hind femur oval , enlarged , length equal 1.77 times of width ,punctate, pubescent punctures on inner side, with small blunt tooth at inner ventral side near base and usually 12 brownish teeth on outer ventral margin. *Abdomen* point posterior, 1st tergite shiny; tergites 2-5 with distinct microsculpture except apical, on ventro-lateral parts and basal 2nd tergit; tergite 6 weakly pitted pipygium compressed from sides; ovipositor sheath visible dorsally, with weakly dense punctuate and thick hairs.

Specimens examined:. Alexandria 13.7. 2013 ; ♀, Baharia Oasis, 20.3.2017 ; ♀ , Cairo, 3 .2014 ; ♀, Giza , 5.5. 2014 ; ♂, Giza 8.6.2014 Watermelons ; ♀, Giza, 17.11.2013 maize ; ♀, Matrouh,28.9.2017 and ♀ , Zigazig, 4. 2014 .

Geographical zone: Costal stripes, Lower Egypt and Upper Egypt.

Distribution: Australia, Europe, India, Iran, Japan, Kazakhstan, Malaysia, Moldova, Montenegro, Netherlands **(Gijswijt, 2003)**, Papua New Guinea, Russia , Syria, Thailand, Turkey, Uzbekistan **(Sychevskaya, 1964) and** Vietnam.

Figure (10): ***Brachymeria minuta*** **Linnaeus (1767)♀.**
a.Dorsal b. Venteral c. Lateral

2.11. ***Brachymeria somalica*** **Masi,1929 (Figure,11):**

Body: Black, length 3.7 – 5 mm, male length less female , covered with white hairs , pubescence and punctuate , some areas glassy and smooth.

Head: Strongly ; with dense and small punctures ; vertex scarcely round ;ocelli rounded , bright brownish and gleaming ; distance between median and lateral ocelli equal twice distance between compound eyes and lateral ocellus ; occiput sloping steeply behind eyes; eyes convex and brownish ; face covered with fine velvet hairs and pitted, genae sculpture distinct; fronto- genal suture quite distinct; preorbital carina visable , postorbital carinae absent ; scrobe not deep, glazy, nearly reaching front ocellus; scape length equal 0.33 of flagellum ; malar speace expanse above the temple; epistomal groove sides indeterminate ;clypeus punctuate producing hair numerous enough impressed; geno temporal margin incomplete prominent, ;antennae blackish, pedicel more wide ; flagellum cylindrical, length equal 0.8 of head breadth, in male thicker; first to third funicle segments elongate, 2nd square; 4th-6th segment slightly wide ; 7th trifle abbreviate; club black brownish ,equal 1.7 times of preceding segment .

Thorax: Higher than abdomen and head ;curved dorsally ; mobilized and expanded ; short coarse areas and other shiny and reticulate ;from dorsal with regular shallow and dense punctures ; pronotum angled with distinct produced lateral carinae, interrupted medially with upper edge, looks like planoconcave; parapsidal furrows marked; scutum width larger than length; scutellum semi circular with long silver hairs, high laterally, length equal width , edges not crenulated ,apical edge complete; metathorax protuberant laterally ; propodeum glazy and coarsely sculptured ;tegulae yellow and triangular shape ; wings hyaline, fore wing with marginal vein less half submarginal vein, and increase twice postmarginal veins; stigma equal one-third of postmarginal vein; hind legs strong ; hind coxae black and glazy, with fine punctures ventrally ; trochanters reddish; hind femur enlarged, width equal three- fifth length, black with small yellow space near tibiae ; clear with dense and finely punctured, ventral margin with ten black triangular teeth, different in thickness and interfaces, first lateral significantly greater, last four apical teeth very close to each other; hind tibia yellowish ,equal femur in length; tarsi yellow with trifle brown, five tarsomeres and last long , ended with brown black claws.

Abdomen: Short , ovate, curved , less thorax length , with pointed end ,1st tergite, large , shiny, finely and densely pitted, carinate laterally and bristle; 2nd

tergite finely and coarsely punctured, sculpture with bristles at lateral or piliferous ; tergites (3-6) coarsely punctured and bristled at upper half, sternite pitted producing hair; ovipositor sheath short , invisible from top ; male abdomen reddish laterally, male genitalia yellow with taper end.

Specimens examined: ♀,Cairo , 18.10.2014; ♀**,** Giza , 29.10. 2014**;** ♂, Giza, 5.12.2014 on halfa **Geographical zone:** Lower Egypt

Distribution: Somalia **(Masi ,1929).**

Figure (11): ***Brachymeria somalica*** **Masi(1929) ♂ and♀.**
a. Dorsal♀ b. Ventral♂ c. Lateral♀

2.12. ***Brachymeria vicina*** **Walker, 1834 (*Chalcis obtusata* Foerster, 1859) (Figure, 12):**

Body: Black**,** length 4-5 mm, shiny, punctuate and hairy.

Head: Heart shape; ocelli yellowish; distance between each compound eye and lateral ocellus equal distance between median and lateral ocellus; eyes brown; fronto-genal suture distinct; pre and postorbital carinae existent; malar area triangular shape,; scrobe smooth; interantennal projection elongate; mandible red brownish with black teeth; antennae black brown, short, thick and width increasing at apex; pedicel brownish and rounded; flagellum with short soft hairs.

Thorax: Black ; shiny, shallow densely and coarsely pitted .Pronotum scapular; scutellum flatten and slopin posteriory ;metanotum lateral blunted ; propodeum coarsely sculptured; tegula yellow and triangular shaped ; wings hyaline; in forewing submarginal vein equal more two times of marginal vein, marginal vein length equal twice of postmarginal vein ;legs black brownish and yellow ; hind coxae large with densely punctured and silver hairs; hind femora elongate , shiny , with one big reddish patch, followed by apical yellowish ring ; length equal one and half times of broad ,with 12 black teeth closed apically; hind tibiae arched ;black with different spots, tarsi 6 segments , white red ; claw and arolium black brownish .

Abdomen: Oval and elongate; 1st tergite smooth ; 2nd tergite smooth with fine punctures and bristles laterally; tergites 3-5 punctured with bristles apically; 6th tergite with coarse dense punctures and covered with bristles; 7th tergite compressed laterally and with coarsely punctured .

Specimens examined: ♀, Giza ,24.10.2013**;** ♀**,**Ismailia , 10.10.2018.

Geographical zone.: Lower Egypt.

Distribution : Austria, Europe , Iran, Iraq, Japan, Moldova, North Africa , Palestine, Turkey and Turkmenistan.

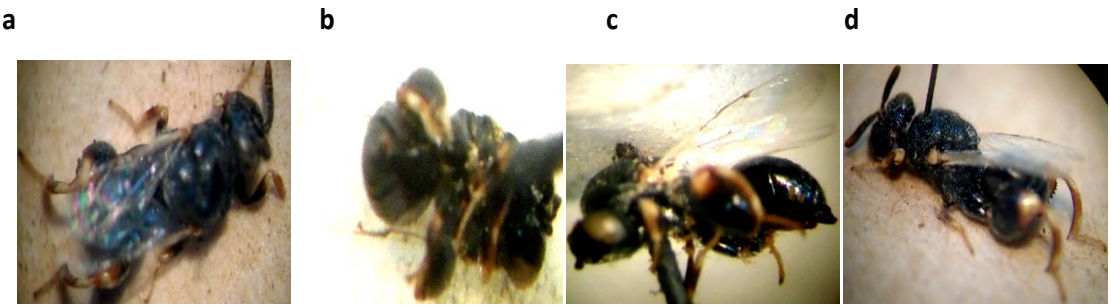


Figure (12): *Brachymeria vicina* Walker, 1834 ♀.
a. Dorsal **b. Ventral** **c and d. Lateral**

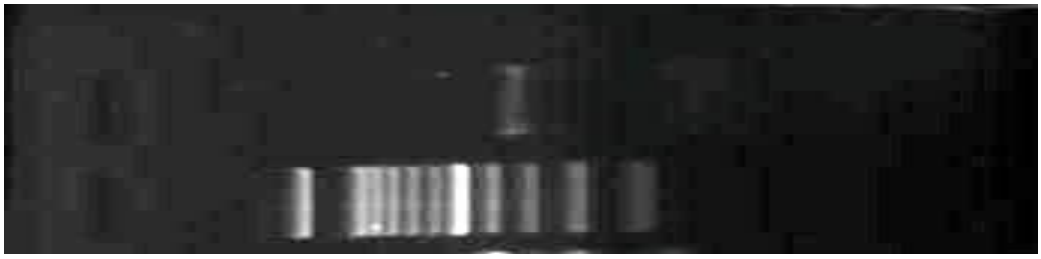
Remarks:

B. ancilla Masi with weak elongate posterior femur, provided with a small long and acute inner tooth; nearly the whole basal two-thirds of the femora red; hind tibia some parts yellow and middle part brownish red**.** *B. femorata* Panzer and *B. minuta* Linnaeus is a very divers or extremely variable species with variations in colouration of hind femora in , the nature of punctuation on the thorax and size especially *B. minuta* and are known as a common species in Mediterranean region as pupal parasitoid of various Lepidoptera. *B. kassalensis* Kirby features in having ,a tooth (not very prominent) on the inner ventral side of hind coxa in female ; the lateral ridges of scrobe only faintly produced in front of the annal toruli ; frist tergit shagreened ; abdomen of female trifle longer than pronotum, mesosctum and scutum combined seems to be related to *B. albicrus* Klug, but *B. kassalensis* differs from *B. albicrus* in having the postorbital carina present and lateral ridges of scrobe distinctly produced in front of the antennal toruli. *B. libyca* Masi claws of the first pair of legs differ from those of the *B.minute* to have the distal tooth obtuse at the apex, and not truncated, and the other teeth, in number of three acute instead of four. *B. minuta* Linnaeus is very close resemblance with *B. fonscolombei* in structure especially in femora, The typical form with reddish femora is easily recognizable, but the hind femora sometimes turn into black even in the female, being very often black in the male. in this case the identification is more or less difficult, but *B. fonscolombei* Dufour hind femora narrow, more than or at least 1.80-2.00 times as long as wide, with apical patch; hind tibia red, with subbasal and apical patches. In typicaly form this apical patch of the hind femora and the subbasal and apical patches of the hind tibia are whitish in *B. minuta* hind femora not more than 1.80 times as long as wide, mostly black, apical yellow; hind tibia mostly black. Apical and prebasal part yellow or brownish-yellow. *B. minuta* , *B. fonsclombei* and *B. vicin* presence of only two teeth in each mandible, the antennal flagellum fusiform, ; the posterior femur with tubercle on the inner side, at the base; the median dorsal part of the fourth targite smooth. Right mandible of *B. excarinara* Gahan with three teeth at apex; fronto-genal sutures completely carinate; genae flat below compound eyes; basal area of hind tibia black.

3.Genetics study and discussion:

The DNA extraction of *Brachymeria* parasitoid in Egypt was diagnostic by PCR in detecting and identifying parasitoids. The DNA sequences of *B. femorata* was obtained included 327 nucleotide (Figure,13) . The amino acid Guanine was the most amino acid found and followed by Adenine, Cytosine and Thymine, respectively. The DNA appeared with amino acid Guanine and ended with amino acid Adenine. The amino acid was arrangement within the DNA in the form of bundles and consisting of 10 amino acids. The information of the sequences was used of congeneric species in GenBank to made relative evaluation of these data. The DNA sequences of *B. femorata* were similar to the available corresponding sequences of congeneric species and the sequence similarity was 99% with *Brachymeria* sp. alignments .The DNA sequences of *Cheiropachus quadrum* , *eupelmus* sp., *Platynocheilus cuprifrons*, *Nasonia vitripennis*, *Epitranus* sp., *Neochrysocharis formosa, Nasonia vitripennis* and *Pteromalus* sp. were also some different (98% sequence similarity) (Table,1) , According Greenstone (2006) determined that the problem of insect parasitism rates was due to the small size and difficult of distinguishing morphological characters for many parasitoid taxa.To solve this problem, entomologists have employed one of four general methods to detect parasitoid protein or nucleic acid markers: serological assay; random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR). Traugott *et al.* (2006) establised the parasitoids key of lepidopteran pests by multiplex PCR. The use of diagnostic polymerase chain reaction (PCR) avaluable approach to study the host–parasitoid interactions. The inherent problems of rearing parasitoids from the collected hosts was a new idea to identify parasitoid and hosts by molecular markers. The useful identify of the host based on multiplex PCR , and screening of field-collected caterpillars. Also they found the *Pl. xylostella*, *P. brassicae*, and P. *rapae* parasitism rates of 33.4% by *D. semiclausum*, 52% by *C. glomerata*, and 53.4% by *C. rubecula*, respectively. Gariepy *et al.* (2007) recorded that the PCR-based techniques today with applications in medical, veterinary, forensic and botanical sciences. Molecular techniques had generally used for insect identification and systematic; however, PCR-based techniques were increasingly becoming recognized as valuable tools in ecological studies. Munro *et al.* (2011) studied a molecular phylogeny of the Chalcidoidea (Hymenoptera). Either Mymarommatoidea or Diaprioidea were the sister group of Chalcidoidea depending on the analysis. Likelihood analyses place Rotoitidae as the sister group of the remaining Chalcidoidea after Mymaridae. Jenkins *et al.* (2012) use molecular techniques for the detection and differentiation of host and parasitoid species of the implications for fruit fly management, Parasitoid rate and identification was a necessary step in the

development and implementation of fruit fly in biological control strategies which employing parasitoid augmentive release. Molecular techniques was also considerable advantage over traditional morphological methods of fruit fly and parasitoid discrimination as well as within-host parasitoid identification



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  1 GAACTCTGTA CGCGAGTCAT TGGGACTTGT TATAAGCCTA AAGGCGCAAT GAAAGTGAAG GTCGGCCATA 70
 71 GCGTCGACCG AGGGAGGATG TCTCGCGTCA CGATGCGAGT CCGCACTCCC GGGGCGTCTC GTGCTCATTG 140
141 CGAGCAGAGG CGCACCTAGA GCGTACACGT TGGGACCCGA AAGATGGTGA ACTATGCCTG GTCAGGACGA 210
211 AGTCAGGGGA AACCCTGATG GAGGTCCGTA GCGATTCTGA CGTGCAAATC GATCGTCGGA ACTGGGTATA 280
            281 GGGGCGAAAG ACTAATCGAA CCATCTAGTA GCTGTTCCCT TCCGAAA 327
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Figure (13): Polymerase chain reaction and Sequence data of *Brachymeria femorata*.

Table (1): Sequences producing significant alignments in Egypt.

Description	Max score	Total score	Query caver	Ident	E value	Accession
Brachymeria sp.	520 - 503	520 - 503	100%	99%	8e-144	JN623581.1
Cheiropachus quadrum	501	501	100%	98%	3e-138	JN624260.1
Epitranus sp.	496	496	100%	98%	1e-136	JN623602.1
Eupelmus sp.	501	501	100%	98%	3e-138	AY599307.1
Nasonia vitripennis	496	496	100%	98%	1e-136	JN623821.1
Neochrysocharis formosa	496	496	100%	98%	1e-136	HM364979.1
Platynocheilus cuprifrons	496	496	100%	98%	1e-136	JN623838.1
Pteromalus sp.	492	492	98%	98%	2e-135	AY552170.1

It is concluded that many species of this genus spread in different places in Egypt and are mostly primary parasitoids in pupae of holometabolous insects, especially of Lepidoptera, but some species attack Diptera, Which contain many of the pests that are harmful to humans, Therefore the precise determination species concerned is highly important in any host –parasite study for biological control. The previous studies cleared that the molecular diagnostic tools had earned their place in taxonomy and biological control research. The last few years had seen a tremendous increase in the number of studies by using diagnostic molecular markers for parasitoid. the diagnostic molecular markers had been used to identify morphologically similar parasitoid species.

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Formulation of 2-methyl-3,1-(4H)-benzoxazin-4-one and evaluation its antifungal activity against some pathogenic fungi

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Abstrcat:

Methyl benzoxazineone was formulated as wettable powder formulation (54% WP). The new local formulation passed successfully all physico-chemical properties of wettable powder formulation. The antifungal activity of both active ingredient and its local formulation was evaluated under laboratory conditions. There are a regration relationship was found between tested concentration of active ingredient and their percentages of inhibition against *Sclerotium rolfsii* Sacc. whereas this indication was not found in case of *Rhizoctonia solani* Kühn. On contrast the above relationship was found with both tested fungi in case of local formulation. Depending on EC_{50} values the effectiveness of active ingredient increased by 37.6% and 100% in case of *Sclerotium rolfsii* and *Rhizoctonia solani* respectively as resulting of formulation.

Introduction

Fungi can grow in almost all habitats, including soil, air, seas, rivers, as well as on organic matter, including food, and other organisms, such as plants, animals, and even human skin (Jampilek, 2016). Many fungal genera including *Fusarium*, *Alternaria*, *Botrytis*, *Helminthosporium*, *Penicillium*, and *Rhizoctonia* have proved harmful pathogenic fungi and cause huge loss of crop yield world-wide (Boyraz and Ozcan, 2006). In Pakistan, all major crops are frequently infected by fungal plan pathogens and cause loss of yield in quality and quantity. Among these diseases, *Rhizoctonia* black scurf and stem canker caused by the fungus *Rhizoctonia solani* Kühn are a severe problem in all potato producing zones of the country (Sneh *et al.*, 1991; Ahmad *et al.*, 1995 and Khan *et al.*, 1995).

Sclerotium rolfsii Sacc. is a soil-borne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species including almost all the agricultural and horticultural crops (Aycock, 1966;

Domsch *et al.*, 1980 and Farr *et al.*, 1989).

Application of fungicides is the most convenient and predominant way for disease control. Their use has made it feasible to enhance crop yields and food production. The efficacy of fungicides is influenced by many biological and environmental factors that directly influence the metabolic activities of fungal cells (Reinprecht, 2010). Sometimes critical concentrations are not effective long-term, as the fungus can become resistant to the fungicide (Neely, 1969 and Brent and Hollomon, 2007). Therefore, it has become an important issue to find alternative control strategies are effective as synthetic pesticides (Javed *et al.*, 2006).

Infectious diseases caused by bacteria and fungi affect millions of people world-wide. Concerted and systematic progresses to discover and develop new antibiotics are always done due to the development of resistance by the microorganisms to the drugs commonly used against them. The rapid rise in bacterial resistance to the traditional antibiotics such as penicillins and tetracyclines had encouraged a continuing search for new classes of compounds with novel modes of antibacterial activity. Quinazolines are considered a very important class of compounds that show a diversity of activities, most prominent of which are antimicrobial and antifungal (Grover and Kini ,2006 ; Girija and Hemalatha, 2010 and Bartroli *et al.*, 1998).

2-methyl-3,1-(4H)-benzoxazin-4-one (Figure,1) was used for the synthesis of quinazolinone derivatives. Quinazolinone and its derivatives are inhibitory to several fungal pathogens of plants, including *Helminthosporium turcicum, Stagonospora nodorum, Microdochium nivale, Fusarium moniliforme, Fusarium culmorum, Gaeumannomyces graminis* and some isolates of *G. graminis, F. culmorum* and *F. moniliforme* (Friebe *et al.*,1998).

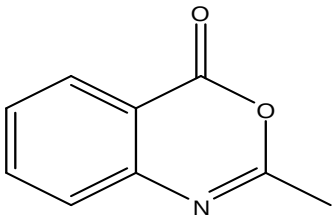

Figure (1): Structure of 2-methyl-3,1-(4H)-benzoxazin-4-one.

Quinazoline derivatives are a class of chemical compounds that have been proved to have antimicrobial activity. The biological activities of quinazoline derivatives such as antitumor, anti-inflammatory, anti-HIV, antihypertensive, anthelminitic and antituberclusis activity and the derivatives of 2-methyl-3- amino-quinazoline-4(3H)-one were synthesized. The antimicrobial activity has been evaluated by micro dilution method. The antifungal activity of the compounds was quite lower than their antibacterial activity (Rezai *et al.*, 2012).

4H-3,1-benzoxazin-4-ones have attracted considerable attention as inhibitors of serine proteases by enzyme acylation due to the nucleophilic attack of the active site serine on the lactone carbon (Gilmore *et al*., 1996 and Gutschow and Neumann, 1997). Benzoxazinone derivatives are also used as antiphlogistic drugs, antifungal and antibacterial agent (Segarra *et al.*, 1998). If a vinyl or phosphate functional group is connected to an aromatic ring located at the position two of the heterocycle, the resulting compounds possess antimuscular contraction properties and can be used as a hypnotic drug. This special reactivity allows this class of compounds to be useful as antimicrobial (Mathew *et al.*, 2010), anti-platelet aggregation (Pritchard *et al.*, 2007),

human leukocyte elastase inhibitors (Pei-Wen Hesieh *et al.*, 2005 and Arcadi *et al.*, 1999), receptor antagonist active (Ward *et al.*, 2007 and Bromidge *et al*., 2009), pesticides (Shakil *et al*., 2010), tissue culture protective and *in vivo* pharmaceuticals 2011, 41033 model of neurodegeneration (Wang *et al.*, 2010) and improve the umbilical vein endothelial cells (Dong *et al.*, 2010).

The successful use of any active ingredient depends on its correct formulation into a preparation which can be applied for crop protection with safety to those applying materials to animal life and to the environment. In general formulation plays an important role in spread over a very large area. Also, it facilitates penetration of the active ingredient to reach its target and achieve its action (El-Kady *et al.*, 2010).

The aim of this is study: 1- Evaluation of fungicidal activity of (methyl benzaxazineone) as active ingredient. 2- Formulation of (methyl benzaxazineone) in suitable formulation form and evaluation their fungicidal activity.

Material and methods

1. Tested chemicals:

1.1. Fine chemicals:

1.1.1. Acetic anhydrid (1), molar mass 122.12 g/ mol^{-1}.

1.1.2. Anthranilic acid (2-amino benzoic acid) (2), molar mass 137.14 g/ mol^{-1} were purchased from Obour Pharmaceutical Industrial Company.

1.2. Surface active agents: Tween 80, polyethylene glycol 600 diolate (PEG 600 diolate), Toximol, Toximol H and Toximol R were supplied by EL-Gomhoria Co., Cairo, Egypt.

1.3. Solvents: Xylene, acetone, absolute ethanol, DMSO (dimethyl sulfoxide) and DMF (dimethyl formamide) were supplied by EL-Gomhoria Co., Cairo, Egypt.

2. Physico-chemical properties of the basic formulation ingredients:

2.1. Physico-chemical properties of active ingredient:

2.1.1. Solubility: It was determined by measuring the volume of distilled water, acetone, xylene, DMSO and DMF for complete solubility or miscibility of one gram of active ingredient at 20 ^{o}C (Nelson and Fiero, 1954). The % solubility was calculated according to the following equation:

$$\% \text{ solubility} = W/V * 100$$

W: active ingredient weight

V: volume of solvent required for complete solubility.

2.1.2. Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

2.1.3. Melting point: It was determined by using electro thermal melting point apparatus 9200A.

3. Surface active agents:

3.1. Solubility: It was determined as mentioned before.

3.2. Free acidity or alkalinity: It was determined according to World Health Organization (WHO) (1979).

3.3. Hydrophilic-Lipophilic balance (HLB): The solubility of surfactant in water was considered as approximate guide to their HLB and usefulness **(**Lynch and Griffin, 1974).

3.4. Critical micelle concentration (CMC): The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentrations, (CMC) of the tested surfactants was determined according to Osipow (1964).

3.5. Surface tension: It was determined by using Cole- Parmer surface tension 21 for solutions containing 0.5 % (W/V) surfactant according to ASTM- 1331 (2001).

4. Carriers: Aswanly clay:

4.1. Free acidity or alkalinity: It was determined according to **WHO specification (1979)**.

4.2. Wettability: It was determined according to CIPAC MT 53.3 (2002).

5. Bulk density: This property was determined according to CIPAC MT 33 (2002).

6. Preparation of methyl benzoxazinone as wettable powder (WP).

This type of formulation is suitable for the active ingredients that did not soluble in water or xylene; several trials were carried out as follow: Different weights from active ingredient were added to other different weights from carrier then mixed together to make a homogenous powder and wetting or dispersing agent single or mixed together with different percentages was added to the mixtures and stirred well using glass rod to ensure homogeneity. After drying, the mixtures were sieving through 590 my sieve to ensure that all particles have the same size. Suspensibilty test was carried out according to CIPAC MT 185 (2002) for all prepared formulations to judge on the success of formulation.

7. Determination of the physico-chemical properties of the local formulated wettable powder:

7.1. Suspensibility: It was determined according to CIPAC MT 184 (2002).

7.2. Wattability: It was determined according to CIPAC MT 53.3 (2002).

7.3. Free acidity or alkalinity: It was determined according to CIPAC MT 31.1 (2002).

8. Determination the physico-chemical properties of the spray solution of the local formulation at the field dilution rate:

8.1. Surface tension: It was determined by using Cole- Parmer surface tensiometer 21, where dyne/cm is the unit of surface tension measurements.

8.2. Viscosity: It was determined by using Brookfield viscometer model DV Π + Pro, where centipoise is the unit of measuring viscosity according to ASTM D- 2196 (2005).

8.3. PH value: It was determined by using Cole-Parmer pH Conductivity meter 1484-44.

8.4. Electrical conductivity: It was determined by using Cole-Parmer pH Conductivity meter 1484-44, where μ/mohs is the unit of electrical conductivity measurement according to Dobrat and Martijn (1995).

9. Fungal strains used:

Pure cultures of *Sclerotium rolfsii* and *Rhizoctonia solani* were supplied from the department of Fungicides, Bactericides and Nematicides, Central Agricultural Pesticides Laboratory (CAPL), Agricultural Research Center (A. R. C.)

10. Effect of active ingredient (methyl benzoxazineone) and its wettable powder formulation on pathogenic fungi:

Antifungal activity of active ingredient (methyl benzoxazineone) and its formulated form (WP 54%) were determined by food poisoned technique (Mohanty *et al.*, 2012). Active ingredient was dissolved in DMSO at concentration 33.3%. Both active ingredient and its wettable powder formulation 54% were added separately to get the required concentrations. The tested concentrations were mixed with 50ml of sterilized PDA medium and transferred equally into three Petri dishes. The media could solidify. Then seven-day old fungal culture disk of 5-mm diameter was taken and inoculated to the center of Petri dishes containing active ingredient (methyl benzoxazineone) and formulated form WP in separate manner. Instead of PDA medium without

active ingredient (methyl benzoxazineone) and formulated form served as control. All dishes were incubated at 27±2°C and radial growth of colony was measured when the mycelia of control had almost filled the Petri dishes. Each test was performed in triplicate.

The fungal growth inhibition which was calculated due to treatment against control using the following formulaaccrding to Satya *et al.* (2014):

Inhibition of growth (%) = R-r/R *100

R is the radial growth of fungal mycelia in the control plate.

r is the radial growth of fungal mycelia in the treated plate.

11. Statistical analysis:

The concentration inhibition regression lines were drawn according to the method of Finney (1971).

Results and discussion

1.Physico-chemical properties of 2-methyl-3,1-(4H)-benzoxazin-4-one as active ingredient:

Data in Table (1) showed that, 2-methyl-3,1-(4H)-benzoxazin-4-one was medium soluble in DMF and DMSO (25 and 33.3%) consequently but completely insoluble in Water, Acetone and xylene. It showed acidic property which appeared from its free acidity (19.6). Taking these results into account, it could be prepared as wettable powder and needs acidic surface-active agents for complete compatibility.

Table (1): Physico-chemical properties of 2-methyl-3,1-(4H)-benzoxazin-4-one as active ingredient.

Solubility % (W/V)					Free acidity as % H_2SO_4
Water	Acetone	Xylene	DMSO	DMF	
N. S	N. S	N. S	33.3	25	19.6

N.S means insoluble.

2.Physico-chemical properties of surface-active agents.

Data in Table (2) showed the physico-chemical properties of toximol, toximol R, toximol H, toximol 500 and Tween 80 as surface active agents. All toximols showed the slight changes in surface tension values, their values were between 36 to 39.2 dyne/cm while for Tween 80 and sds it was 39.2 and 31. Also all the tested surfactants had the same hydrophilic-lipophilic balance, except sds. Also, there was no differences between toximol and sds in CMC values that showed 0.3 while Tween 80 showed 0.5 %. On the other hand for the free acidity or alkalinity, all tested surfactant showed acidic property, Tween 80 showed the highest value (0.61), followed by Toximol R (0.49), toximol 500 (0.39) then toximol H (0.2) at the finally toximol has lowest value (0.03). Depending on the values of free acidity for the five surface active agents, any of them can be used for formulating this active ingredient in the form of wettable powder, but the main factor that determined the best surfactant for this formula was their stability and compatibility with the required properties of required formulation.

Data in Table (3) showed that physico-chemical properties of aswanly clay as carriers were 7.86 wettabillity per second, 0.87 density and 0.8 bulk density.

Table (2):Physico-chemical properties of the tested surface-active agents.

Surface active agent	Surface tension dyne/cm	HLB	CMC %	Free acidity as % H_2SO_4	Free alkalinity as NaOH
Toximol	36	8-10	0.3	0.03	-
Toximol R	37.02	8-10	0.3	0.49	-
Toximol H	39.2	8-10	0.3	0.2	-
Toximol 500	36	8-10	0.3	0.39	-
Tween 80	39.2	8-10	0.5	0.61	-
Sds	31	>13	0.3	-	0.026

Table (3):Physico-chemical properties of carriers.

	Wettabillity Second	Density	Bulk density
Aswanly clay	7.86	0.87	0.8

3.Physico-chemical properties of the local % wettable powder formulation before and after accelerated storage.

Table (4) show that physico-chemical properties of the 54 % Wettable powder formulation before and after accelerated storage (50 ±3 °C for three days). All physico-chemical properties of the formulation did not show any valuable changes, it showed acidic property before and after storage by relatively close values; in addition, it was completely suspensibility in both cases. Generally, there were no effective changes in the physico-chemical properties of the new formula before and after accelerated storage.

Table (4): Physico-chemical properties of local formulation before and after accelerated storage.

Before storage			Cold storage	After storage		
Suspensibility		Free acidity as H_2SO_4		Suspensibility		Free acidity as H_2SO_4
Hard water	Soft water			Hard water	Soft water	
100%	100%	21.56	pass	100%	100%	22.54

4.Physico-chemical properties of spray solution at field dilution rate.

The biological activity of a pesticide to the target pest species is greatly influenced by its physical and chemical properties. The physical properties of a pesticide determine the pesticide mode of action, dosage, mode of application and the subsequent environmental chemodynamics. The physical properties of pesticides vary greatly according to their chemical nature and formulation. The spray solution showed a decrease in surface tension and pH, while an increase in electrical conductivity and viscosity was observed (Table,5) . Decreasing in surface tension of spray solution cause improving in wettability and spreading on the treated surface then increasing deposit and activity of pesticide (Osipow, 1964). The decrease in PH value with increasing electrical conductivity can result in an increase in pesticide efficacy according to Tawfik and El-Sisi (1987) who stated that, retention and effectiveness of pesticides spray solution increased with decreasing in pH values with increasing its conductivity. The relation between increasing viscosity and increasing the pesticidal efficiency could be explained according to Richardson (1974) who reported that, increasing viscosity of spray solution caused a reduction in drift and an increase in the retention and sticking of spray solution on the surface of plant.

Table (5): Physico-chemical properties of spray solution at field dilution rate 0.5%.

Viscosity centipoises	Electrical conductivity µ/mhos	PH	Surface tension dyne/cm
12.72	720	5.05	39.5

5.Effect of active ingredient (methyl benzoxazineone) on pathogenic fungi:

Data in Table (6) indicated that there are a regration relationship was found between tested concentration of (methyl benzoxazineone) and their inhibition effect against *S. roffsii*. On contrast no inhibition effect was found with all tested concentration in case of *R. solani.* The effect of the active ingredient may be due to it mode of action (Gilmore *et al*., 1996) told that 4H-3,1-benzoxain-4-ones have attracted considerable attention as inhibitors of serine proteases by enzyme acylation due to the nucleophilic attack of active serine on the lactone carbon. On the other hand, *R. solani* may be possessed barriers prevent the active ingredient from reach to location effect.

Table (6): Effect of active ingredient (methyl benzoxazineone) on pathogenic fungus.

Concentration of (ppm)	*Sclerotium rolfsii*		*Rhizoctonia solani*	
	Radial growth (mm)	% of inhibition	Radial growth (mm)	% of inhibition
1000	32.6	67.3	90.0	0.0
500	52.3	47.6	90.0	0.0
250	60.3	39.6	90.0	0.0
125	79.6	20.3	90.0	0.0
62.5	90.0	0.0	90.0	0.0
*Control no solution	90.0	0.0	90.0	0.0
**Control DMSO	90.0	0.0	90.0	0.0

Each number represents the mean of 3 replicates.

***Control without active ingredient (medium free of any solvent and discs were cut from the pathogen only on PDA).**

****Control DMSO (medium mixed DMSO and discs were cut from the pathogen grown on PDA).**

6. Effect of formulated (methyl benzoxazineone) 54% WP on pathogenic fungus:

Data in Table (7) indicated that there is a positive relationship were found between tested concentrations of local formulation and its inhibition percentages. On the other hand, local formulation increased the activity of active ingredient against *R. solani*. This indication may be due to the wetting and depressing agents that used in formulation.

Table (7): Effect of formulated (methyl benzoxazineone) 54% WP on pathogenic fungus.

Concentration of (ppm)	*Sclerotium rolfsii*		*Rhizoctonia solani*	
	Radial growth (mm)	% of inhibition	Radial growth (mm)	% of inhibition
2000	-	-	0.87	99.1
1500	-	-	7.6	92.4
1250	-	-	15	85
1000	26	74	55.3	44.7
500	41	59	90.0	0.0
250	52	48	90.0	0.0
125	68	32	90.0	0.0
62.5	77	23	90.0	0.0
*Control	90.0	0.0	90.0	0.0

Each number represents the mean of 3 replicates, (-): disappear

***Control without active ingredient (medium free of any solvent and discs were cut from the pathogen only on PDA).**

Data in Table (8) showed that clearly the percent of EC_{50} and EC_{90} values and slop value for the tested fungi *S. roffsii* and *R. solani*. Depending on EC_{50} values, local formulation increased the effectiveness of active ingredient against both tested fungi by 37.6% and 100%. Also *S. roffsii* was more sensitive to local formulation in case of *S. roffsii* than *R. solani.* On the other hand. the slope values showed the nearest value with both tested fungi.

Table (8): The EC_{50}, EC_{90} and slope values for *Rhizoctonia solani* and *Sclerotium rolfsii* with the active ingredient and formulated (methyl benzoxazineone) 54% WP.

Treatment	*Sclerotium rolfsii*			*Rhizoctonia solani*		
	EC_{50}	EC_{90}	Slope	EC_{50}	EC_{90}	Slope
Active ingredient	477.5	4401	1.3±1989	-	-	-
Formulation	298.1	3880	1.1499± 0.1420	1018	1390	9.4745± 1.2275
Increased effectiveness	37.6%			100%		

(-): disappear

It is concluded that methyl benzoxazineone was formulated as wettable powder formulation. Both active ingredient and local formulation were evaluated as fungicidal against *S. rolfsii and R. solani* under laboratory condition. The local formulation increased the effectiveness by 37.6% and 100% respectively.

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Population dynamics of the cabbage aphid *Brevicornae brassicae* (Hemiptera: Aphididae) infesting canola in El-Minia Governorate

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Abstract:

The present studies were carried out throughout the period from 2016-2017 to 2017-2018. The main objectives were studying population dynamics of the cabbage aphid *Brevicornae brassicae* L. (Hemiptera: Aphididae) infesting canola in Malawi, El-Minia Governorate. Data showed that the migration of aphid from the overwintering site into canola field occurred after about 26 days (nearly during the second week of December). Maximum population density of the cabbage aphid occurred after about 97 days. Therefore, the peak of abundance could expect around the end of February and the beginning of March. The population then vanished from the canola field in 122 days (toward the end of March). Also, the present results indicated that the number of cabbage aphid was significantly higher in the second season 2018 than that of first 2017. The differences in levels of infesting between the seasons might be attributed to the differences in weather factors (temperature and relative humidity) and / or the effect of the common natural enemies in each season.

Introduction

The cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), a pest on many cruciferous crops is distributed throughout all the temperate and warm temperate regions of the world. This aphid is considered one of the most damaging and consistently present pests on cabbage crops (Theunissen, 1989). *B. brassicae* causes direct damage, resulting from searching for food, which may induce plant deformation and indirect damage caused either by honeydew or by transmission of viruses. The cabbage aphid is a vector of 20 virus diseases in a large range of plants (Liu and Yue 2001; Lotfalizadeh, 2003; Ahmed, 2006; Almeida *et al*., 2007 and Ponti *et al*., 2008).

The present studies were oriented to obtain better knowledge about the cabbage aphid population infesting canola at Malawi area, El-Minia Governorate.

Materials and Methods

The present studies were carried during two successive growing seasons, 2016-2017 and 2017-2018. An area of about half feddan (2100m^2) was cultivated with canola plants (cultivar pactol). Plants were normally planted at

first half of November. Regular conventional agricultural practices were normally performed, and no chemical control was used during the study period. Weeds were removed by hand.

Regular samples consisted of 50 plants of canola were randomly collected and brought back in transparent polyethylene bag to the laboratory for counting aphid species and their natural enemies. Samples were taken weekly when the migration of aphids onto the crops from overwintering sites began and continued through the time till when aphid population and their natural enemies declined to low or undetectable levels. The number of aphids (nymphs and adults) and the associated natural enemies were counted and recorded at each inspection date.

Temperature (maximum and minimum) and relative humidity (maximum and minimum) were obtained from a meteorological station located at 100 m away from the experimental site in the field.

Results and Discussion

The population of the aphid species infesting canola plants was studied in the experimental farm of Malawi, El-Minia Governorate during 2016-2017 and 2017-2018 seasons. Data on the population densities of the cabbage aphid species expressed in terms weekly numbers / plant in Table (1). In 2016-2017 season, the changes in the population densities of *B. brassicae* on canola plants are presented in Table (1). Data indicate that the nymphs and adults of the pest were detected on canola plants in a relatively low level (5.60 aphids / plant) during the end week of January when the plants were in the bud stage. Thereafter, the population tended to increase gradually through February and first half of March. The maximum level (235.41 aphids / plant) was attained during first half of March when the plants were in the end of flowering stage. The number of aphids then showed a sharp decrease and approximately vanished from the field during meddle of April when the plants were in the end of ripening stage. Data in Table (1) showed that the seasonal abundance of the cabbage aphid during 2017- 2018 season. The aphid started to appear on canola plants in extremely low numbers (0.56 aphid / plant) during meddle of December when the canola plants were in the seedling stage. Its population reached a peak of 2510.34 aphids / plant during the third week of February when the plants were in the flowering stage. The populations continued in relatively high numbers in the next month and vanished from the field during meddle of April when the plants were in ripening stage. In general, the cabbage aphid appeared in the period lasted from the third week of December up to meddle of April with a peak number during the middle of February when the plants were in the flowering stage.

It could be generally concluded that the population of the pest appeared with a few numbers during the third week of December. In this time the plants were in the seedling stage coincided with a plant age of 59 days, temperature ranged from 4.80 to 23.38°C, relative humidity ranged from 33.00 to 100.00%. Predators and mummies were recorded in low density during this phase. The data revealed also that the population of cabbage aphid increased markedly by the progress of canola plant growth toward flowering stage and the maximum population densities of aphids occurred when the plants were in the third week of February. In this point plant age was in an average of 122 days. This period (third

week of February) however, coincided with a maximum temperature ranged from 18.08 to 20.74°C, maximum RH. ranged from 99.85 to 100.00%. These conditions seem to be the favorable range for the reproduction and multiplication of the cabbage aphid. However, the rapid increase in the population of aphid in this period might be related to suitability of the host plant. The data however showed a decline in the aphid population during the end of April. This period coincided with the end of ripening growth stage of canola plants. The prevailing maximum temperature ranged from 23.37 to 30.57°C, the relative humidity ranged from 93.42 to 100.00%, however, the number of predators and mummified aphids progressively increased to exhibit a peak as the aphid populations declined. However, the eventual decline of aphid populations later in the growing season results from a combination of rapid drop in the suitability of the crop in this time, accompanied by much alate emigration and the action of the natural enemies of aphids (Tables, 2 and 3).

The relationship between incidence of the cabbage aphid infesting canola plants and selected abiotic and biotic factors were statistically analyzed using multiple regression analysis. The selected abiotic factors were air temperatures and relative humidity; and three biotic factors i.e. plant age (in days), number of predators and mummified in relation to the population of *B. brassicae* during 2016-2017 (Table,2) and 2017-2018 (Table,3) growing seasons. The present results indicate that the number of cabbage aphid was significantly higher in the second season 2017- 2018, than that of 2016-2017 season. The differences in levels of infesting between the seasons might be attributed to the differences in weather factors (temperature, relative humidity) and / or the effect of the common natural enemies in each season.

2.1. Biotic factors

2.1.1. Plant age

Data in Tables (4 and 5) showed that plant age has a coefficient of determination of about 28.21% out of 72.40% and 22.15% out of the total efficiency 66.89%. This evidence indicated that about 28.21% and 22.15% of the variability of the infestation was due to plant age under the studied variables (7 variables). Also, Tables (4 and 5) showed that the rating sort of the plant age cams in number one.

2.1.2. Predators

Data in Tables (4 and 5) showed that predators seemed to be responsible for about 7.82% during 2017 season and 10.24 during 2018 season in the changes *B. brassica* population. Predators came in the rating sort in number four and two.

2.2. Abiotic factors

2.2.1. Air temperature and humidity

It was found that the effect of maximum and minimum temperature on the infestation of canola plants by *B. brassicae* has a coefficient of determination of about 6.24% and 11.25% out of 72.40% during 2016-2017 season and 9.32 and 5.31% out of 66.89% during 2017-2018 season of the total efficiency (7 variables). The rating sort of the maximum and minimum temperature came in number five and two (2016-2017) and three and six (2017-2018) (Tables 4 and 5).

The maximum and minimum relative humidity was found to be responsible for 9.04% and 3.62% during 2017 and 7.22 and 4.34 during 2017-2018 seasons of the variability of number of cabbage peach aphid infesting canola, respectively.

Canola is one of the newly introduced oil crops in Egypt to contribute in reducing oil shortage; especially it could be cultivated in soils affected by salinity. Rapeseed has a bright future in Egypt because of its ability to grow in the new reclaimed lands under wide soil variation as drought and salinity as revealed by some Egyptian (Kandil *et al*., 1996). The pests inhabiting canola plants in certain countries of the world i. e. India, Pakistan, USSR, China, Italy, Canada, Poland, Bulgaria, UK, Australia, Turkia, Germany, Brazil, North America, USA, Denmark, Estonia, South Africa and Egypt, illustrated that the main pests of canola plants are certain species of insects belonging to different orders (Lamb., 1989). Various authors in certain parts of the world i. e. Warner *et al*., 2000; Carcamo *et al.,* 2001; Mosiane *et al.,* 2003; Hansen, 2004 and Pontoppidan *et al*., 2005, discussed pests inhabiting canola from an economic viewpoint. The cabbage aphid is distributed throughout all the temperate and warm temperate regions of the world. This aphid was considered one of the most damaging and consistently present pests on cabbage crops (Theunissen, 1989). *B. brassicae* caused direct damage, resulting from searching for food, which may induce plant deformation (Oatman and Platner, 1969), and indirect damage caused either by honeydew or by transmission of viruses. The cabbage aphid was a vector of 20 virus diseases in a large range of plants (Chan *et al.,* 1991).

The cabbage aphid, *B. brassicae* is a polyphagous sap sucking pest of canola throughout the world causing a significant problem in the field followed by economic losses (Ahmed, 1980; Pontoppidan *et al.*, 2003 and Mohamed, 2011). Pontoppidan *et al.*, (2003) reported that cabbage aphid is specialized on cruciferous plants and constitutes a worldwide problem with a substantial negative impact on agriculture and horticulture. When aphids form dense colonies on developing flowers, yield losses of up to 70% have been reported if infestations are left untreated. They added that canola should be sown as early as practical within the sowing window to avoid both yield and oil penalties induced by a contribution of aphid population density and fluctuation: The cabbage aphid infestations can occur at two stages of canola crop cycle; during autumn / winter establishment stage and again during spring when the crop is in flowering and pudding (Aslam *et al*., 2007) studied population abundance of cabbage aphid, *B. brassicae* and mustard aphid, *L. erysimi* on Sultan Raya variety of Indian mustard, *Brassica juncea* L. in Pakistan. In Egypt, cabbage aphid, *B. brassicae* is known to be the most abundant and destructive species of aphididae on canola crop during the flowering and podding stage (Sayed and Teilep, 2013; Mahmoud and Shebl, 2014; Mahmoud and Osman, 2015 and Abu Omira, 2017). At Ismailia, the mean population of aphid demonstrated that the greatest numbers of aphid among dates of observations were 6.85 and 4.53 individuals/plant which were recorded on the 2nd week of April and on the 1st week of March, whereas, the minimum populations of aphids were 0.21 and 0.25 individuals/plant that were recorded on the 2nd week of February and on the 1st week of May (Sayed and Teilep, 2013). Mohamed (2016) reported that, *B. brassicae* seems to be the most important economic pest infesting canola as indicated by the highest value of dominance and abundance degrees (81.82 and 100%). The peak of abundance was around the end of February and the beginning of March.

Table (1): Population fluctuation of *Brevicornae brassicae* infesting canola plants, Malawi, El-Minia Governorate, 2016-2017 and 2017-2018 seasons.

Sampling date	Growth stage	Mean no individuals / plant		
		2016-2017	2017-2018	Average
Dec. 4	Seedling	0.00	0.00	0.00
11	Seedling	0.00	0.56	0.28
18	Seedling	0.00	3.24	1.62
25	Rosette	0.00	6.32	3.16
Jan. 1	Rosette	0.00	19.25	9.625
8	Rosette	0.00	75.84	37.92
15	Rosette	0.00	160.24	80.12
22	Rosette	0.00	382.64	191.32
29	Bud	5.60	632.65	319.12
Feb. 5	Bud	14.72	665.24	339.98
12	Flower	26.00	1055.26	540.63
19	Flower	28.15	2510.34	1269.24
26	Flower	73.83	1820.24	947.03
March 5	Flower	102.65	730.12	416.38
12	Flower	235.41	520.64	378.02
19	Ripening	163.49	300.25	231.87
26	Ripening	165.80	165.25	165.52
April, 2	Ripening	87.55	80.44	83.99
9	Ripening	36.87	25.00	30.93
16	Ripening	0.00	0.00	0.00
Total	---	940.07	9153.52	10093.59
Mean	---	9.31	90.68	100

Table (2): Population of Brevicornae brassicae infesting canola in relation to some factors (abiotic and biotic) Malawi, El-Minia Governorate, 2016-2017.

Sampling date	Mean no aphids / plant	Plant age (days)	Temp. (°C)			R.H. (%)			Predators	Parasitoids
			Max.	Min.	Avg.	Max.	Min.	Avg.		
Dec. 4	0.00	45	24.34	8.54	16.44	100.00	37.57	47.21	0.00	0.00
11	0.56	52	21.10	7.44	14.27	95.85	33.28	31.28	0.00	0.00
18	3.24	59	23.37	7.97	15.67	100.00	34.00	33.00	0.28	0.00
25	6.32	66	23.51	10.30	16.90	100.00	39.71	30.14	1.52	0.00
Jan. 1	19.25	73	19.35	6.61	12.98	100.00	49.57	25.21	5.17	0.00
8	75.84	80	20.40	5.55	12.97	100.00	41.57	29.21	3.40	0.00
15	160.24	87	22.38	2.74	12.56	100.00	35.57	32.21	5.06	0.00
22	382.64	94	20.08	3.90	11.99	100.00	40.28	29.85	6.40	0.00
29	632.65	101	17.70	4.98	11.34	100.00	40.42	29.78	8.39	0.00
Feb. 5	665.24	108	23.20	5.30	14.25	100.00	38.00	31.00	4.48	0.00
12	1055.26	115	28.51	9.67	19.09	100.00	33.14	33.42	3.92	0.00
19	2510.34	122	20.74	7.63	14.18	99.85	44.00	27.92	5.57	0.14
26	1820.24	129	26.65	11.12	18.89	100.00	30.28	34.85	5.84	0.30
March 5	730.12	136	27.35	9.77	18.56	98.00	25.28	36.35	1.69	2.19
12	520.64	143	29.37	11.80	20.58	95.00	27.00	34.00	1.24	4.16
19	300.25	150	28.38	10.34	19.36	95.57	23.00	36.28	0.49	13.17
26	165.25	157	29.32	11.01	20.17	88.14	19.00	34.57	0.34	18.50
April, 2	80.44	164	24.34	8.54	16.44	100.00	37.57	47.21	0.54	11.58
9	25.00	171	21.10	7.44	14.27	95.85	33.28	31.28	0.00	19.97
16	0.00	178	23.37	7.97	15.67	100.00	34.00	33.00	0.00	0.00

Table (3): Population of Brevicornae brassicae infesting canola in relation to some factors (abiotic and biotic) Malawi, El-Minia Governorate,2017-2018.

Sampling date	Mean no aphids/plant	Plant age (days)	Temp. (°C)			R.H. (%)			Predators	Parasitoids
			Max.	Min.	Avg.	Max.	Min.	Avg.		
Dec. 4	0.00	45	20.62	8.00	14.31	100.00	41.25	70.62	0.00	0.00
11	0.00	52	21.74	7.52	14.63	100.00	45.14	72.57	0.00	0.00
18	0.00	59	19.98	4.80	12.39	100.00	40.85	70.42	0.00	0.00
25	0.00	66	17.31	4.18	10.75	100.00	49.85	74.92	0.00	0.00
Jan. 1	0.00	73	18.42	3.60	11.01	100.00	43.57	71.78	1.28	1.00
8	0.00	80	18.12	1.50	9.81	99.85	36.42	68.14	2.00	1.00
15	0.00	87	19.27	3.21	11.24	100.00	33.57	66.78	2.10	1.00
22	0.00	94	20.31	5.10	12.70	100.00	43.42	71.71	2.87	1.00
29	5.60	101	20.52	5.27	12.90	99.71	33.85	66.78	0.04	0.14
Feb. 5	14.72	108	19.25	2.91	11.08	100.00	33.85	66.92	2.93	0.09
12	26.00	115	22.42	3.57	13.00	100.00	29.28	64.64	5.01	0.05
19	28.15	122	18.08	3.61	10.85	100.00	35.85	67.92	6.04	0.02
26	73.83	129	22.15	5.91	14.03	100.00	37.00	68.50	3.93	0.03
March 5	102.65	136	22.15	5.91	14.03	100.00	37.00	68.50	9.07	0.03
12	235.41	143	25.21	8.07	16.64	100.00	33.85	66.92	13.36	0.01
19	163.49	150	26.11	9.43	17.77	90.28	26.42	58.35	19.57	0.02
26	165.80	157	23.38	8.70	16.04	96.00	32.28	64.14	15.68	0.02
April, 2	87.55	164	25.68	9.24	17.46	96.14	26.42	61.28	15.41	0.01
9	36.87	171	28.40	9.78	19.09	90.57	21.85	56.21	7.08	0.04
16	0.00	178	30.57	9.71	20.14	93.42	19.00	56.21	0.00	0.00

Table (4): Multi factors affecting population of *Brevicornae brassicae* infesting canola plants during 2016-2017 growing season.

Factors			Simple correlation	Relative efficiency	Rating
Biotic	Plant age (days)		0.64	28.21	1
	Predators		٠,٦١	7.82	4
	Parasitoids		٠,٧٢	6.22	6
Abiotic	Air temp. (ºC)	Max.	0.65	6.24	5
		Min.	0.55	11.25	2
	R. H (%)	Max.	0.91	9.04	3
		Min.	0.42	3.62	7
Co-efficient			72.40		

Table (5): Multi factors affecting population of *Brevicornae brassicae* infesting canola plants during 2017-2018 growing season.

Factors			Simple correlation	Relative efficiency	Rating
Biotic	Plant age (days)		0.67	22.15	1
	Predator		٠,٦٥	10.24	2
	Parasitoids		٠,٥٥	٨,٣١	4
Abiotic	Air temp. (ºC)	Max.	0.71	9.32	3
		Min.	0.52	5.31	6
	R. H (%)	Max.	0.72	7.22	5
		Min.	0.49	4.34	7
Co-efficient			66.89		

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Insect pests and the associated natural enemies in the cultivation of canola in El-Minia Governorate

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Abstract:

The present studies were oriented during 2016-2017 and 2017-2018 growing seasons of canola plants at Malawi, El-Minia Governorate. Results indicated that the presence of 26 species of arthropods belonged to 22 families and 14 orders. From the species collected, 5 species are considered the main pests causing great damage, 4 slightly harmful, and 8 beneficial arthropods as well as unidentified species of true spiders. The identified species were listed and classified to pests, parasitoids, predators, pollinators and visitors.

Introduction

Canola is one of the newly introduced oil crops in Egypt to contribute in reducing oil shortage; especially it could be cultivated in soils affected by salinity. Rapeseed has a bright future in Egypt because of its ability to grow in the new reclaimed lands under wide soil variation as drought and salinity as revealed by some Egyptian (Kandil *et al.*, 1996). Literature review for the pests inhabiting canola plants in certain countries of the world i. e. India, Pakistan, USSR, China, Italy, Canada, Poland, Bulgaria, UK, Australia, Turkia, Germany, Barazil, North America, USA, Denmark, Estonia, South Africa and Egypt, illustrated that the main pests of canola plants were certain species of insects belonging to different orders (Lamb, 1989). Various authors in certain parts of the world i. e. Warner *et al.*, 2000; Carcamo *et al.*, 2001; Mosiane *et al.*, 2003; Hansen, 2004; Pontoppidan *et al.*, 2005 and Ahmed, 2006 discussed pests inhabiting canola from the economic point of view.

The present study aims to survey the pests and the associated natural enemies inhabiting canola plants and to determine their abundance and dominance degrees in an attempt of planning successful control programme for these pests under Malawi, El-Minia Governorate condition.

Materials and methods

The present studies were conducted at the experimental farm of Malawi, Agricultural Research Station during the period from 2016-2017 and 2017-2018 canola growing seasons. An area of about one feddan (4200 m^2) was

divided into equal plots. Each plot [1/400 of feddan (6 rows / plot)] was cultivated with canola (baktol variety) in a randomized complete block design. All recommended agricultural practices were performed, and no chemical treatments were used during the study period.

1. Survey of pests and the associated natural enemies inhabiting canola:

In order to survey the pests and the associated natural enemies inhabiting canola plants, sweep-net technique and whole plant examination were used as sampling methods.

1.1.Sweep net sampling:

The sweep-net consisted of a wooden handle 100cm in length; the rim was about 38cm in diameter and 75cm deep. Ten sweeps repeated ten times were taken weekly. Each collected sample was emptied into labeled cage and transferred to the laboratory. Specimens were anaesthetized by Chloroform and examined under stereomicroscope. Number of species and number of individuals of each species was recorded and unidentified species were kept in vials containing 75% ethyl alcohol for later identification. Samples were taken weekly and continued throughout the growing season until the end of the season. Samples were taken, whenever possible, from the same plot but never from the same plant. The number of species and the numbers of individuals each species within each sample were counted and recorded at each inspection date.

1.2. Whole plant sampling:

Weekly samples of 50 canola plants were taken early in the morning (8.00-10.00 Am) at random from the area. The number of adults of the insect's pests and associated natural enemies were carefully counted. The number of the immature stages of the insect pests and mites were counted using a binocular microscope. Inspection was made from the beginning of the vegetative stage to flowering and fruiting stage up to the end of season. Specimens of unknown spices were kept in glass vials contain 75% ethyl alcohol for later identification.

2. Statistical analysis:

Dominance (%) and abundance (%) degrees of the identified species were calculated according to the formula of Facylate (1971).

2.1. Dominant degrees (D):

$D = t/T \times 100$, where

(t) = total number of each species during the collecting period. (T) = total number of all species during the collecting period.

2.2. Abundant degrees (A):

$A = n / N \times 100$, where,

(n) = total number of samples in which each species appeared.

(N) = total number of samples taken all over the season.

Results and Discussion

1.Survey of pests and their associated predators recorded on canola plants:

Data presented in Table (1) showed a partial taxonomic list of arthropod pests and the associated natural enemies recorded by whole plant and sweeping sampling from canola plants cultivated in Malawi Agricultural Research Station during 2016-2017 and 2017-2018 growing seasons. Results indicated that the presence of 26 species of arthropods belonged to 22 families and 14 orders as well as some species of true spiders (unidentified). From the species collected, 5 species are considered as abundant pests causing great damage, 4 species are considered as pests' species

slightly harmful, 8 beneficial arthropods as well as unidentified species of true spiders and 3 species are considered as pollinators and visitors.

1.1. Pests:

Intensive and extensive observations indicated that the collected species can be classified as piercing-sucking pests, leaf feeders, and leaf miners. In general 6 orders (Orthoptera, Thysanoptera, Hemiptera, Lepidoptera, Diptera and Acari) and 11 families (Acridiidae, Gryllotalpidae, Gryllidae, Thripidae, Miridae, Pentatomodae, Aleyrodidae, Aphidadae, Cicadellidae, Noctuidae, Agromyzidae, as well as four families of Acari order (Tetranychidae) were recorded inhabiting canola plants during 2016- 2017 and 2017-2018 growing seasons. Species belonging to order Lepidoptera were collected as larvae by direct observations on the plants and presented by family Noctuidae. Two species were belonged to this order, *Agrotis ipsilon* (Rott.) and *Spodoptera littoralis* (Boisd.) (Noctuidae). Three species of order Orthoptera were recorded during the present study. These species were grasshopper, *Heteraacris littoralis* (Rumb.) and *Acrotylus insubricus* (Scopli) which pertaining to family Acridiidae. The mole cricket, *Gryllotalpa gryllotalpa* L., (Family: Gryllotalpidae) and *Gryllus domestich* (L.) (Family: Gryllidae) have no serious damage to the crop. Collected species belonging to the group of arthropods, which pierce the tissue and suck the sap of canola plants are belonging to order Hemiptera , Heteroptera and Thysanoptera as well as the two-spotted spider mite of the order Acari. The most important serious pricing sucking pests were the plant bug, *Campylomma impicta*, Stink bug, *Nezara veridula* L. (Pentatominae); whitefly, *Bemisia tabaci* (Genn.) (Aleyrodidae); cabbage aphid, *Brevicorene prassicae* L.*; green* peach aphid, *Myzus persicae* (Sulzer.) (Aphididae); the leafhopper, *Empaosica* spp. (Cicadellidae) and the onion thrips, *Thrips tabaci* (Thripidae) as well as the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Laboratory examination of the randomly collected canola leaves revealed the presence of the twospotted spider mite *T. urticae* which causes heavy infestation to the canola leaves throughout the whole growing season.

These results are in accordance with those obtained by El-Dabi (1999) and Amro (2008) who reported a taxonomic list of arthropode pests and predators recovered from some plantation. However, Ahmed (2003), Hagrass *et al*. (2008), Ghallab *et al*. (2011), Abd El-Wahab *et al*. (2012) and Gameel (2013), Metwally *et al*.(2013) reported that *B. tabaci; A. gosspyii; T. tabaci; Empoasca* spp. are the most important piercing sucking insects of cucumber crops. Two spotted spider mite, *T. urticae* was found to be as an economic pest infesting cucurbit plants (Farrag *et al*., 1982; Abou-Taka and Zahdy, 1990; El-Maghraby *et al*., 1994; Ali, 1995 and Abou El-Saad, 2015).

1.2. Natural enemies:

This group of beneficial insects included parasitoids and predators.

1.2.1. Predators:

As shown in Table (1), nine species were identified as entomophagous in addition to true spider (unidentified). They are belonging to 6 orders (Dermaptera, Hemiptera, Neuroptera, Coleoptera and Diptera as well as some of the unidentified species of true spiders. Results also indicated that these species are belonging to 6 families (Labiduridae, Anthocoridae, Chrysopidae, Coccinellidae, Staphylinidae and

Syrphidae). The green lacewing, *Chrysoperla carnea* (Stephens) ; the hover fly, *Syrphus corolla* Fabricius and the lady beetles, *C. undecimpunctata*, were the most abundant predator species. Species such as *Orius* sp., *P. alferii* and some unidentified species of true spiders were collected occasionally and in scarce numbers.

1.2.2. Parasitoids:

Two species of parasitoids were recorded and identified attacking aphid species infesting canola plants belonging to the order Hymenoptera and the family Aphidiidae namely: *Diaeretiella rapae* (Mclntoch) and *Praon necans* Mackauer.

1.2.3. Pollinators and visitors:

Among the survived insects, certain species, pertaining to the orders Diptera and Hymenoptera, were recorded and classified as visitor and pollinator insects. These include 3 species of order Diptera, belonging to three Families, Drosophilidae and Muscidae. However, *Vespa orientales* classified as a visitor to canola plants. The previously results showed that, onion thrips, *T. tabaci*, whitefly, *B. tabaci* and cotton aphid, *B. prasicae* and *M. persicae* in addition to the two-spotted spider mite, *T. urticae* are the most important piercing-sucking arthropod pests infesting canola plants. The most important pricing sucking insects, *T. tabaci, B. tabaci, B. prasicae* and *M. persicae* were recorded as common pests infesting canola plants in many parts of the world as recorded by Abd El-Kareim, 1980; Mukhamediev and Akhmedov, 1984; Omar *et al.*, 1988; Hilije *et al*., 1993; Mineo *et al*., 1994; Tonhasca *et al*., 1994; Kamel *et al*., 2000; Gameel and Sayed, 2008 and Younes *et al.*, 2010. The common spider mite, *T. urticae* was found to be as an economic pest infesting canola plants (Farrag *et al*., 1982; Perring, 1987; El-Maghraby *et al*., 1994; Ali, 1995; Kamel *et al.*, 2000 and Balkema *et al.*, 2003). The present results are generally agreeing with those of El-Maghraby *et al.* (1994); Ali (1995) and Bachatly and Sedrak (1997).

2.Dominance and abundance degrees of sucking pests and the associated predators on canola plants:

2.1. Pests:

The field studies through the period extended from2016- 2017 and 2017-2018 seasons; show that seven species were the most serious pests on canola plants. These species were: Stink bug, *N. veridula*; whitefly, *B. tabaci* ; cabbage aphid, *B.brassicae;* green peach aphid, *M. persicae*; the leafhopper, *Empaosica* spp. and the onion thrips, *T. tabaci* as well as the two-spotted spider mite, *T. urticae.* In 2017 season, data in Table (2) show that *B.brassicae*, and *M. persicae* seems to be the most important economic pests as indicated by the highest value of dominance and abundance degrees (39.97 and 36.59% and 90.00 and 85.00%). However, *T. tabaci* had the relatively high abundance degrees (80.00%) with low dominance degrees (16.99%) indicating that this species could be of economic importance if the environmental conditions changed in their favour. Meanwhile, the species of *B. tabaci. Emposica* spp. and *N. viridula* which had low values of abundance and dominance degrees (30.00 and 0.009%, respectively) is expected to be of little economic importance as it may cause a minor role as a pest in cantaloupe plantations. As for dominance and abundance degrees of aphid species infesting canola plants during 2017-2018 season. Data in Table (3) show that also *B. tabaci* seems to be the most important economic pests as indicated by the highest value of dominance and abundance degrees (98.73 and 100%). However, both *A. gossypii* and *M. persicae* had

moderately abundance degrees (70.00%) with also low dominance degrees (0.194 and 0.012%) indicating that these species could be of economic importance if the environmental conditions changed in their favor. Meanwhile, the species of *Empoasca* spp. and *T. tabaci* which had low values of abundance and dominance (50.00 and 30.0% and 0.004 and 0.189%, respectively) are expected to be of little economic importance as they may cause a minor role as pests in cantaloupe plantations.

In general, from the above-mentioned results it could be concluded that *B. tabaci* and *T. urticae* seem to be the most important economic pests infesting cantaloupe as indicated by the highest value of dominance and abundance degrees. However, the high abundance degrees of *M. persicae* and *A. gossypii* which had low dominance degrees indicate that these species could be of economic importance if the environmental conditions changed in their favour. Meanwhile, the species of *Empaoascae* and *T. tabaci* which had low values of abundance and dominance are expected to be of little economic importance as they may cause a minor role as pests in cantaloupe plantations in Mallawi, El-Minia.

2.2. Predators:

Data presented in Tables (2) and (3) showed that there are four species of predators recorded on canola plants through the period extended from 2016-2017 to 2017- 2018 seasons. These species were: lion aphid, *Chrysoperla carnea* Steph, eleven spotted lady beetle, *Coccinella undecimpunctata* (L.) , *Paederus alferii* Koch. and *Syrphus corolla* F. In 2016-2017 season, the *C. undecimpunctata* seemed to be the most important economic predator as indicated by the highest value of dominance degree (70.69%). However, high abundance degrees of *P. alferii* (80.00%) which had low dominance (7.76%), also, moderately abundance degrees (35.00%) of *C. carnea* and *S. corolla* which had low dominance degrees (10.34% and 7.76%) indicated that this species could be of a little economic importance. indicated that these species could be of a little economic importance. During 2018 season, data in Table 3 show also that the *C. undecimpunctata* seemed to be the most important predators as indicated by the relatively high value of dominance and abundance degrees (93.86% and 85.00%). However, *P. alferii* and *C. carnea* which had lower values of dominance degrees (4.24% and 1.23%) are expected to be of little economic importance.

Although the predators, *C. carnea* and *C. undecimpuncta*a seem to be the

most numerous predators recovered in this survey (Tables, 2 and 3), the lower dominance degrees of predators than those of pests indicate that the natural enemies may be subjected to unfavorable conditions, which affect their efficiency in managing pests existed in the experimental area. Modifying the environment in favor to natural enemies should be studied.

The present investigations were carried out during two successive of canola growing seasons (2016 – 2017 and 2017 – 2018). Owing to field survey studies three species of aphids were detected on canola plants. These species were: Cabbage aphid, *B. brassicae*; green peach aphid, *M. persicae* and turnip aphid, *Lypaphis erysimi* (Kalrenbach). Previous studies in Egypt and abroad showed that canola plants are subjected to attack by these aphid species (Sarwar,2013 and Ahmed , 2006). In general, data show that *B. brassicae* seems to be the most important economic pests infesting canola as indicated by the greatest value of dominance and abundance degrees. However, the high abundance degrees (79.17%) of *M. persicae* which had low dominance degrees (9.98%) indicates that this species could be of economic importance if the environmental conditions changed in their favour. Meanwhile, the species of *L. erysimi* which had low values of abundance and dominance (58.33 and 8.19%, respectively) is expected to be of little economic importance as they may cause little role as a pest in canola plantations. The cabbage aphid has become one of the three primary pests of winter-seeded canola in Egypt. Cabbage aphid pressure just prior to and during bloom aborts flower buds, deforms developing pods, and generally saps vigor from plants resulting in yield losses of up to 40 percent in untreated fields. Colonies of more than 300 aphids per raceme are common each season. These aphid species were distributed throughout all the temperate and warm temperate regions of the world. Also, were considered of the most damaging and consistently present pests on cabbage crops (Theunissen, 1989) and caused direct damage, resulting from searching for food, which may induce plant deformation (Oatman and Platner, 1969), and indirect damage caused either by honeydew or by transmission of viruses (Chan *et al.*, 1991).

Table (1): A partial taxonomic list of arthropod pests and the associated natural enemies inhabiting canola plants, Malawi, El-Minia Governorate during 2016-2017 and 2017-2018 growing seasons.

Order	Family	Scientific name	Common name	الاسم العربي	Frequency	Notes
I – Pests						
Orthoptera	Acridiidae	*Heteraacris* (*Thisoicetrus*) *littoralis* (Rumb.)	Grasshopper	نطاط البرسيم المتشابه	**	S
		Acrotylus insubricus (Scopli)		نطاط ذو الجناح الاحمر	**	S
	Gryllotalpidae	*Gryllotalpa gryllotalpa* (L.)	Mole cricket	الحفار	*	S
	Gryllidae	*Gryllus domestic*	Field cricket	صرصور الغيط	*	S
Thysanoptera	Thripidae	*Thrips tabaci* Lind.	Onion thrips	تربس البصل	***	P+S
Hemiptera-Heteroptera	Miridae	*Campylomma impicta* (Wagnar)	Plant bug	بق النبات	**	P+S
	Pentatomidae	*Nezara veridula* L.	Stink bug	البقه الخضراء	*	P+S
	Aleyrodidae	*Bemisia tabaci* (Genn.)	Whitefly	الذبابه البيضاء	***	P
	Aphidadae	*Brevicorene prassicae* L.	Cabbage aphid	من الكرنب	***	P+S
		Myzus persicae (Sulzer.)	Green peach aphid	من الخوخ الاخضر	***	P+S
	Cicadellidae	*Empoasca* discipiens Paoli.	Leaf hopper	الجاسيدز	**	P+S
Lepidoptera	Noctuidae	*Agrotis ipsilon* (Rott.)	Cut worm	الدوده القارضه	*	S
		Spodoptera littoralis (Boisd.)	Egyptian cotton leaf worm	دوده ورق القطن	*	S
Diptera	Agromyzidae	*Agromyza pussilla* Meig	Leaf miners	صانعه الانفاق	*	P
Acari	Tetranychidae	*Tetranychus urticae* Koch	Two spotted spider mite	اكاروس العنكبوت الاحمر	***	P
II – Parasitoids						
Hymenoptera	Aphidiidae	*Diaeretiella rapae* (Mclntoch)	Aphid parasitoid	*طفيليات من*	**	P
		Praon necans Mackauer				
III – Predators						
Dermaptera	Labiduridae	*Labidura riparia* Pall.	Giant earwig	ابره العجوز	*	S
Hemeptera - Heteroptera	Anthrocoridae	*Orius* sp.	Flower bug	بق الاوريس	*	P
Neuroptera	Chrysopidae	*Chrysoperla carnea* (Steph.)	Lace wing	اسدالمن	*	S
Coleoptera	Coccinellidae	*Coccinella undecimpunctata* L.	eleven-spotted lady beetle	ابو العيد١١	***	P+S
	Staphylinidae	*Paederus alferii* Koch		الرواغة	*	P+S
Diptera	Syrphidae	*Syrphus corolla* F.	Hover fly	ذبابه السرفيس	*	P+S
True spider		Unidentified species	True spider	عناكب حقيقية	*	P+S
IV – Pollinators and visitors						
Diptera	Dorsophilidae	*Drosophila* sp.	Vinegar fly	ذبابه الدروسوفلا	*	S
	Muscidae	*Musca domestica* L	House fly	الذبابه المنزليه	**	S
Hymenoptera	Vespidae	*Vespa orientales*	Oriental hornet	دبور البلح الاحمر	*	S

Frequency = * = Rare, ** = Common, *** = Abundant Notes = P = Plant sampling, S = Sweeping

Table (2): Dominance and abundance degrees of the pests and the associated predators inhabiting canola plants, Malawi, El-Minia Governorate during 2016-2017 season,

Species	Dominance		Abundance (%)
	Mean No. / plant	(%)	
Pests			
B. tabaci	186	3.71	70.00
B. brassica	2004	39.97	90.00
Empoasca spp.	103	2.05	60.00
M. persicae	1835	36.59	85.00
N. viridula	23	0.46	70.00
T. tabaci	852	16.99	80.00
T. urticae	11	0.23	3.57
Total	5014	100.00	-----
Predators			
C. carnea	12	10.34	35.00
C. undecimpunctata	82	70.69	25.00
P. alferii	13	11.21	80.00
S. corolla	9	7.76	20.00
Total	116	100.00	-----

Table (3): Dominance and abundance degrees of the pests and the associated predators inhabiting canola plants, Malawi, El-Minia Governorate during 2017-2018 season,

Species	Dominance		Abundance (%)
	Mean No. / plant	(%)	
Pests			
B. tabaci	128	1.79	55.00
B. brassica	2544	35.68	80.00
***Empoasca* spp.**	348	4.88	75.00
M. persicae	2966	41.59	85.00
N. viridula	49	0.69	55.00
T. tabaci	1084	15.20	85.00
T. urticae	12	0.17	30.00
Total	7131	100.00	-------
Predators			
C. carnea	11	1.23	35.00
C. undecimpunctata	841	93.86	85.00
P. alferii	38	4.24	65.00
S. corolla	6	0.67	15.00
Total	896	100.00	------

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Bioactivity and chemical composition of anise (*Pimpinella anisum*) on *Rhyzopertha dominica* (Coleoptera: Bostrichidae) compared to malathion.

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Abstract:

In recent years, the focus has been on using alternatives to control stored grain pests to overcome resistance to pesticides. It reduced the costs, providing good control and safe to use. Ways to protect grain storage include the use of safe alternatives such as plant products. Lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) is one of the most important insect pests of stored products in the world. The adult emergence may spend several days within the grain attacking wheat grains causing before making exit holes to emerge. This cause weight loss and reduced nutrition values. The present study was conducted in laboratory to evaluate the efficacy of anise (oil and powder) compared to malathion as a recommended insecticide for controlling stored grain pests. The tested compounds evaluated by mixing with medium assay to study the parameters of mortality %, emergence % of *R. dominica* adults, on the repellent activity, germination %, weight loss % beside the identification of chemical components of anise oil by GC/MS analysis. The results showed that anise oil and powder and malathion were effective in reducing insect infestations. The tested materials significantly increased the insect mortality % and reduced the emergence % with increasing concentrations and exposure time. Also, the weight loss % of wheat grains decreased with increasing concentrations of all tested compounds compared to control. Moreover, the tested materials showed a good repellent activity on *R. dominica* adults especially at the highest concentration. The effect of anise oil was higher than that of the powder and malathion for repellent activity. Furthermore, anise oil slight inhibited the germination percentage of wheat grains followed by anise powder. While, malathion had no effect on germination percentage compared with control, after three months post treatment. Considering the results of current study, it could be suggested that the anise oil and powder have the potentiality to be used as an alternative to chemical insecticides for protecting stored grains against *R. dominica* in the integrated pest management program.

Introduction

Storage of grains is part of the post-harvest system through which food material passes on its way from field to consumer. It is generally accepted that 5–15% of the total weight of all cereals, oil seeds, and pulses is lost after harvest (Anonymous, 1989). Cereals are the staple and nutritive food but their storage is not safe due to the attack of certain stored grain insect pests. Insect infestation alone has been noted for the causes of over 5-10% losses of stored grains in the temperate countries and 20-30% in the tropical zones (Dubey *et al*., 2008).

Lesser grain borer *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) is one of the most important insect pests of stored products in the world. Newly emerging adults may spend several days within the grain before chewing exit holes to emerge (Benhalima *et al*., 2004). Feeding by *R. dominica* larvae and adult can reduce weight by as much as 75% (Dal bello *et al*., 2001),and also reduce nutritional and aesthetic value of the grain. Moreover, the lesser grain borer reduce germination (Moino *et al.*, 1998). Controlling of stored product insect populations is primarily depended upon continued applications of insecticides. However, the implications of these are serious problems of toxic residues, health and environmental hazards, development of insect strain resistant to insecticides, increasing cost of application (Sighamony *et al*., 1986; Okonkwo and Ewete, 1999 and Dubey *et al*., 2008). Malathion-resistant phenotype has almost completely replaced the susceptible strain. Moreover, many of the stored product insects have developed resistance to the commonly used chemicals (Subramanyam and Hagstrum, 1995 and Srivastava and Singh, 2002).

The use of plant oils for controlling insect pests in stored grains is a sustainable alternative because the oils are derived from natural resources. Such oils could function as contact toxin, fumigant, repellent, antifeedant and oviposition inhibitor (Tapondjou *et al*., 2002 and Isman, 2008).

Furthermore, the use of plant materials as traditional protectants of stored products is an old practice used allover the world (Aslam *et al*., 2002). The protection of stored products generally involves mixing grain with plant-based protectants (Tapondjou *et al*., 2002 and Udo *et al*., 2011). It is an age-old practice of traditional farmers in the tropics to mix a local plant with seeds of legumes. Using plant with insecticidal properties is therefore an attractive alternative to the more explosive synthetic insecticides. Various plant byproducts have been tried recently with a good degree of success as protectants against number of stored grain insect pests (Ketoh *et al*., 2005; Hosny *et al*., 2007; Ziga *et al*., 2012 and Wanida *et al*., 2012). Therefore, the goal of this study to evaluate the efficiency of anise oil and powder compared to the recommended compound malathion against *R. dominica* with the respect to adult mortality and progeny reduction, to identify the chemical components of anise oil and also losses of grain weight, repellency and germination of wheat grains has been evaluated.

Materials and methods

1.Materials:

1.1.Tested insects:

Lesser grain borer, *R. dominica* used in this study was reared free of insecticidal contamination at 28 ± 2^{oc},

70±5±R.H.% of the laboratory of Stored Product Pests Research Department, Plant Protection Research Institute, Sakha Agricultural Research Station. The culture was maintained under the same conditions, insects were to emplace in glass jar (1000 gm) containing 500 g of sterilized wheat grain and 400-500 of *R. dominica* insects. Adult insects were left for two weeks for eggs laying in the jar and kept again at the untreated conditions in the rearing laboratory. The newly emerging adults (1-2 weeks-old) of *R. dominica* were used for experimental work.

1.2.The stored product:

Wheat grains used were Masr 1, which obtained from Sakha Agriculture Research Station Farm. The grains were used to culture *R. dominica* and to evaluate the efficacy of anise oil and powder against *R. dominica* compared to malathion insecticide.

1.3.Collection and preparation of plant powder:

The plant powders used (*Pimpinella anisum*) anise seeds were collected from local market. The target plant seeds were dried at the room temperature (25-28^{oc}), the dried seedes powdered mechanically by using an electric blender, then sieved through 300 mesh size. The resulting fine powders were maintained in tightly closed dry bags until used for the experimental work.

1.4.Plant Oil used:

The oil of anise was obtained from Hashem Brothers Company for Essential Oils and Aromatic Products (Kafr Elsohbya, Qalyoubeya, Egypt).

1.5. Analysis of anise essential oil:

The constituents of anise plant oil was analyzed by gas chromatography-mass spectrometry (GC/MS) using HP5890 system with HP column (60 meter x 0.25 millimeter, 0.25 m film thickness). Detector was flame ionization detector (FID). The mobile phase was nitrogen and hydrogen was the stationary phase. Initial temperature was 60^{oc} and maximum temperature was 250^{oc}. The injector temperature was 240^{oc}. Relative percentage amounts were calculated from peaks total area by apparatus software. The compounds were identified by matching the mass spectra data with those held in a computer library (Wiley 275 L). All steps of sample preparation, extraction and analysis procedure were carried out in the Analysis Laboratory of Hashem Brothers for Essential oils and Aromatic Products, Abdel-Moneim Riad St., Giza, Egypt.

1.6.The chemical insecticide (Malathion):

Chemical name: O,O dimethyl-S-(1,2 dicarboxy-ethyl) ethyl phosphorodithioate

The applied formulation: odorless malathion (dust 1%)

Source: Kafr El-Zayat Pesticides and Chemical Company, Egypt

2.Methods:

2.1.Toxicity activity of tested materials against *Rhizopertha dominica* adults:

Mixing with feeding medium technique was used to determine the insecticidal effects of anise oil and powder and malathion against *R. dominica*. The considerable concentrations used were (1.0, 2.0, 3.0 and 4.0%) w/w for anise oil, (0.5, 1.5, 3.0 and 5.0% w/w) for anise powder and (0.04, 0.06, 0.08 and 0.1% w/w) for malathion insecticide. These concentrations of each tested materials were separately mixed with 20 g of wheat grains and were introduced in 250 ml and the jar was shaken hand to mix the grain with all tested concentrations. The jars without any tested materials were used as

control. Each concentration and untreated control replicated three times. Twenty of newly emerged adults of *R. dominica* (1-2 weeks old) were added to each jar, the jars covered with muslin cloth and kept under laboratory conditions. Mortality counts were recorded after 7 and 15 days. All results were corrected with Abbott's formula (1925).

Data were then analyzed using Probit analysis Litchfield and Welcoxon (1949), to estimate LC50, slope value and 95% confidence limits (CL).,Toxicity index.

2.2.Biological activity of tested materials against *Rhizopertha dominica*:

The biological effect of anise (oil and powder) and malathion were evaluated after recording mortality. The desirable concentrations of anise (oil, powder) and malathion were (1.0, 2.0, 3.0 and 4.0% w/w) dissolved in acetone (0.5, 1.5, 3.0 and 5.0% w/w) and (0.04, 0.06, 0.08 and 0.1% w/w) for anise oil, marjoram powder and malathion, respectively. Each concentration was applied in three replicates and in each replicate there were 20 g of wheat grains in 250 mL glass jars. For oil the treatment was carried out by adding 1 ml of each concentration to the wheat grains, mixing well and then left in jars for suitable time until the solvent evaporated before using them in experiment. However, for anise powder and malathion dust the treatments were carried out by mixing powder and dust with wheat grains and were shaken thoroughly to ensure uniform coverage by the different treatments. The untreated treatment was used as control and was replicated three times. After that, 20 adults unsexed (1-2 week-old) of *R. dominica* were transferred to the treated wheat grains in glass jars (250 mL) and kept at 28±1^{oc} and 70±5 R.H. according to the method described by El-Lakwah *et al*. (1992). Mortality counts were recorded after 7 and 15 days. Then the adults were sieved out and discarded after twenty days. The newly adult emergence were used to calculate the reduction percentages in *R. dominica* progeny.

$$\text{Reduction \%} = \frac{\text{No. of adults emerged in control}}{\text{No. of adults emerged in treatment}} \times 100$$

2.3. Repellency activity of tested materials against *Rhizopertha dominica*:

The repellency effect of anise (oil and powder) and malathion against *R.dominica* adult was conducted using the modified apparatus according to Helen (1989). It consists of a metallic ring (6 cm diameter x 1 cm height) was placed at the center of Petri-dish (12 cm diameter x 2.5 cm height). Concentrations of anise oil, powder and malathion (1.0, 2.0, 3.0 and 4% w/w), (0.5, 1.5, 3.0 and 5.0% w/w) and (0.04, 0.06, 0.08 and 0.1% w/w) for anise oil, powder and malathion, respectively. anise oil only was dissolved in 1 ml acetone. However, the treatment was carried out by mixing the anise powder and malathion dust with wheat grains and were shaken thoroughly to ensure uniform coverage with the different treatments. The untreated treatment was used as control. The treatments and control were replicated three times. After that, ten grams of treated wheat grains were put inside the metallic ring. Twenty unsexed adults (1-2 weeks-old) of *R. dominica* were released separately at the center of the ring. The Petri dishes were covered and were kept at 28±1^{oc} and 70±5% R.H. Repellency percentage (PR) values were estimated after 6, 12, 24, 48 and 72 hours according to the following equation of Helen (1989).

% Repellency (PR):

$$P.R. = \frac{\text{No. of adults outside ring}}{\text{Total No. of adults used}} x100$$

2.4. Wheat grains weight loss:

The weight loss of wheat grains due to infestation with *Rhizopertha dominica* was determined three months post treatment by sieving the insects from the wheat grains. Three replicates were done for each treatment and control. The weight loss of wheat grains was calculated as dry weight loss according to the equation of Harris and Lindblad (1978):

$$\% = \frac{\text{initial dry weight of seeds - dry seeds weight after three months}}{\text{Initial dry weight of seeds}} x100$$

2.5. Germination test:

The germination tests for anise (oil and powder) and malathion were accomplished on wheat grains of each treatment according to Qi and Burkholder (1981), with slight modification. Sixty wheat grains of each treatment were divided into three replicates, placed in Petri-dishes containing cotton layers (instead of filter paper) soaked with tap water and covered with tissue. Grains germination percentages were recorded four days after treatment after three months post-treatment. % germination percentages were calculated

2.6. Statistical analysis:

The data were statistically analyzed according to Duncan's multiple range test (**Duncan, 1955)** using SPSS software (1995).

Results and discussion

Results obtained in Table (1) showed that malathion was the most effective agent against *R. dominica* followed by oil powder of anise plant with LC_{50} (0.053 and 0.035), (0.930 and 0.721) and (1.521 and 0.924) for malathion, oil and powder of anise plant after one and two weeks, respectively.

Table (1): Toxicity of malathion, anise oil and powder against *Rhizopertha dominica*.

Total materials	7days					15days				
	LC_{50} %	Confidence limits		S.V.	Toxicity index	LC_{50} %	Confidence limits		S.V.	Toxicity index
		Upper	Lower				Upper	Lower		
Malathion	0.053	0.0735	0.0220	1.8	100	0.035	0.0512	0.0331	2.3	100
Anise oil	0.93	0.978	0.526	1.7	5.69	0.721	0.971	0.563	1.9	4.88
Anise powder	1.521	2.751	0.643	1.3	3.48	0.924	1.211	0.533	1.2	3.62

Also, anise oil was more effective than its powder against *R.dominica*. The LC_{50} values of the tested materials were positively correlated with the time of exposure under all treatments, since the LC_{50} values after 7days were higher than this after 15days in the all treatments. Results in Table 1 were in agreement with those of Derbalah and Ahmed (2011) who found that spearmint oil and powder were effective on the mortality percentage of *Sitophilus oryzae* compared to malathion and mortality increased with increasing exposure time and concentrations with the all tested materials.Gonzalez *et al*. (2014) demonstrated that the geranium and bergamot oils had the highest effective on mortality against *T. castaneum* and *R. dominica* . Adel *et al*. (2015) demonstrated that the higher concentration of basil, fennel, and geranium essential oils achieved 100% mortality resulted in contact toxicity against S. *oryzae* and *C. maculates* adults. However, for *S. oryzae* adults only fennel oil exhibited the lowest LC_{50} followed by basil oil. Geranium oil evoked no detectable mortality of *S. oryzae* adults. Fennel oil induced the

highest mortality rate to *C. maculates* followed by geranium, and basil oils. Akunne and Ononye (2015) recorded high mean mortality of adult S. oryzae in the rice grains treated with (10g/20g) *Piper guineense* and *Citrus sinensis* powder.

1.Biological activity of tested materials against *Rhizopertha dominica*:

The results in Table (2) indicated to the differences in the mortality percentages of *R. dominica* between treatments at 7 and 15 days post treatment. Malathion was the most effective followed by oil and powder of anise plant against adult emergence of *R. dominica*.

Table (2): Effect of malation, anise oil and powder on mortality, reduction % in progeny and wheat grain loss % against *Rhizopertha dominica*.

Tested materials	Conc. w/w %	Morality		Mean no. of adult emergence	Reduction in progeny %	Weight loss of wheat grains
		7 days	15 days			
Malathion	0.04	60.0	70.0	55.0	71.0e	10.0d
	0.06	80.0	86.7	47.0	75.2d	7.1e
	0.08	86.7	93.3	32.0	83.2b	5.0f
	0.10	88.3	98.3	8.0	95.7a	2.7g
Anise oil	1.0	45.0	55.0	75.0	60.5g	14.2 c
	2.0	60.0	70.0	66.0	65.3f	10.2d
	3.0	66.7	75.0	54.0	71.6 e	8.1e
	4.0	80.0	90.0	29.0	84.7 b	4.7 f
Anise powder	0.5	41.3	50.0	91.0	52.1i	20.0 b
	1.5	50.0	61.7	80.0	57.9 h	14.0 c
	3.0	66.7	75.0	60.0	68.4f	10.3d
	5.0	75.0	83.3	36.0	81.0c	7.2e
Control				190.0		46.0 a

Moreover, malathion and anise oil were the most effective treatment on progeny of *R. dominica* followed by anise powder with % reduction values of 95.7, 84.7 and 81.0%,at the highest concentration respectively. In this respect anise oil was more effective than anise powder at all concentration levels. In addition, treatments significant reduced the weight loss with increasing the concentrations. The lowest loss of grain weight was found with the highest concentration. Results also demonstrated that malathion was the premier agent for reducing weight loss followed by anise oil and powder with % values of 2.7, 4.7and 7.2%, respectively. Generally, malathion had the highest effect on the all aspects of the present study. Several studies were conducted on the effect of essential oils on biology of stored product insects. Abo-Arab *et al*. (1998) found that *Nigella sativa* oil at level of 16 ml/kg grains completely prevented adult emergence of *S. oryzae.* Similarly, Abd El-Aziz (2011) found that the marjoram essential oil completely prevented emergence adults of *T. castaneum* and *S. oryzae*. Gamal (2016) mentioned that malathion and the tested plant oils reduced adult emergence of *Callosobruchus maculatus*. Norambuena *et al*. (2016) found that the emergence (F1) was reduced reaching maximums of 60% in the case of *S. granarius* and *S. oryzae*, and 36% in *S. zeamais* by the essential oil *Laureliopsis philippiana*.

2.Repellency activity of tested materials against *Rhizopertha dominica*:

Results shown in Table (3) cleared the repellent effect of tested materials at 6, 12, 24, 48 and 72 hours post treatment. The lowest repellent

effect values were recorded with malathion insecticide. In contrast, the repellent effect of anise oil was more effective than anise powder and malathion against *R. dominica* adults. The repellent effect for all tested materials increased with increasing concentration against *R. dominica* adults. Also, data in Table (3) showed that the tested materials exhibited repellent activity at the highest rate ranged between (96.0-100.0) and (84.0-100.0) and (30.0-44.0) percent of repellent for anise oil,powder and malathion, respectively. Results also showed that the repellent effect decreased with the increasing of exposure time. The obtained results did agree with those of Zapata and Smagghe (2010) reported the repellent activity of the elaves and bark of *Laurelia sempervirens* and *Drimys winters* against *T. castaneum*. The oils tested had a very strong repellent activity towards *T. castanneum* when tested in filter paper arena test. After 4 hrs exposure >90% repellency was achieved Lashgari *et al.* (2014) found that repellency effect was increased with increasing concentration and the highest repellency effect was belonged to the highest concentration. Essential oils of *Mentha piperita* and *Cuminum cyminum* caused 61.2 and 66.4 repellency on *T. castaneum*. Meanwhile their effect was found to be 55.2 ad 60.4% repellency on *S. oryzae* at the highest concentration. Norambuena *et al*. (2016) reported that all treatments of the oil *Laureliopsis philippiana* had a repellent effect against adults of *S. oryzae, S. zeamais,* and *S. granaries.*

Table (3): Repellent effect of the malathion, anise oil and powder against *Rhizopertha dominica.*

Materials	Conc. w/w %	% Repellency				
		Hours post treatment				
		6	12	24	48	72
Malathion	0.04	8.7 h	13.0 i	12.0 i	10.0 i	8.6 i
	0.06	22.0 g	24.0 h	26.0 h	21.0 h	20.0 h
	0.08	27.0 f	32.0 g	34.0 g	25.0 g	22.0 g
	0.10	40.0 e	44.0 f	40.0 f	32.0 f	30.0 f
Anise oil	1.0	72.0 c	78.0 d	70.0 e	61.0 e	58.0 e
	2.0	85.0 b	85.0 b	83.0 c	75.0 c	66.0 d
	3.0	100.0 a	100.0 a	95.0 b	90.0 b	77.0 c
	4.0	100.0 a	100.0 a	100.0 a	97.0a	96.0 a
Anise powder	0.5	66.0 d	73.0e	70.0 e	65.0 d	60.0 e
	1.5	73.0c	77.0 d	78.0 d	60.0 e	61.0 e
	3.0	86.0 b	86.0c	84.0 c	70.0 d	66.0 d
	5.0	100.0 a	97.0 b	97.0 b	86.0b	84.0b

3.Identification of chemical components of anise oil:

The chemical composition of essential oils extracted from anise oil (Table, 4) was determined by GC-MS. The Chromatogram profile of anise oil, *P. anisum* was showed in Figure (1), the highest components were trans-anisole (86.74%), estragole (4.08%) and methyl-chavicol (1.68%).

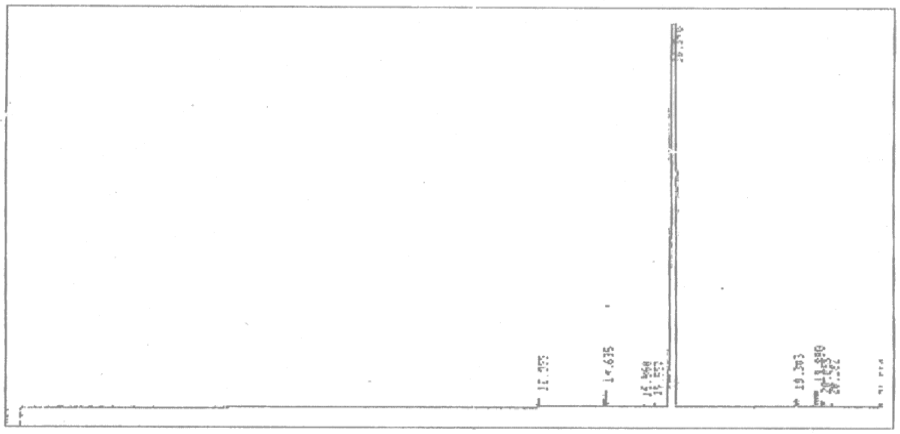

Figure (1): Chromatogram profile of anise seed (*Pimpinella anisum*) essential oil obtained by GC/MS analysis.

Table (4) : Chemical compositions of anise oil, *Pimpinella anisum*.

Main components	Component rate %	Retention time (min.)
Methyl chavicol	1.68	14.61
Trans-anisole	86.74	18.94
Estragol	4.08	25.68

Different studies established the composition of the essential oil of by GC-MS (Özcan and Chalchat (2006) found that the main constituents of *P. anisum* L oil trans-anethole (93.9%) and estragole(2.4%). Soliman *et al*. (2009) reported that the constituents of *O. majorana* L. included terpinen-4-ol (37.4%, 20.5%, 16.3%) was a major component in the summer, autumn and winter oils, resp. and α-terpinene (up to 13.3% in summer). Abd El-Aziz (2011) reported the major constituents of marjoram essential oil plant growing in Egypt are 4-terpineol (29.96%) and β-terpinene (11.34%). Sarrou *et al*. (2013) found that the constituents of *C. aurantium* included limonene (0.53%–94.67%). Ullah *et al*. (2014) reported that the constituents of *P. anisum* L.) oil was trans-anethole (82.1%) . Zarrad *et al*. (2017) found that the constituents of *C. aurantium* included limonene (87.52%).Monoterpenes have insecticidal toxicity including contact and antifeedant action on stored product insect pests. (Lee *et al*., 2003; Rozman *et al*., 2007 and Abdelgaleil *et al*., 2009). The mode of action of bioactivity natural monoterpinoids (hydrocarbons, alcohols and ketones) from spearmint oil may be due to inhibition of acetylcholinesterase. Miyazawa *et al*. (1997) reported that 1, 8-cineole was most potent inhibitor of AChE among the monoterpenes tested. This inhibition may be a mode of action for essential oil and monoterpene. The compounds may prove toxic when penetrating the insect body via the respiratory system (Shaaya *et al*., 1997 and Park *et al*., 2003).

4.Germination tested:

The effect of anise oil and powder and malathion on wheat grains germination percentages after three months post treatment was shown to understand the bioactivities of any essential oil. It is important to know the main chemical composition of the target oil in the research for example all constituents of *C. cyminum* were monoterpenes. In Table (5) the results showed that anise (oil and power) had a slight effect on germination of wheat grains while malathion exhibited non-significant effect on the germination compared to the untreated control.

Table (5): Germination of wheat grains with malation and anise oil and powder after 3 months post-treatment.

Materials	Conc. w/w g/kg	% Germination
Malathion	0.04	100.0 a
	0.06	100.0 a
	0.08	100.0 a
	0.10	100.0 a
Anise oil	1.0	90.0 e
	2.0	86.0 f
	3.0	80.0 g
	4.0	77.0 h
Anise powder	0.5	96.0 b
	1.5	96.0 b
	3.0	94.0 c
	5.0	93.0 d
Control		100.0 a

Anise oil was the highest treatment that reduced germination percentages of wheat grains followed by anise powder. The obtained results agree those of Derbalah and Ahmed (2011) who found that the efficacy of plants evaluated relative to malathion as standard compound to protect wheat against *S. oryzae*. The spearmint oil was the highest treatment that reduced the germination percentage of wheat grains followed by spearmint powder and malathion, respectively. Arya and Tiwari (2013) found that mustard oil at 2% concentration clearly reduced the wheat grains germination.

The current study demonstrated that malathion had distinctive effect on the most investigated parameters compared to anise oil and powder. Since, it achieved the highest effect against *R. dominica* with respect to adult mortality and emergence. However, malathion had many disadvantages serious hazards on human and environment. Furthermore, the development of natural insecticides (anise oil and powder) may help to reduce the negative impact of chemical insecticides malathion because of their low toxicity, no development of resistance of insect and safety to the environment, biodegradable, non-toxic un-target organism, ecofriendly, easily and many plant derived natural products acting against insects could be produced from locally available raw materials. So, the present findings suggest application of anise oil and powder as protectants against the infestation of *R. dominica* as alternatives to the chemical control of *R.dominica*.

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Aluminum and silica oxides nanoparticles as a new approach for control the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) on wheat grains

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Abstract:

Two nano-particles, silica oxide (SiO_2) and aluminium oxide (Al_2O_3) were used as stored product insect protectants compared to malathion as standard reference, by mixing with grains against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Results obtained cleared showed that malathion had the highest adverse effect on all parameters of *T. castaneum* adults. Also, the results indicated that mortality % of *T. castaneum* adults increased gradually and reduction in wheat weight loss % by increasing both concentration and exposure period. In addition, results accentuated that the two nano-particles (SiO_2 and Al_2O_3) significantly inhibited the number of progeny of *T. castaneum*. In addition to Al_2O_3 was had the most effect than SiO_2 nano-particles. SiO_2 and Al_2O_3 nano-particles were gave good result in this study. It could be concluded that use SiO_2 and Al_2O_3 nano-particles are adequate for protection stored grains as alternative method to chemical insecticides because are relatively safe for human compared to malathion. Further research is needed in order to obtain information regarding the practical effectiveness and lack of side effects of nanoparticles in protecting stored products.

Introduction

The red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is an economically important stored grain pest that has been widely used as a model organism in pesticide and ecotoxicology research (Silver *et al.*, 2014). This beetle has high reproductive potential, short life cycle, many generations per year, and is easy to rear in laboratory settings (Strobl *et al.*, 2015). Furthermore, *T. castaneum* is a globally distributed crop pest, infesting a wide variety of stored products worldwide (Opit *et al.*, 2012) with impairment of their quality and quantity (Arthur *et al.*, 2006). Stored grain insect

pests result in economic heavily losses infesting stored agricultural products. According to an estimate, stored grain insect pests are caused damage about from 10 to 40% of the annual worldwide loss (Matthews, 1993). Also, reported that stored grain insect pests because high risks to grains and seeds in storage include weight loss, less germination and reduced nutrition values of grains (Tefera *et al.*, 2011) and possible toxic effects on mammals and health hazards (Domínguez and Marrero, 2010). *T. castaneum* infestation is primarily controlled relying on the use of synthetic insecticides (Aktar *et al*., 2009), especially in countries producing large quantities of cereals for domestic consumption and export (Kim *et al*., 2015). However, the frequent and massive use of pesticides lead to some shortcomings on human health and the environment, including the development of cross- and multi-resistance in targeted insects (Isman, 2006). To avoid these drawbacks, novel eco-friendly control tools are needed (Athanassiou *et al.*, 2018). Nanoparticles help to produce new pesticides, insecticides and insect repellent (Owolade *et al*., 2008). Also, researchers believe that nanotechnology will revolutionize agriculture including pest management soon (Bhattacharyya *et al*., 2010). Although there have been numerous studies 100 nm or less (Auffan *et al*., 2009), other authors refer to NPS as colloidal particulate systems with size ranging between 10 and 1000 nm. Nanomaterials hold great promise regarding their application in plant protection and nutrition due to their size-dependent qualities, high surface to volume ratio and unique optical properties (Puoci *et al*., 2008). Young-Min *et al*. (2009) expressed that nanoparticles loaded with garlic essential oils is efficacious against *T. castaneum* (Herbst). Stadler *et al*. (2010) showed that nano-alumina could be successfully used to control stored grain pests.

The present study was to investigate the entomotoxicity of silica nanoparticles (SiO_2) and luminumoxide nanoparticles (Al_2O_3) compared to malathion against *T. castaneum* under laboratory conditions.

Materials and methods

1.Insects used:

The red flour beetle, *T. castaneum* was used in the laboratory experiments. *T. castaneum* was reared on broken wheat grains mixed with 5% dried yeast in incubator at 28 ± 1 °C and 60 ± 3 r.h. %, and L: D 10 :14 photoperiod. Unsexed adults used in the experiments were 7-14 days old.

2. Insecticides:

2.1. Malathion:

Common name: Malathion

Chemical name: O, O dimethyl 1-5 (1, 2 dicarboxyelhyl) ethylphos-phorodithioate.

Formula: $C_{10}H_{19}O_6PS_2$

The applied formulation: odorless malathion (dust 1% w/w)

Source: Kafr El-Zayat pesticides and chemical co., Egypt.

2.2. Nanoparticles:

2.2.1. Silica nanoparticles (SiO2):

Supplier: Nano Tech. Egypt for photo-electronics.

Appearance color: white

Appearance form: powder

Solubility: Dispersion into water or ethanol

Avg. Size (TEM): 40 nm

Synthesis of silica nanoparticle

A sequential method has been used, for the first time to prepare monodisperse and uniform-size silica nanoparticles using ultrasonication by sol-gel process. The silica particles were obtained by hydrolysis of tetraethyl orthosilicate

(TEOS) in ethanol medium. Rao *et al*., (2005) reported a pioneering method for the synthesis of spherical and monodisperse silica nanoparticles from aqueous alcohol solutions of silicon alkoxides in the presence of ammonia as a catalyst and different sizes of silica nanoparticles were prepared ranging from 50 nm to 10 µm with a narrow size distribution. The size of particles depends on the type of silicon alkoxide and alcohol user sized experiments were 40 nm.

2.2.2. Aluminum oxide (Al_2O_3):

Synthesis of aluminium oxide (Al_2O_3) nanoparticles, chemical routes for production of these materials include Sol-gel hydrothermal processing and control precipitation of boehmite obtained from aluminum salts, alkoxides and metallic powder. Gamma alumina nanoparticles was prepared by Sol-gel method using aluminum nitrate precursor and ammonium carbonate route posses spherical nano-sized particle (Ruihong *et al.*, 2006) user sized experiments were 10+2 nm

Supplier: Nano-tech. Egypt for photo-electronics.

Appearance color: White

Appearance (form): powder.

Solubility: Dispersed in ethanol or water

Avg. Szie (TEM): 10±2 nm

Shape (TEM): Spherical like shape.

3. Preparation of grains for experiment:

Enough quantities of broken wheat grains were firstly sieved to remove stone, dusts and insects the broken wheat grains then sterilized by heating at 70^{oc} for one hour, then the wheat grains were left to cool and reabsorb moisture. The broken wheat grains were sterilized before experiment.

4. Treat wheat grains against *Tribolium castaneum*:

Toxic effect:Different concentration of nanoparticles and malathion were admixed with broken wheat grains to determine their effect concentrations 0.3, 0.5, 1.0, 1.5, 2.0 and 2.5% w/w for nanoparticles and 0.04, 0.06, 0.08 and 0.1% w/w of malathion of each prepared concentration was added to twenty gm of treatment broken wheat grains were infested with 20 newly emerged adults (1-2 weeks old) of *T. castaneum.* Experiments were applied in jars (250 ml) with three replicates for each treatment and the untreated control. All replicates were kept at 28±1^{oc} and 70±5 R.H. for all treatment and control. Mortality percentage was recorded after one and two weeks post-treatment. All obtained results were corrected for natural mortality by using Abbott's formula (1925). And was statistically computed by Litchfield and Wilcoxon (1949) LC_{50}, confidence limit and slope value were calculated after one and two weeks post treatment.

5. Biological effect of nanoparticles and malathion:

The broken wheat grains were treated with same concentrations used with the toxic effect methods mentioned above. After two weeks post-treatment insects of *T. castaneum* was removed and the emerged adult's insects were recorded. The reduction % of emerged adults was calculated according to the method mentioned by Henderson and Tilton (1955) as the following formula:

$$\% \text{ Reduction} = \frac{\text{M.C.} - \text{M.T.}}{\text{M.C.}} \text{x} 100$$

MC = Number of adult emerging in control.

MT = Number of adult emerging in treatment

6. Weight loss %.

The weight loss of wheat grains against *T. castaneum* was determined three

months post-treatment by sieving the dusts and insects from the broken wheat grains. The weight loss of wheat grains was calculated as dry weight loss according to the following equation of **Harris and Lindblad (1978)**.

$$\%\,\text{Loss} = \frac{\text{Initial dry weight of grains} - \text{dry grains weight after 2 months}}{\text{Initial dry weight of grains}} x100$$

7. Germination tests:

The germination tests were accomplished on wheat grains of each treatment according to **Qi and Burkholder (1981)** with slight modification. Sixty seeds after 3 months post-treatment of each treatment were divided into three replicates, placed on Petri dishes containing cotton layers (instead of filter paper) soaked with tap water and covered with paper. Grain germination percentages were recorded four days after treatment of wheat grains with water after three months post-treatment according to the following equation:

Total number of tested seeds (100) /number of germination seeds x100

Results and discussion

Toxicity of silica aluminum nano-particles and malathion against *T. castaneum*. Results obtained in (Table, 1) showed that tested nano-particles materials (silica and aluminum), and malathion the mortality percentages of *T. castaneum* after treatment, mlathion was the most effective treatment against *T. castaneum* followed by aluminum and silica nano-particles with LC_{50} (0.054 and 0.036), (0.66 and 0.285) and (1.2 and 0.64) for malathion, aluminum and silica nanoparticles, after one and two weeks, respectively. The LC_{50} values of the tested materials were positively correlated with the time of exposure under all treatments. These findings agree with those Abo-Arab *et al*. (2014) and Salem *et al*. (2015). They found that the LC_{50} values for Al_2O_3 and ZnO nano-particles on adults of *T. castaneum* increased with increasing in exposure periods.

Data in Table (2) indicated that differences in the mortality percentages of *T. castaneum*, among treatments as recorded one and two weeks post-treatment, reduction of emerged adults and the loss weight of wheat grains. The mortality percentage increased with increasing the concentration and exposure time. The results showed that malathion resulted in the highest concentration 0.1% g/kg was highest mortality 93.8%. Reduction percentages of progeny were increased with increasing of concentration. The highly reduction was illustrated with concentration of 0.1w/w% g/kg for malathion 93.8%. Yet the increased concentration reduced the loss weight percentage from 7.6% at 0.04 mg/kg to 1.3% at 0.1 g/kg wheat grains for malathion compared to control 23.0%.This findings are in agreement with those Goswami *et al*. (2010), it has been revealed that the control efficacy against adult *T. castaneum* was about 80%, presumably due to the slow and persistent release of the active components from the nano-particles. (Leiderer and DeKorsy, 2008). They found that nano Al_2O_3 and amorphous nano SiO_2 were found to be highly effective and nano ZnO was moderately effective against *S. oryzae.* Also, the results in Table (3) indicated that accumulative mortality percentages of *T. castaneum*. increased gradually by increase the exposure time and the number of mortality scored higher mortality reached to 40.0% and 65.0% individuals after one and two weeks for treated with silica oxide nano-particles at the concentration of 1.5 gm/kg,

respectively. Also, results obtained manifested that the silica oxide nano-particles significantly inhibited the number of progeny and weight loss of wheat grains against *T. castaneum*.

Table (1) :Toxicity of silica aluminum nano-particles and malathion against *Tribolium castaneum.*

Total materials	One week				Two weeks			
	LC_{50} w/w%	Confidence limits		S.V.	LC_{50} g/kg	Confidence limits		S.V.
		Upper	Lower			Upper	Lower	
Malathion	0.054	0.681	0.381	3.9	0.036	0.361	0.0150	3.8
Silica nano SiO_2	1.20	1.78	1.03	1.6	0.64	0.961	0.543	1.3
Aluminum nano Al_2O_3	0.66	0.927	0.505	0.9	0.285	0.389	0.182	0.8

Table (2) : Biological activity of the malathion against *Tribolium castaneum.*

Malathion conc. w/w %	% Mortality		Mean no. of adult emergence	% reduction	% loss of wheat grains weight
	1 week	2 weeks			
0.04	36.7	60.0	95.0	48.1d	7.6b
0.06	53.3	75.0	72.0	63.1c	5.3c
0.08	66.7	91.7	32.0	83.6b	3.1d
0.1	81.3	95.0	12.0	93.8 a	1.3 e
Control			195.0		23.0 a

The highly reduction in F1 progeny was observed with concentration 2.5 g/kg for silica oxide nanoparticles 62.6%. In addition, the increased concentration reduced the weight loss percentage from 16.3 at 0.30 g/kg to 4.3 at 2.5 g/kg wheat grains compared to control 23.0%. These findings are in agreement with those of recommended for commercially available insecticidal dusts (Arthur, 2000 and 2002; Athanassiou *et al*., 2003 and 2004 and Vayias and Athanassiou, 2004). Stadler *et al*. (2010) applied successfully nano-aluminum against two stored pests. As the Al_2O_3 nano-particles gave mortality percentage at concentration 1 g/kg (95.33 $\pm$ 0.33).

Table (3) : Biological activity of the silica oxide (SiO_2) nanoparticles against *Tribolium castaneum.*

SiO_2 nanoparticles conc. w/w%	% Mortality		Mean no. of adult emergence	% reduction	% loss of wheat grains weight
	1 week	2 weeks			
0.30	6.6	13.3	121.0	37.9e	16.3b
0.50	10.3	20.0	107.0	45.1 d	13.2 c
1.000	15.0	28.7	97.0	50.3 d	11.6 d
1.50	23.3	36.7	89.0	54.4 c	9.3 e
2.00	28.7	40.0	78.0	60.0 b	7.1 f
2.50	36.7	53.3	73.0	62.6 a	4.3 g
Control			195.0		23.0 a

Data in Table (4) demonstrate the differences in the mortality percentages of *T. castaneum* among treatments as recorded one and two weeks increased gradually by increasing the concentration and exposure time of aluminum oxide (Al_2O_3) nano particles. The number of mortality scored slight mortality reached to 30.0 and 40.0 individuals after one and two weeks at the concentration of 2.5 g/kg, respectively.

Table (4) : Biological activity of the aluminum oxide (Al_2O_3) nanoparticles against *Tribolium castaneum*.

Al_2O_3 nanoparticles conc.. w/w%	% Mortality		Mean no. of adult emergence	% reduction	% loss of wheat grains weight
	1 week	2 weeks			
0.30	6.6	10.0	122.0	37.4 f	17.2 b
0.50	9.6	13.3	110.0	43.6 e	13.6 c
1.00	12.6	20.0	99.0	49.2 d	11.3 d
1.50	20.0	28.7	90.0	53.8 c	9.1 e
2.00	23.3	33.3	70.0	64.1 b	7.0 f
2.50	30.0	40.0	**62.0**	**68.2 a**	4.6 g
Control			195.0		23.0a

The results obtained manifested that the Al2O3 nanoparticles significantly inhibited the number of progeny and weight loss of wheat against T. castaneum. The highly reduction in progeny was observed with concentration 2.5 g/kg 62.0%. IN addition, the increased concentration reduced weight loss percentage from 17.2% to 4.6 % at 0.3 g/kg to 2.5 g/kg, respectively, compared to control 23.0%. Salem *et al*. (2015) found that malathion achieved the the highest effect on mortality of progeny and weight loss against *T. castaneum* compared to Al_2O_3 and ZnO nano-particles. In addition, they indicated that Al_2O_3 had higher effect than that of ZnO against *T. castaneum*.

Data in Table (5) demonstrated the effect of malathion, silica oxide nanoparticles and aluminum oxide nanoparticles on the wheat grains germination percentage after three months post-treatment, malathion and silica oxide (SiO_2) has no effect on the germination of wheat grains after three months post-treatment.

Table (5): Effect of malathion, silica oxide (SiO_2) and aluminum oxide (Al_2O_3) nanoparticles on germination.

Tested materials	Conc. w/w%	% After 3 months post-treatment
Silica nano (SiO_2)	0.3	99.0a
	0.5	100.0a
	1.0	99.0a
	1.5	98.0a
	2.0	100.0a
	2.5	100.0a
Aluminum nano (Al_2O_3)	0.3	94.0b
	0.5	92.0b
	1.0	88.0c
	1.5	80.0e
	2.0	77.0f
	2.5	76.0f
Malathion	0.04	100.0 a
	0.06	100.0 a
	0.08	99.0 a
	0.1	100.0 a
Control		100.0 a

A slight effect in germination of wheat grains with the aluminum oxide Al_2O_3 nanoparticles compared to control. Aluminum oxide nanoparticles were the

highest treatment that reduced the germination percentage of wheat grains .These results are in accordance with those of Leiderer and DeKorsy (2008). They found that nano Al_2O_3 and amorphous nano SiO_2 were found to be highly effective and nano ZnO was moderately effective against *S. oryzae*, but nano Al_2O_3 has deleterious effects on seeds, whereas non-crystalline nano-SiO_2 has no adverse effect on rice seeds. Here, we present the first report showing that nanocides, especially nano SiO_2 can be effective used to control insect pests.

Malathion had the highest effect followed by Al_2O_3 and SiO_2. The tested nanoparticles are promising and require to improve some of their physical properties. It is known that malathion formulation comprise adjuvant materials beside the active ingredient while nanoparticles does not have any additive materials, where it acts only by their natural properties. So, the present study suggests that the distinction of malathion effect may be due to the adjuvants. However, the safety of studied nanoparticles on human and the environment make it the best for the control of stored product insect pests, if compared with malathion, while cause severe hazards on human and the environment, make it the best for the control of stored product insect pests if compared with malathion which cause severe hazards on human and the environment.

The insecticidal activity of silica and aluminum oxides nano-particles against *T. castaneum* indicate the potential using of this nanoparticles as a natural source of insecticidal materials. Insecticidal activity was confirmed in nano-particles, although the results showed that silica and aluminum nano-particles varied in their effectiveness against *T. castaneum*. Malathion had the highest effect followed SiO_2 and Al_2O_3 nanoparticles. The ability of using SiO_2 and Al_2O_3 nano-particles as alternatives to the chemical control of *T. castaneum* is possible. This approach can help reducing the estimation of insecticides applied and subsequently minimize its hazards to human health and environment. Nanoparticles are promising and require improving some of their physical properties. Further research is needed to identify its mode of action and its non-target toxicity, and to determine the potential of other nano-structured materials as pest control options for insects.

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Field evaluation of methoxyfenozide and chromafenozide, ecdysone agonists against cotton leaf worm, sugar beet moth and preservation their predators

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Abstract:

Due to the significant economic losses of the sugar beet crop caused by the cotton leaf worm *Spodoptera* sp. (*littoralis* and *exigua*) and the sugar beet moth *Scrobipalpa ocellatella* (Boys.) (Lepidoptera : Gelechiidae), as well as the desire to reduce the use of traditional insecticides for their harms, this study was conducted during seasons; 2017/2018 and 2018/2019 at shenno village, Kafr El-Sheikh Governorate .The aim was to evaluate alternatives to traditional insecticides represented by five ecdysone agonists. It also assesses its role in maintaining the presence of arthropod predators associated with these pests in the field. Results showed that the tested ecdysone agonists and the tested insecticides were similar in reducing the number of *Spodoptera* sp. larvae. The all tested insecticides induced above 92% reduction in *Spodoptera* sp. larvae number in the both study seasons. As for the arthropod predators associated with *Spodoptera* sp., the maximum overall mean reduction was 31.59% and 11.57% during the first and second seasons respectively Compared to traditional insecticides (99.38% and 98.68% in 2017/2018 and 2018/2019 seasons respectively). Ascendancy reducing the number of *S. ocellatella* larvae, overall mean of reductions to all tested insecticides took the same trend. They caused above 87% reduction in *S. ocellatella* larvae numbers. Concerning the arthropod predators numbers associated with *S. ocellatella*, the maximum overall mean of reduction caused by the tested ecdysone agonists were 12.41% and 14.40% in the first and second seasons respectively. While, that recorded when using traditional insecticides 99.20%in 2017/2018 season and 99.54% in 2018/2019.

Introduction

Sugar beet *Beta vulgaris* L. (Family : Chenopodiaceae) attacks by several insect species beginning from seed germination up to harvest (Abo–Saied, 1998; Bazazo, 2005; Saleh *et al*.,2009; Bazazo, 2010; El–Dessouki, 2014; Bazazo *et al*., 2016; Khalifa 2018 and El-Dessouki, 2019). These insect

pests proved to reduce the crop quality (Sugar Percent) and quantity (roots weight per feddan) (Shalaby, 2001; Bazazo, 2010; Shalaby *et al*., 2011; Rashed, 2017 and Abbas, 2018). Lepidopteran pests of sugar beet cause severe yield reduction in most growing areas of the world (Jafari *et al*., 2009). The cotton leaf worms, *Spodoptera littoralis* Boisd and *Spodoptera exigua* Hub. (Lepidoptera: Noctuidae) and the beet moth Scrobipalpa *ocellatella* (Boys.) (Lepidoptera: Gelechiidae) are destructive insects and causing high economic losses to sugar beet crop in Egypt.

Severe infestation of sugar beet with *S. cellatella* larvae was caused significant reductions of 38.20 and 52.40% in root weight and sugar percentages, respectively (Abo-Saied,1987). Bassyouny *et al*. (1991) found that the younger plants were highly infested with cotton leaf worms, the greater damage was caused in both sugar beet leaves and roots, consequently a considerable reduction in sugar percentages. Also, Mesbah (2000) concluded that one larva of *S.littoralis* consumed 183.6 cm^2 of sugar beet leaf tissues throughout the entire larval stage, Causing large bare batches. All the farmers spray the conventional insecticides in controlling these insects. But, the intensive use of conventional insecticides led to several important drastic problems, i.e. environmental pollution, sedtruction of the natural enemies and incidence insect resistance to these insecticides (Awad *et al*., 2014).

Over the past four decades, efforts have been made to develop novel insecticides with selective properties that are designed to act on specific biochemical sites or physiological processes of the target pest. Insect Growth regulators (IERs) are bio-rational insecticides with novel modes of action which disrupt the physiology and development of the target pest, such compounds tend to be selective and generally less toxic to natural enemies than conventional insecticides (Gurr *et al*., 1999). Ecdysone agonists are one of the most important groups of IGRs, and widely used against many lepidopteran pests. Methoxfenozide and Chromafenozide are important members of ecdysone agonist they highly specific to lepidopteran pests all over the world (Pineda *et al.,* 2009). They were reported to be safer for natural enemies than conventional products (Schneider *et al*., 2008). They favorable eco-toxicological profile and short period of persistence in the environment mad their good choice for integrated pest management (IPM) programs in various crops (Pineda *et al*., 2006).

Therefore, the current study was conducted for field evaluation of the five ecdysone agonists efficiency (methoxyfenozide and chromafenozide) in reducing the number of cotton leaf worm and sugar beet moth larvae. In addition to assess their role in maintaining the presence of the associated predators compared to conventional ones.

Materials and methods

The current study was conducted during two successive seasons; 2017/2018 and 2018/2019. Farida cultivar was planted at Shenno village, Kafr El-Sheikh Governorate. The early plantation was sown on 5th August and the late plantation was sown on 30th October in both seasons. Five ecdyson agonists and five traditional comparison insecticides are listed in Table (1) were used. Each treatment was replicated four times (10 x 4 = 40 plots) in randomized

block design. Each plot was measured $42m^2$, in additional to four plots as control. The experimental plots were separated from each other by untreated belts to avoid spray drift. Each sample was consisted of 10 plants/plot (40 plants/ treatment). The primary examination was done before treatment. The treatments were applied on 5th September at the early plantation and on 10th March at the late plantation against *Spodoptera* sp. and *S. ocellatella* larvae, respectively in both seasons. Knapsac sprayer (20 L Volume) was used in applying the treatments. Number of *Spodoptera* sp and *S. ocellatella* larvae was simultaneously counted at the early and the late plantations respectively. The associated arthropod predators were distinguished and accounted. The visual examination was done three, seven and 10 days after tested ecdysone agonists application. While it was achieved one, seven and 10 days after traditional tested insecticides application according to Anonymous (2019). Also, arthropod fauna of predators was sampled using visual examination and sweep net. In each replicate, ten single strokes were made at diagonal direction (Kandil *et al*., 1991). The Reduction in the *Spodoptera* sp., *S. ocellatella* larvae and associated arthropod predators number were calculated by Henderson and Tilton formula (1955).

Differences between mean numbers of the *Spodoptera* sp. and *S. ocellatella* larvae after the tenth day of treatment were analyzed using Duncan test (1955).

Table (1): List of the tested insecticides and their rats per feddan.

Insecticide		Category	Rate
Common name	**Trade name**		
Methoxyfenozide	Raner 24% Sc	Ecdysone agonist	75 cm^3 /fed.
Methoxyfenozide	Abhold 36% Ec	Ecdysone agonist	125 cm^3 /fed.
Chromafenozide	Ferto 5% Sc	Ecdysone agonist	400 cm^3 /fed.
Methoxyfenozide	Xtreme 36% Ec	Ecdysone agonist	125 cm^3 /fed.
Methoxyfenozide	Methobiet 24% SC	Ecdysone agonist	75 cm^3 /fed.
Chlorpyrifos	Dora 48% EC	Conventional	1L./fed.
Carbosulfan	Marshal 20% Ec	Conventional	250 cm^3 /fed.
Chlorfenapyr	Fanty plus 36% EC	Conventional	90 cm^3 /fed.
Methomyl	Diracomel 90% Sp	Conventional	300 gm /fed.
Pyridalyl	Pelo 5% Ec	Conventional	100 cm^3 /fed.

Results and discussion

1. Effects on *Spodoptera* sp. larvae and their associated arthropod predators:

Data shown in Table (2) indicate that the reduction percentages for the five ecdysone agonists insecticides; raner, abhold, ferto, xtreme and methobiet in *Spodoptera* sp. larvae number were close. High reduction percentages were achieved after the third, seventh and tenth days of treatment. Overall mean of reduction percentages in *Spodoptera* sp. larvae number were 92.04, 93.29, 94.01, 93.78 and 94.12%, respectively in 2017/2018 season. As well in 2018/2019 season, over all mean of reduction percentages were 95.87, 95.93, 95.53, 94.34 and 95.70%, respectively. Concerning the conventional insecticides, dora, marshal, fanty plus, diracomel and pelo recorded high reduction percentages after the first, seventh and tenth days of treatment. Overall mean of reduction percentages was 97.15, 95.61, 95.44, 95.30 and 95.61%, respectively in 2017/2018 season. Also, it was 95.34, 96.09, 97.56, 97.38 and 97.76%, respectively in 2018/2019 season.

Overall mean of reductions to the all tested insecticides ranged between

92.04 – 97.15% in the first season and 94.34 – 97.76% in the second season. This means that the all tested insecticides induced above > 92% reduction in *Spodoptera* sp. larvae number.

The arthropod predators associated with *Spodoptera* sp were true spiders, formicidae, *Chrysoperla carnea* (Stephens). Data in Table (3) reveal that the treatment of five ecdysone agonists insecticides resulted in a low decrease in reduction percentages in the number of predators. The mean number of predators ranged between 8.25 to 9.75 and 7.80 to 11.75 individuals /10 plants during seasons 2017/2018 and 2018/2019 respectively. The overall mean reduction percentages were ranged between 23.16 to 31.59% and 10.84 to 11.57% during the first and second seasons respectively. While the treatment with conventional insecticides led to a high reduction in the number of predators. The mean number of predators ranged between 0.00 to 0.75 individuals /10 plants in the two study seasons. The overall mean reduction percentages were ranged between 95.59 to 99.38% and 96.72 to 98.68% in 2017/2018 and 2018/2019 seasons respectively. After the tenth day of treatment, the effect of the tested ecdyson agonists and the tested traditional insecticides differed significantly in the mean number of predators during the two successive seasons.

Table (2): Reduction percentages in *Spodoptera* sp. larvae number during 2017/2018 and 2018/2019 seasons.

Season 2017/2018										
Compound	**Before spray**	**After one day**		**After 3 days**		**After 7 days**		After 10 days		Overall mean of reduction
	M	**M.**	**% Red**	**M.**	**% Red**	**M.**	**% Red**	M.	% Red	
Raner	25.25	-	-	2.5	92.15	3.25	91.7	3.75	92.28	92.04
Abhold	26.25	-	-	2.25	93.2	3	92.63	3	94.06	93.29
Ferto	25.75	-	-	2	93.84	2.5	93.74	2.75	94.45	94.01
Xtreme	26	-	-	1.75	94.66	2.75	93.18	3.25	93.5	93.78
Methobiet	25.5	-	-	2	93.78	2.5	93.68	2.5	94.9	94.12
Dora	26.25	0.25	99.11	-	-	1.5	96.31	2	96.04	97.15
Marshal	26.25	1.25	95.56	-	-	1.75	95.73	2.25	95.54	95.61
Fanty plus	26.5	1	96.48	-	-	1.75	95.74	3	94.11	95.44
Diracomel	25	0.75	97.2	-	-	1.75	95.48	3.25	93.24	95.3
Pleo	25.25	0.75	97.23	-	-	1.75	96.8	3.5	92.8	95.61
Control	26.75	28.75	-	33.75	-	41.5	-	51.5	-	-
Season 2018/2019										
Compound	**Before spray**	**After one day**		**After 3 days**		**After 7 days**		After 10 days		Overall mean of reduction
	M	**M.**	**% Red**	**M.**	**% Red**	**M.**	**% Red**	M.	% Red	
Raner	21.5	-	-	0.75	97.59	1.75	95.44	2.25	94.58	95.87
Abhold	21.75	-	-	0.75	97.62	1.5	96.13	2.5	94.05	95.93
Ferto	22.25	-	-	1	96.89	2	94.96	2.25	94.76	95.53
Xtreme	22.5	-	-	1.25	96.16	2.5	93.78	3	93.1	94.34
Methobiet	21.25	-	-	1.25	95.94	1.5	96.04	2	95.12	95.7
Dora	22.75	1	96.04	-	-	1.75	95.69	2.5	94.31	95.34
Marshal	22.25	0.5	97.97	-	-	2	94.96	2	95.34	96.09
Fanty plus	22	0	100	-	-	1.25	96.81	1.75	95.88	97.56
Diracomel	22	0	100	-	-	1	97.45	2.25	94.7	97.38
Pleo	21.75	0	100	-	-	0.75	98.06	2	95.24	97.76
Control	22.25	24.75	-	32.25	-	39.75	-	43	-	-

Table (3): Reduction percentages in arthropod predators number associated with *Spodoptera* sp. during 2017/2018 and 2018/2019 seasons.

Season 2017/2018										
Compound	Before spray	After one day		After 3 days		After 7 days		After 10 days		Overall mean of reduction
	M	M.	% Red	M.	% Red	M.	% Red	M.*	% Red	
Raner	10	-	-	9	23.12	8.75	31	8.25a	40.65	31.59
Abhold	9.75	-	-	9.75	14.58	9.5	23.17	9.25a	31.75	23.16
Ferto	10	-	-	9.5	18.85	9.5	25.09	9.25a	35.72	26.55
Xtreme	9.25	-	-	9	16.89	9	23.28	8.75a	31.95	24
Methobiet	9.5	-	-	9.25	16.83	9.25	23.22	9.25a	29.96	23.33
Dora	10.25	0	100	-	-	0.5	96.15	0.75b	90.64	95.59
Marshal	9.75	0	100	-	-	0.25	97.97	0.50b	96.31	98.09
Fanty plus	9.75	0	100	-	-	0	100	0.25b	98.15	99.38
Diracomel	9.5	0	100	-	-	0.25	97.92	0.75b	94.32	97.41
Pleo	9	0	100	-	-	0	100	0.50b	96	98.66
Control	10.25	11.25	-	12	-	13	-	14.25	-	-
Season 2018/2019										
Compound	Before spray	After one day		After 3 days		After 7 days		After 10 days		Overall mean of reduction
	M	M.	% Red	M.	% Red	M.	% Red	M.*	% Red	
Raner	12.25	-	0	12	7.8	11.75	11.45	11.75a	14.73	11.32
Abhold	12	-	0	11.75	7.84	11.5	11.53	11.50a	14.81	11.39
Ferto	11.75	-	0	11	11.88	11.5	9.65	11.50a	13	11.51
Xtreme	11.75	-	0	11.5	7.88	11.25	11.62	11.25a	13.04	10.84
Methobiet	11.5	-	0	11	9.97	11	11.7	11.25a	13.04	11.57
Dora	11	0	100	-	-	0.25	97.9	0.50b	95.95	97.95
Marshal	11.25	0	100	-	-	0	100	0.50b	96.04	98.68
Fanty plus	11.25	0	100	-	-	0	100	0.57b	94.07	98.02
Diracomel	12	0	100	-	-	0.25	98.07	0.50b	96.29	98.12
Pleo	11.5	0	100	-	-	0.5	95.98	0.75b	94.2	96.72
Control	12	12.5	-	12.75	-	13	-	13.5	-	-

The Duncan test at level of 5% probability was applied, the mean followed by the same letter do not differ significantly.

2. Effects on *Scrobipalpa ocellatella* larvae and their associated arthropod predators:

Concerning the relation between the number of *S. ocellatella* larvae and the ten tested insecticides was shown in the Table (4). The tested ecdyson agonists caused a considerable decrease in the number of larvae during 2017/2018 and 2018/2019 seasons. The minimum overall mean reduction percentages were 89.25and 88.76% in the first and second seasons respectively. While the maximum overall mean reduction percentages were 90.86 and 92.08% in the first and second seasons respectively. About traditional insecticides recorded the minimum overall mean reduction percentages which were 89.89 in 2017/2018 season and 87.71% in 2018/2019 season. As for the maximum records were 91.23% in the first season and 88.18% in the second season. In the two seasons of the study, effects of the tested ecdyson agonists were like those of the traditional insecticides in reducing the number of sugar beet moth larvae. Overall mean of reductions to all tested insecticides ranged between (89.25 – 91.23%) for the first season and (87.71 – 92.08%) for the second season, this means that all tested insecticides caused above > 87% reduction in *S. ocellatella* larvae numbers.

On the other hand, the arthropod predators associated with *S. ocellatella* were true spiders, formicidae, and

Coccenilla undecimpunctata (L.). Results in Table (5) clarify that the tested ecdysone agonists were caused overall mean of reduction percentages (ranged between 8.30 to 12.47%) in these predators' numbers less than that recorded when using traditional insecticides (ranged between 98.36 to 99.20%) in 2017/2018 season. The results of the second season took the same trend as the previous season. The lowest and highest overall mean of reduction percentages were 7.97 and 14.3% respectively in case of the tested ecdyson agonists treatment. Whereas the traditional insecticides treatment achieved 98.11 and 99.54% as the lowest and highest records respectively. Statistical analysis showed significant differences between the average numbers of predators after the tenth day of the treatment in the both study seasons.

In conclusion, the current study presented that the tested ecdyson agonists have converged with conventional insecticides in their highly reduced impact on the tested insect pests' larvae numbers. As for its effect in reducing the number of predators, it is minimal compared to traditional insecticides. This means its safe effect on natural enemies and their survival under field conditions.

These results are agreement with Sparks (2001) who reported that the diacylhydrazines are novel class of IGRs which in the Lepidoptera function as ecdysone agonists which disrupting the molting process by mimicking the action of 20 – Hydroxy ecdysone. As well as good selectivity towards beneficial insects. Smagghe *et al*. (2003) reported that the compound methoxyfenozide was the newest member of this new group of moulting hormone accelerating IGRs to reach the marketplace against Lepidoptera. Yanagi and Kawagishiu (2006) demonstrated that Toxic effects of chromafenozides against lepidopteran larvae mainly via digestion. The treated larvae stopped the feeding within 10 – 12 hr. after treatment to toxic doses of the agent and inducing the molting process. A treated larva slipped its head out of the old head capsule prematurely to attempt to molt. Furthermore, several authors i.e. Gurr *et al*. (1999), Moulton *et.al*.(2002), , Pineda *et al.* (2006), Schneider *et al.* (2008), Pineda *et al.* (2009), Shahout *et al*. (2011) and Rani *et al.* (2018) concluded that ecdysone agonists (methoxyfenozide and chromafenozide) are promising insecticides with high efficacy against various lepidopteran insects, at the same time almost non-toxic to pollinators, predators, parasitoids, mammals and has minimum impact on the environment. Consequently, it would be an ideal agent for integrated pest management (IPM).

Table (4): Reduction percentages in *Scrobipalpa ocellatella* larvae number during 2017/2018 and 2018/2019 seasons.

Season 2017/2018										
Compound	Before spray	After one day		After 3 days		After 7 days		After 10 days		Overall mean of reduction
	M	M.	% Red	M.	% Red	M.	% Red	M.	% Red	
Raner	19.75	-	-	4.75	80.8	2.5	91.3	1.5	95.65	89.25
Abhold	19.75	-	-	4.5	81.81	2.75	90.43	1.5	95.65	89.29
Ferto	20	-	-	4.5	82.04	2.25	92.27	1.25	96.42	89.57
Xtreme	20	-	-	4.25	83.04	2	93.13	1.25	96.42	90.86
Methobiet	19.5	-	-	4.25	82.6	2.25	92.07	1.5	95.59	90.08
Dora	19.5	4.25	80.65	-	-	2	92.95	1.25	96.33	89.97
Marshal	19.25	4.25	80.4	-	-	1.75	93.75	1.5	95.53	89.89
Fanty plus	19.25	4.25	80.4	-	-	1.75	93.75	1.5	95.53	89.89
Diracomel	20.25	4	82.46	-	-	1.75	94.06	1	97.17	91.23
Pleo	19	4	81.31	-	-	1.5	94.57	1	96.98	90.95
Control	19.75	22.25	-	24.75	-	28.75	-	34.5	-	-
Season 2017/2018										
Compound	Before spray	After one day		After 3 days		After 7 days		After 10 days		Overall mean of reduction
	M	M.	% Red	M.	% Red	M.	% Red	M.	% Red	
Raner	17.5	-	-	4.75	81.05	2.5	98.66	1.25	96.55	92.08
Abhold	17.25	-	-	5	79.77	2.75	90.71	1.5	95.8	88.76
Ferto	17.25	-	-	4.75	80.78	2.75	90.71	1.25	96.5	89.33
Xtreme	16.75	-	-	4.75	80.78	2.75	90.43	1.25	96.4	89.2
Methobiet	16.75	-	-	4.5	81.25	2.5	91.3	1.25	96.4	89.65
Dora	17.75	4.75	76.36	-	-	2.75	90.97	1.5	95.8	87.71
Marshal	17	4.5	76.66	-	-	2.5	91.43	1.5	95.74	87.94
Fanty plus	17	4.5	76.66	-	-	2.5	91.43	1.5	95.74	87.94
Diracomel	16.5	4.5	75.95	-	-	2.5	91.17	1.25	96.34	87.82
Pleo	17	4.5	76.66	-	-	2.5	91.43	1.25	96.45	88.18
Control	16.75	19	-	24	-	28.75	-	34.75	-	-

Table (5): Reduction percentages in arthropod predators number associated with *Scrobipalpa ocellatella* during 2017/2018 and 2018/2019 seasons.

Season 2017/2018										
Compound	Before spray	After one day		After 3 days		After 7 days		After 10 days		Overall mean of reduction
	M	M.	% Red	M.	% Red	M.	% Red	M.*	% Red	
Raner	19.75	-	-	19	7.31	18	14.28	18.00a	15.29	12.29
Abhold	19.75	-	-	19	7.31	19	9.52	19.50a	8.23	8.35
Ferto	20	-	-	19.5	6.06	19.5	8.3	19.25a	10.54	8.3
Xtreme	20	-	-	18.75	9.67	19	10.65	19.00a	11.7	10.67
Methobiet	19.5	-	-	18	11.06	18	13.18	18.25a	13.01	12.41
Dora	19.5	0	100	-	-	0	100	0.59b	97.61	99.2
Marshal	19.25	0	100	-	-	0.25	98.77	0.75b	96.37	98.38
Fanty plus	19.25	0	100	-	-	0	100	0.75b	96.37	98.79
Diracomel	20.25	0	100	-	-	0.25	98.83	0.75b	96.55	98.46
Pleo	19	0	100	-	-	0.25	98.76	0.75b	96.33	98.36
Control	19.75	20	-	20.5	-	21	-	21.25	-	-
Season 2018/2019										
Compound	Before spray	After one day		After 3 days		After 7 days		After 10 days		Overall mean of reduction
	M	M.	% Red	M.	% Red	M.	% Red	M.*	% Red	
Raner	17.5	-	-	16	12.48	12.25	12.37	16.25a	13.59	12.81
Abhold	17.25	-	-	16.25	59.83	16.5	9.73	16.50a	10.99	10.18
Ferto	17.25	-	-	15	16.77	16	12.47	16.00a	13.68	14.3
Xtreme	16.75	-	-	16.25	7.14	16.25	8.45	16.50a	8.33	7.97
Methobiet	16.75	-	-	14	2	15	15.49	15.00a	16.66	11.38
Dora	17.75	0	100	-	-	0.25	98.68	0.50b	97.37	98.68
Marshal	17	0	100	-	-	0.25	98.61	0.50b	97.26	98.62
Fanty plus	17	0	100	-	-	0.25	98.61	0.50b	97.26	98.62
Diracomel	16.5	0	100	-	-	0.25	98.57	0.75b	95.77	98.11
Pleo	17	0	100	-	-	0	100	0.25b	98.63	99.54
Control	16.75	17	-	17.5	-	17.75	-	18	-	-

The Duncan test at level of 5% probability was applied, the mean followed by the same letter do not differ significantly.

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Evaluation of two different pesticides sprayer equipment techniques on squash plants

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Abstract:

The main experiments were carried out during 2017 and 2018 seasons at the New Salheia, Sharkia Governorate to investigate and evaluate two techniques (pressure or hydraulic atomization and centrifugal atomization) to apply pesticides and their effect on volume median diameter, number of droplets/cm², L and loss and drift outside treatment or contamination of applicator, pesticides efficiency. This work was tested three equipment, the equipment used were ULVA sprayer, electric battery sprayer fitted with flat fan nozzle Ss-83 and conventional motor sprayer with variable spraying rates. In addition, pesticides buprofezin and imidacloprid against the aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) and the tomato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and pests infested squash plants where be used. Current study was determined the effect of each technique and pesticide of reduction of pests and determined the contamination of applicator, L and loss and Drift outside treatment caused by each technique. The result obtained during the two seasons showed that the spray with high volume was gave low percent reduction of pests and high contamination of applicator or Losses on land if compared with low volume spraying. No drift spray was recorded by ULVA sprayer and electric battery sprayer fitted flat fan (Ss-83) nozzle, while there is drift spray was occurred with the use conventional motor sprayer on distance 1m, 2m and 3m.

Introduction

Squash is one of the most important vegetables in Egypt, it cultivates under summer and winter conditions, although still not widely used by the food industry, squashes are consumed worldwide. Fruits are consumed as vegetables or dessert (pie) and seeds as nuts and, to a lesser extent, as cooking oil (Lazos, 1986 and 1992). Because of their resistance to drought and the high protein (23-35%) and oil (25-55%) contents of their seeds, squashes

have attracted the attention of many growers and plant breeders within the past 50 years (Curtis, 1946; Bemis *et al*., 1978 and Scheerens *et al*., 1991). According to Food and Agricultural Organization (FAO) (2012), the Egyptian production for squash was 658.234 metric tons. The cultivated area with this crop increased during the last two decades especially in new reclaimed regions in both open and protected plantation. Throughout the growing season, cucumber plants are suffering from severe infestation with different phytophagous insect pests such as the aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) and the tomato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) , which considered the most common and dangerous insect pests of cucumber plants. In case of heavy infestation, these pests are causing serious damage to plants, leading to great reduction in the final yield (Hanafy, 2004). Squash crop is infested by many pests, these are aphid, *A. gossypii*., whitefly, *B. tabaci* and thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Mohamed ,2011).

Therefore, pest control through chemical spraying in highly needed in Egypt to reduce the annual losses in crops caused particularly by pests. Two types of insecticides have been recommended to control sucking aphid and whitefly. The insecticide effect of droplets sprayed is dependent on spectrum droplets (Palti and Ausher, 1986). The performance of pest control dependent on the proper choose of suitable technique to use of spraying. So, this study compared with two techniques centrifugal atomization technique(ULVA sprayer) with 18.4L/fed., pressure or hydraulic atomization (Electric battery sprayer fitted with flat fan nozzle Ss-83) with 89.3 L/fed. and pressure or hydraulic atomization (Conventional motor sprayer) with 330L/fed. Therefore, the main objective of this study is to evaluate some techniques (pressure or hydraulic atomization and centrifugal atomization) used to apply pesticide in Egypt and their effects on spraying efficiency volume median diameter, number of droplets/cm², L and loss and drift outside treatment or contamination of applicator, pesticides efficiency were also conducted.

Materials and methods

1. Squash crop:

The variety was used in this study for manual planting were planted in ridges the distance between each ridge was 80 cm and row spacing between the plants was 50 cm. this variety is recommended in Egypt.

2. Field layout:

The experiment was carried out in a rectangular shape area about 2 Feddans. Squash area were planted by hand in ridges. The experiment area divided into nine plots area of plot 1056m² (44x24m) and left between each plots (treatment) and the other (44 x 8m) for measure the drift sprayer has two plots, as shown in Tabel (1). Six plots for treatment and one for control.

Table (1): Designed showing the field experiment.

3kerates	6kerates	ULVA Sprayer		Electric Battery Sprayer fitted with flat fan nozzle (Ss-83)		Conventional Motor Sprayer		3 kerates
		Buprofezin	Imidacloprid	Buprofezin	Imidacloprid	Buprofezin	Imidacloprid	
		6 kerates	6 kerates	6 kerates	6 kerates	6 kerates	6 kerates	

3. Pesticide used:

3.1. Buprofezin (Applaud 25% SC) suspension concentrate with recommended rate 600cm/fed.

3.2. Imidacloprid (Avenue 70%WG) water dispersible granules with recommended rate 120gm/fed.

4. Equipment Used:

Spraying machinery used in this investigation and specification of equipment as follow:

4.1. ULVA sprayer (Centrifugal atomization technique):

The sprayer has aluminum tube 1.30m long contain five batteries 1.5 volt located in the section of the tube and connected via an on/off switch to a 7.5-volt motor located at the rear of the tube. The sprayer has one-liter plastic bottle concentrate liquid (pesticide) which was fed by gravity to reach the spinning disc. The sprayer attached with back tank ten liters and is led to the spinning disc through plastic pipe (food house) to increase performance. The sprayer made in England.

4.2. Electric battery sprayer (Pressure or hydraulic atomization) with flat fan Ss83 nozzle:

The electric battery sprayer has 20-liter liquid tank capacity, and diaphragm pump motor operated without air chamber. The power consumption for 4.5-hour continuous operation is Battery12 volt, motor speed 2800- 3200 rpm., and operating pressure is 3 bar.

4.3. The Conventional motor sprayer (Pressure or hydraulic atomization):

This equipment is local manufacturing, it consists of 600 liters tank capacity, spray gun connecting with the pump by 40 – 80m long rubber house, reciprocating pump with air chamber, the power is 5 hours while, the operating pressure is 3 bar. This equipment works with hydraulic agitation, with cooled air.

5. Measurements instrument:

5.1. Tape: For measuring the distance cut by operator of each replicate.

5.2. Stopwatch: It used to calculate the average forward speed and flow rate with accuracy too sec.

5.3. Graduated cylinder: Graduated Cylinder was used to calibrate the volume of the spraying solution.

5.4. Water sensitive paper: Ciba Geigy sensitive paper (76 x 26mm) to receive spray droplets from sprayers during their operation.

5.5. Wind meter: Wind meter was used to measure the wind velocity (m/s).

5.6. Strubin® lens (X15)**:** This lens used to measure the number and volume of deposited droplets on sensitive paper.

6. Measurements: -

6.1. Flow rate (L/min):

Flow rate was the first test made to calibrate the equipment. The researcher was filled the sprayer tank with water and regulated the required pressure and height of nozzle. The flow rate was measured by collecting the water in a graduated cylinder for one minute, and repeated this step for three times, and calculated the average to achieve accurate result. Then we consider the flow rate was achieved as expressed the sprayer.

6.2. Swath width of the sprayers (m):

The Patternation test by means of only one nozzle, as well as the pass of ground sprayer over sensitive cards. The sensitive cards technique was found to be less accurate but easier and quicker technique than the former one. Therefore, the pass spray technique will be selected to determine the swath width of the tested sprayer, at two spray heights and two walking speed with the use of water and sensitive cards calculated are presented in Table (2).

Table (2): Laboratory technical data of sprayer techniques used by three tested sprayers.

Item	ULVA Sprayer	Electric battery sprayer with flat fan nozzle(ss83)	Conventional motor sprayer
Type of sprayer	Rotary	Hydraulic	Hydraulic
Spray tank, (L).	10	20	600
Flow rate, (L/min.)	0.175	0.850	2.36
Rate of application, (L/fed.)	18.4	89.3	330
Spray height, (m)	0.50	0.50	0.50
Swath width, (m)	1	1.00	0.75
Working speed, (Km/h.)	2.4	2.4	2.4
Type of spray used	Target	Target	Target
Productivity, (fed/h.)	0.57	0.57	0.43
Rate of performance (fed/day.)	2.28	2.28	1.72

Productivity, (fed/h.) = $\frac{60*speed*swaghwides}{4200}$ **Rate of performance/day= Productivity, (fed/h.) * (6 and 8) *** $\frac{2}{3}$**).**

7. Description of sampling line:

Six plots were sprayed, and one was left for control. The sampling line consisted of 5 wires holders fix at one (m). In diagonal line inside each treatment to collected sprayer chemicals. Sensitive paper cards double with the wire holder were fixed in "L" shape on the top of wire holders to measure the distribution ratio on the upper and lower surface of the sensitive paper. Three sensitive paper cards double were distribution on some plants (right, middle, left) at distance of one meter to measure the distributed on the upper and lower surface at five plants. In addition to, one sensitive paper card was placed under each plant to measure loss of land. While, sensitive paper cards were fixed on the applicator (Head, Thorax, abdomen and legs (right and left)) for measure the contamination deposit. All cards were numbered, collected and transferred carefully to the laboratory for measurement the volume and number of deposited droplets per cm^2 by the above-mentioned Strobing lens. Therefore, calculate the VMD of droplets. Results were then recorded, in ten successive classes with a range of 50microns. Volume Median Diameter (VMD) value was calculated according to the following equation **(Gabir, 1978).**

$$V.M.D = [\Sigma_i^n = 1(nixi3)/\Sigma_i^n = 1ni]^{\frac{1}{3}}$$

Xi= droplet diameter for a given size class (1) µm

$\sum^{n} i$ = total number of droplet, in all droplets categories

8. Laboratory coverage for used equipment:

The table (3) is conducted the laboratory coverage of used equipment.

Table (3): Spray coverage on artificial targets as produced by electric battery sprayer and ULVA sprayer.

Equipment				Electric Battery Sprayer (Ss-83)			ULVA Sprayer		
Spray Volume (L/fed)				89.3			18.4		
Droplets spectrum / Spray parameter				VMD µm	N/cm²	% N	VMD µm	N/cm²	% N
Working Speed (2.4km/h)	Spray height	0.30 m	Upper	170	55	69	99	93	72
			Lower	73	25	31	87	36	28
		0.50 m	Upper	174	63	70	107	97	69
			Lower	78	27	30	81	43	31
Working Speed (3.0 km/h)		0.30 m	Upper	174	55	75	91	72	65
			Lower	88	18	25	90	39	35
		0.50 m	Upper	128	53	76	118	81	59
			Lower	80	17	24	93	56	41

9. Weather conditions:

Weather conditions during the experimental periods were measured, measurements will be taken by the method described by **(Barry, 1978)** Table (4). A simple anemometer has a pith ball which moves up at vertical tube according to the strength of the wind "Dwyer's anemometer"

Table (4): Average of meteorological conditions during experiments execution.

Experiments	Season	Date of Experiment	Governorate	A.T (°C)*	R.H (%)**
In Laboratory	2017	28. 2.2017 5. 3.2017	Spray technology Departement, EL- Dokki, Giza	24.0 27.0	68.0 71.0
Insecticides on squash plants fields	2017	3. 6. 2017	New Salhia, Sharkia	31.0	68.0
	2018	10. 5. 2018		34.0	73.0

Notations: * Air Temperature (ºC). ** Relative Humidity (R. H.%).

10. Experimental treatments:

The chemical pest control treatments were conducted during squash cultivated seasons 2017&2018**.** Chemical applications were started 37days at season 2017 and 39 days at season 2018 after the sowing of squash plants.

11. Determination of spray deposit

Number and size spots (droplets) on sensitive cards will be measured with a special scaled monocular lens (**Struben®**) with a magnification of X 15. This is a hand lens which gives a direct measurement because it magnifies both the spot and scale at the same rate, scales 6 mm in 60 parts, and diameter 7 mm. The area of its field *=0.432 cm².* Obtained data was corrected (by knowledge of the spread factor) and is calculated to obtain the Volume Median Diameter of droplets (VMD) and the number of these droplets in one square centimeter (N/cm²), according to **Gabir (1975/95).**

The volumetric diameter droplets on Ciba-Geigy sensitive paper can be calculated as follows:

Actual droplet diameter= $\frac{\text{stain diameter of droplet}}{\text{spraed factor}}$ µm.

12.Spread factor:

The values of spread factor cited from Ciba Geigy Company, were followed here (Table,5) **(Gehan, 2000).**

Table (5): The values of spread factor.

Stain diameter of droplet in (µm)	Spread factor	Droplet diameter actual in (µm)
100	1.7	050
200	1.8	100
300	1.9	155
400	2.0	200
500	2.0	243
600	2.1	285

13. The wind velocity:

Face the wind hold meter in front of you in vertical position and with scale side toward. Do not block bottom holes. Height of ball indicates wind velocity for high scale, cover hole at extreme top with finger.

14. The Drift:

Outside treatment of squash plants only wire holders were fixed in the distance 1, 2, and 3 m to measure drift spray lost by air.

15. Biological whitefly and Aphids infesting:

Imidacloprid and buprofezin evaluated at a recommended rate (120gm/fed. and 600cm³/fed.), respectively, against squash plants insects. Samples of 25 plants were chosen at randomly from each replicate before treatment and at 1, 3, 7 and 15 days after pesticides application. The number of target insects was counted. Percentage of the insect population was calculated according to Henderson and Tilton (1955). Comparing differences mean, the main effect and Independent factors interaction were analyzed throughout Spss version 19.

Results and discussions

The data obtained from the field experiment with the purpose of evaluating some techniques (pressure or hydraulic atomization, centrifugal atomization) to apply pesticides in Egypt and their effect on spraying efficiency (droplet size and spray distribution pattern), environmental pollution.

1. Field performance:

The performance of some techniques (pressure or hydraulic atomization, centrifugal atomization) with two types of pesticides were tested and evaluated according to the following aspects: a. Volume medium diameter. b. Number of droplets. c. Land loss. d. Drift. e. Contamination of applicator. f. Pesticides efficiency.

2. The evaluation of techniques:

The evaluation of techniques was based on volume median diameter (VMD) (µm) and number of droplets (N/cm²), this well be on both a horizontal card (on wire and the cards on the surface of leaves for the plant right, middle and left, (is calculated as follows upper and lower of surface the card while on the land (is calculated as follows upper surface the card).

2.1. ULVA sprayer (Centrifugal atomization technique):

Generally, were the obtained results showed that the volume medium diameter and number of droplets there was discrepancy between the two surfaces upper and lower for artificial and plants when using avenue 70% WG (Imidacloprid), while when using applaud 25% SC (Buprofezin), the volume medium diameter and number of droplets decreased on lower surface and increasing on upper surface for artificial and plants, meanwhile increases the volume medium diameter and decreased the number of droplets on the land loss with two types pesticides.

2.1.1. Effect using ULVA sprayer on volume median diameter and number of droplets with avenue 70% WG (Imidacloprid):

Table (6) showed that the effect using ULVA sprayer on volume median diameter (µm) and number of droplets (N/cm²) at 18.4 L/fed. spray rate. The volume median diameter and number of droplets on upper and lower surface for the card on artificial was (72 and 88 µm) and (106 and 48 N/cm²), respectively. While, the upper and lower surface for the cards on leaves of the plant (right, middle and left) was (82, 78 and 84µm), (86, 77 and 75 µm) and (62, 59 and 58 N/cm²), (42, 44 and 44 N/cm²), respectively. Meanwhile, the volume median diameter and number of droplets on upper surface for the card on the land 131µm and 33 N/cm², respectively.

Table (6): The effect using ULVA sprayer on volume median diameter (VMD) and number of droplets (N/cm²) with avenue 70% WG (Imidacloprid).

Spray receptors	Spray direction	Place the spray card	VMD (µm)	N/cm²	N %
Wire	Artificial	Upper	72	106	69
		Lower	88	48	31
Plants	Right	Upper	82	62	59
		Lower	86	42	40
	Middle	Upper	78	59	57
		Lower	77	44	43
	Left	Upper	84	58	57
		Lower	75	44	43
Land			**131**	**33**	**100**

2.1.2. Effect using ULVA sprayer on volume median diameter and number of droplets with applaud 25% SC (Buprofezin):

Table (7) showed that the effect using ULVA sprayer on volume median diameter (µm) and number of droplets (N/cm²) at 18.4 L/fed. spray rate. The volume median diameter and number of droplets on upper and lower surface for the card on artificial was (78 and 78 µm) and (81 and 53 N/cm²), respectively. While, the upper and lower surface for the cards on leaves of the plant (right, middle and left) was (89, 76 and 90µm), (63, 73 and 54 µm) and (64, 76 and 54 N/cm²), (40, 48 and 44 N/cm²), respectively. Meanwhile, the volume median diameter and number of droplets on upper surface for the card on the land 109 µm and 26 N/cm² respectively.

Table (7): The effect used ULVA sprayer on volume median diameter (VMD) and number of droplets (N/cm²) with applaud 25% SC (Buprofezin).

Spray receptors	Spray direction	Place the spray card	VMD (µm)	N/cm²	N%
Wire	Artificial	Upper	78	81	60
		Lower	78	53	40
Plants	Right	Upper	89	64	62
		Lower	63	40	38
	Middle	Upper	76	76	61
		Lower	73	48	39
	Left	Upper	90	54	55
		Lower	54	44	55
Land			**109**	**26**	**100**

2.2. Electric battery sprayer (Hydraulic atomization technique):

Generally, the obtained results showed that the volume medium diameter and number of droplets decreased on lower surface and increasing on upper surface for artificial and plants, while increases the volume medium diameter and decreased the number of droplets on the land loss with two types of pesticides.

2.2.1. Effect using electric battery sprayer technique on volume median diameter and number of droplets with avenue 70% WG (Imidacloprid):

Table (8) showed that the effect using electric battery sprayer technique (Battery –operated knapsack motor sprayer) on volume median diameter (µm) and number of droplets (N/cm²) with using flat fan nozzle at 89.3 L/fed. spray rate. The volume median diameter and number of droplets on upper and lower surface for the card on artificial was (171 and 89µm), (58 and 30 N/cm²). While, the upper and lower surface for the cards on leaves of the plant (right, middle and left) was (165, 132 and 154µm), (80, 106 and 85 µm) and (58, 54 and 55 N/cm²), (27, 26 and 25 N/cm²), respectively. Meanwhile, the volume median diameter and number of droplets on upper surface for the card on the land was 215µm and 28 N/cm².

Table (8): The effect of using electric battery sprayer with flat fan nozzle (Ss83)) on volume median diameter (VMD) and number of droplets (N/cm²) at use Avenue 70% WG (Imidacloprid)

Electric Battery sprayer fitted flat fan nozzle (Ss83)					
Spray receptors	**Spray direction**	**Place the spray card**	**VMD (µm)**	**N/cm²**	**N%**
Wire	Artificial	Upper	171	58	66
		Lower	89	30	34
Plants	Right	Upper	165	58	67
		Lower	80	27	33
	Middle	Upper	132	54	68
		Lower	106	26	32
	Left	Upper	154	55	69
		Lower	85	25	31
Land			**215**	**28**	**100**

2.2.2. Effect using Electric Battery sprayer technique on volume median diameter and number of droplets with applaud 25% SC (Buprofezin):

Table (9) showed that the effect using hydraulic atomization technique (Battery–operated knapsack motor sprayer with flat fan nozzle) on volume median diameter (µm) and number of droplets (N/cm²) 89.3 L/fed. spray rate. The volume median diameter and number of droplets on upper and lower surface for the card on artificial when using flat fan nozzle was (163 and 80µm), (64 and 33 N/cm²) respectively. While, the upper and lower surface for the cards on leaves of the plant (right, middle and left) was (152, 154 and 158µm), (87, 85 and 85 µm) and (58, 53 and 56 N/cm²), (31, 31 and 36 N/cm²) respectively. Meanwhile, the volume median diameter and number of droplets on upper surface for the card on the land was 224 µm and 20 N/cm².

Table (9): The effect of used Electric sprayer atomization with flat fan nozzle (Ss83) on volume median diameter (VMD) and number of droplets (N/cm²) at use applaud 25% SC (Buprofezin).

Electric Battery sprayer fitted and flat fan nozzle (Ss83)					
Spray receptors	**Spray direction**	**Place the spray card**	**VMD (µm)**	**N/cm²**	**N%**
Wire	Artificial	Upper	163	64	66
		Lower	80	33	34
Plants	Right	Upper	152	58	65
		Lower	87	31	35
	Middle	Upper	154	53	63
		Lower	85	31	37
	Left	Upper	158	56	61
		Lower	85	36	39
Land			**224**	**20**	**100**

2.3. Conventional motor sprayer (Hydraulic atomization technique) with rate spray 330 L/fed.:

2.3.1. Effect using conventional motor sprayer on volume median diameter and number of droplets at use Avenue 70% WG (Imidacloprid):

Table (10) showed that the effect using Conventional Motor Sprayer technique on volume median diameter (µm) and number of droplets (N/cm²).The volume median diameter and number of droplets on upper and lower surface for the card on artificial was (520 and 162 µm) and (28 and 21 N/cm²), respectively. While, the upper and lower surface for the cards on leaves of the plant (right, middle and left) was (483, 459 and 460 µm), (135, 156 and 148µm) and (28, 24 and 27 N/cm²), (18, 20 and 17 N/cm²) respectively. Meanwhile, the volume median diameter and number of droplets on upper surface for the card on the land 518 µm and 19 N/cm², respectively.

Table (10): The effect used conventional motor sprayer technique on volume median diameter (VMD) and number of droplets (N/cm²) at use avenue 70% WG (Imidacloprid).

Spray receptors	**Spray direction**	**Place the spray card**	**VMD (µm)**	**N/cm²**	**N%**
Wire	Artificial	Upper	520	28	57
		Lower	162	21	43
Plants	Right	Upper	483	28	61
		Lower	135	18	39
	Middle	Upper	459	24	55
		Lower	156	20	45
	Left	Upper	460	27	61
		Lower	148	17	39
Land			**518**	**19**	**100**

2.3.2. Effect using conventional motor sprayer on volume median diameter and number of droplets at use applaud 25% SC (Buprofezin):

Table (11) showed that effect using Conventional Motor Sprayer technique on volume median diameter (µm) and number of droplets (N/cm²). The volume median diameter and number of droplets on upper and lower surface for the card on artificial was (504 and 167 µm) and (30 and 21 N/ cm²), respectively. While, the upper and lower surface for the cards on leaves of the plant (right, middle and left) was (478, 474 and 494 µm), (170, 184 and 152 µm) and (26, 18 and 28 N/cm²), (19, 21 and 21 N/cm²), respectively. Meanwhile, the volume median diameter and number of droplets on upper surface for the card on the land 519 µm and 18 N/cm², respectively.

Table (11): The effect used conventional motor sprayer technique on volume median diameter and number of droplets at use applaud 25% SC (Buprofezin).

Spray receptors	Spray direction	Place the spray card	VMD (µm)	N/cm²	N%
Wire	Artificial	Upper	504	30	59
		Lower	167	21	41
Plants	Right	Upper	478	26	58
		Lower	170	19	42
	Middle	Upper	474	18	46
		Lower	184	21	54
	Left	Upper	494	28	57
		Lower	152	21	43
Land			**519**	**18**	**100**

3. Drift:

The drift into adjacent land during application different techniques atomization on squash field was studied. Drift deposits of pesticide determined by volume median diameter (µm) and number of droplets (N/cm²). The determination was assayed on the cards land positioned at various distances from treated squash field (1, 2, and 3m). These results could be easily explained on the basis that wind speed during spray was 4 m/sec, relative humidity was (68-73%) and air temperature was (31-34°C). Tables (12 a and b) show that the greater drift within adjacent land showing detestable residues was observed during spray application followed by that of Hydraulic atomization (Conventional Motor Sprayer) and no drift ULVA Sprayer and Electric Battery sprayer with Ss83 with two types pesticides. Also, note that the drift tends to be greater with smaller droplets than with large droplets.

Table (12a): Effect used sprayer technique on drift work experiences at use avenue 70% WG (Imidacloprid).

Techniques	Equipment	Drift outside treatment								
		1m			2m			3m		
		VMD µm	N/cm²	% N	VMD µm	N/cm²	% N	VMD µm	N/cm²	% N
Centrifugal atomization	ULVA sprayer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydraulic atomization	Electric battery sprayer with Ss83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Conventional motor sprayer	80	33	51	76	18	28	35	14	21

Table (12b): Effect used sprayer technique on drift work experiences at use applaud 25% SC (Buprofezin).

Techniques	Equipment	Drift outside treatment								
		1m			2m			3m		
		VMD µm	N/cm²	% N	VMD µm	N/cm²	% N	VMD µm	N/cm²	% N
Centrifugal atomization	ULVA sprayer	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydraulic atomization	Electric Battery sprayer with Ss83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Conventional Motor Sprayer	95	29	48	88	22	37	46	9	15

4. The amount of pesticide deposits of the applicator:

The evaluation of the amount of pesticide deposits operator body for spray techniques was based on number of droplets on the operator body legs, chest (right and left) and head.

4.1. Effect using spray techniques on the amount of pesticide deposits of the operator's body with Avenue 70% WG (Imidacloprid).

Results obtained in Table (13) indicated that, the highest average number of droplets per cm² operator's head was 21 N/cm² for hydraulic atomization (Conventional motor sprayer), while the other machines are zero N/cm². While, the highest average number of droplets per cm² operator's chest (right and left) was (32 and 58 N/cm²) and (11 and 20 N/cm²) for hydraulic atomization (Conventional Hydraulic Sprayer), (Electric Battery sprayer with Ss83), respectively. The obtained results showed also, the highest average number of droplets per cm² on applicator legs, right and left were 53 and 45 droplets respectively by Electric Battery sprayer with Ss83. In case of the ULVA sprayer were 35 and 40, respectively. While, the Conventional Motor Sprayer (right 28 and left 30).

Table (13): Contamination of applicator produced by spray different techniques with avenue 70% WG (Imidacloprid) at (2017 – 2018) seasons.

Equipment	Spray Volume (L/fed)	N/cm² (on head)	N/cm² (on chest)		N/cm² (on legs)	
			Right	Left	Right	Left
Electric battery sprayer with Ss83	89.3	0.0	11	20	53	45
		0.0%	8.53%	15.51%	41.08%	34.88%
ULVA sprayer	18.4	0.0	0.0	0.0	35	40
		0.0%	0.0%	0.0%	46.67%	53.33%
Conventional motor sprayer	330	21	32	58	28	30
		12.43%	18.93%	34.32%	16.57%	17.75%

4.2. Effect using spray techniques on the amount of pesticide deposits of the operator's body with applaud 25 % SC (Buprofezin):

Results obtained in Table (14) indicated that, the highest average number of droplets per cm² operator's head was 15 No./cm² for hydraulic atomization (Conventional Motor Sprayer) while the other machines are zero No./cm². While, the highest average number of droplets per cm² operator's

chest (right and left) was (33and 68 N/cm²) and (0.0 and 11 N/cm²) hydraulic atomization (Conventional Motor Sprayer), (Electric Battery sprayer with Ss83) respectively. The obtained results show also, the highest average number of droplets per cm² on applicator legs, right and left were 48 and 59 droplets respectively by Electric Battery sprayer with Ss83. In case of the ULVA sprayer were 44 and 37 respectively. While, the Conventional Motor Sprayer (right 31 and left 33).

Table (14): Contamination of applicator produced by spray different techniques with applaud 25 % SC (Buprofezin) at (2017 – 2018) seasons.

Equipment	Spray Volume (L/fed)	N/cm² (on head)	N/cm² (on chest)		N/cm² (on legs)	
			Right	Left	Right	Left
Electric battery sprayer with Ss83	89.3	0.0	0.0	11	48	59
		0.0%	0.0%	9.3%	40.7%	50%
ULVA sprayer	18.4	0.0	0.0	0.0	44	37
		0.0%	0.0%	0.0%	54.3%	45.7%
Conventional motor sprayer	330	15	33	68	31	33
		8.3%	18.3%	37.8%	17.2%	18.3%

5. Comparison between spray techniques:

Reviewing the obtained results for the tested spray techniques, cleary show that the spray was mainly number of droplets (spray deposit) on the upper surface of the leaves, while the lower surface the least received number of droplets (spray deposit) comparing between three spray techniques had values of percentage spray coverage on squash plants, loss on land, drift and contamination. Results obtained in Table (15) indicated that, the highest percentage coverage squash plants were 57.5 and 47 for centrifugal atomization (ULVA sprayer) and hydraulic atomization (Electric battery sprayer with Ss83) respectively. While, the highest percentage of drift with Conventional Motor Sprayer was 13.6 % the percentage of contamination was 14.3 and 32.2 % for centrifugal atomization (ULVA sprayer) and hydraulic atomization (Electric battery sprayer with Ss83) respectively.

Table (15): Percent of number of droplets /cm² on targets produced by ground sprayer with applaud 25 % SC (Buprofezin) insecticide against whitefly and aphid at (2017 – 2018) seasons.

Equipment	ULVA Sprayer	Electric Battery sprayer with Ss83		Conventional Motor Sprayer	
Spray volume (L/fed)	18.4	89.3		330	
Dose rate	Recommended rate				
Droplets spectrum / Target	N/cm²	% N	N/cm²	% N	N/cm²	% N
Artificial	134	23.6	97	17.2	51	11.5
Plants	326	57.5	265	47	133	30.1
Loss of land	26	4.6	20	3.6	18	4.1
Drift	-	0.0	-	0.0	60	13.6
Contamination	81	14.3	182	32.2	180	40.7

Results obtained in Table (16) indicated that, the highest percentage coverage squash plants were 54.1 and 50% for centrifugal atomization (ULVA sprayer) and hydraulic atomization (Electric battery sprayer with Ss83)

respectively. While, the highest percentage of drift with conventional motor sprayer and electric battery sprayer with Ss83 was 14.9 and 0% respectively. Meanwhile, the lowest percentage of contamination was 13.1 and 26.3% for centrifugal atomization (ULVA sprayer) and hydraulic atomization (Electric battery sprayer with Ss83) respectively.

Table (16): Percent of number of droplets /cm² on targets produced by ground sprayer with Avenue 70% WG (Imidacloprid) insecticide against whitefly and aphid at (2017 – 2018) seasons.

Equipment	ULVA Sprayer		Electric battery sprayer with Ss83		Conventional motor sprayer	
Spray Volume (L/fed.)	18.4		89.3		330	
Dose rate	Recommended rate					
Droplets spectrum / Target	N/cm²	% N	N/cm²	% N	N/cm²	% N
Artificial	154	27	88	18	49	11.2
Plants	309	54.1	245	50	134	30.7
Loss of land	33	5.8	28	5.7	19	4.4
Drift	-	0.0	-	-	65	14.9
Contamination	75	13.1	129	26.3	169	38.8

6. Efficiency of the applied insecticides against aphids and whitefly infesting squash plants:

Data in Table (17) showed that applaud 25% SC (Buprofezin) and avenue 70% WG (Imidacloprid) exhibited the same trend. The two insecticides achieved similar reduction present against aphid *A.gossypii*. In addition, Buprofezin showed slight increase in reduction percent compared to imidacloprid. Table (17) showed that the initial effect (after 24 hours) conducted the least reduction percent compared to the other periods. While the greatest effect was obtained after 7 days of application, followed by 15 day and 3 day, respectively. Similar trend was observed with *B. tabaci* whitefly, two tested insecticides showed similar reduction percent profile. With the least reduction percent initial and greatest reduction percent after 7 days. Table (18) illustrated the significant difference between buprofezin and imidacloprid in both *gossypii* and *B. tabaci*. The date in the table clarified that, there were no significant difference between the applied insecticides in both insects (at level. 0.05).

6.1. Effect of the used equipment on insecticides efficiency:

Based on the mean effect of the tested insecticides with reference to the used equipment, the obtained data were tabulated in Table (19). The results clarified that, ULVA sprayer was the most efficiencies sprayer and proved the heighted reduction percent. followed by electric battery sprayer with Ss83 nozzle and finally the conventional motor sprayer. The previous finding was consistent with the two applied insecticides with either A *gossypii* or *B. tabaci*: our finding gave a similar trend in relation to the impact of application equipment.

Table (17): Effect of applaud 25% SC (Buprofezin) on aphid *Aphis gossypii* and whitefly *Bemisia tabaci* infesting squash plants with various ground application techniques during Seasons (2017/ 2018).

Pesticide	Date	ULVA Sprayer	Electric battery sprayer with Ss83	Conventional motor sprayer
Avenue 70% WG (Imidacloprid)	**Aphids**			
	1 day	54.30± 3.79	47.73±7.03	46.6± 2.70
	3 day	76.95± 2.19	69.40± 5.68	67.1±1.34
	7 day	91.75± 1.11	85.51 ±4.25	82.6±2.62
	15 day	82.50± 3.88	73.31±5.20	62.0±5.83
	Whitefly			
	1 day	52.71±9.49	46.88±4.67	44.43±5.48
	3 day	73.67±7.47	64.46±12.26	66.38±5.93
	7 day	87.97±3.71	75.83±13.50	80.63±3.08
	15 day	81.16±3.83	78.79±8.93`	61.03±6.07
Applaud 25% SC (Buprofezin)	**Aphid**			
	1 day	54.01±3.34	47.69±10.16	46.38±6.75
	3 day	74.99±8.15	70.13±5.39	69.07± 4.77
	7 day	88.46±4.39	88.54±2.92	82.84±2.90
	15 day	80.98±3.34	73.79±7.39	64.52±3.34
	Whitefly			
	1 day	51.56±9.56	46.54±7.40	43.73±7.23
	3 day	72.80±7.84	68.60±5.32	68.60±5.32
	7 day	86.83±4.64	80.06±3.93	80.06±3.93
	15 day	80.43±4.17	61.05±2.28	61.05±2.28

Table (18): Significant difference between buprofezin and imidacloprid.

Pests	Pesticide		Sig*
Aphid	Avenue 70% WG (Imidacloprid) 71.47(70.25-72.69)	Applaud 25% SC (Buprofezin) 72.43(71.12-73.64)	0.27
whitefly	Avenue 70% WG (Imidacloprid) 70.14(68.65-71.63)	Applaud 25% SC (Buprofezin) 70.68(69.20-72.16)	0.61

Table (19): Interaction between buprofezin pesticide and equipment on the mortality.

Pesticide	Equipment	Mean ± S. E	95% Confidence Limit
Applaud 25% SC (Buprofezin) aphid	ULVA Sprayer	74.61±1.33	(71.96-77.25)
	Electric Battery sprayer with Ss83	70.04±1.37	(67.32-72.75)
	Conventional Motor Sprayer	65.70±1.37	(62.99-68.42)
Applaud 25% SC (Buprofezin) whitefly	ULVA Sprayer	72.90±1.67	(69.59-76.22)
	Electric Battery sprayer with Ss83	68.03±1.67	(64.72-71.35)
	Conventional motor sprayer	63.36±1.67	(60.05-66.68)
Avenue 70% WG (Imidacloprid) aphids	ULVA Sprayer	76.62±1.33	(73.98- 79.27)
	Electric battery sprayer with Ss83	68.99±1.37	(66.27-71.70)
	Conventional motor sprayer	64.55±1.37	(61.83-67.26)
Avenue 70% WG (Imidacloprid) whitefly	ULVA sprayer	73.88±1.63	(70.64-77.11)
	Electric battery sprayer with Ss83	66.49±1.67	(63.18-69.81)
	Conventional motor sprayer	63.12±1.67	(59.80-66.43)

Table (20) explained the significant difference between the used equipment the statistical analysis explained that, there were significance difference between all the tested equipment. As a result, to the previous finding, the used equipment's affect and contribute significantly in the success of pest control.

Table (20): Significant difference between the used equipment.

(I) equipment	(J) equipment	Sig.[b]	
		Aphid	whitefly
Conventional motor sprayer	Electric battery sprayer with Ss83	.002	.018
	ULVA sprayer	.000	.000
Electric battery sprayer with Ss83	Conventional motor Sprayer	.002	.018
	ULVA sprayer	.000	.000
ULVA sprayer	Conventional motor sprayer	.000	.000
	Electric battery sprayer with Ss83	.000	.000

6.2. Significance of the main factors:

Table (21) showed that in either aphids or whitefly the tested pesticides exhibited non-significance difference with P value of 0.276 and 0.611 for aphids and whitefly, respectively.

Oppositely, equipment exhibited high significance difference with P value of 0.00 and 0.00 for the insects. Similarly, Time showed the same degree of significance with both insects.

The interaction between pesticides, equipment and time Table (21) exhibited non-significant difference with both aphid and whitefly with P value of 0.10 and 0.24 for aphids and whitefly, respectively.

Table (21): Significant difference between machines, time and pesticide.

Pesticide	0.276	0.611
Equipment	0.000	0.000
Time	0.000	0.000
Pesticide * Equipment * Time	0.10	0.24

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Effects of neonicotinoids and sulfoxaflor application against sucking pests infesting watermelon and their associated predators

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Abstract:

Field studies were carried out during 2018 and 2019 summer seasons, at Nubarya district, El-Beheira Governorate to evaluate the effects of some neonicotinoid insecticides (Imidacloprid, clothianidin, thiamethoxam and acetamiprid) compared with sulfoxaflor, at recommended rates against sucking pests infesting watermelon and their associated predators. Results showed that, all treatments exhibited excellent and fast action activity against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and the least reduction percentages were recorded by acetamiprid at both seasons. Under the same conditions, neonicotinoid insecticides have toxic effect on predators; *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Paederus alfierii* Koch. (Coleoptera: Staphylinidae) and *Coccinella* spp.) (Coleoptera: Coccinellidae) while, sulfoxaflor has slightly toxic effect. The present study suggests neonicotinoid insecticides can be disruptive to natural and biological control by reducing insect predators populations, so the population of *Tetranychus urticae* Koch. (Acari: Tetranychidae) will be increased during both seasons. Yet, sulfoxaflor is also reported as being slightly harmful to biological control agents, it as a preferred insecticide, with less harmful effects on the fitness components of natural enemies, for integrated pest management of sucking insects (*B. tabaci*) on watermelon plantations.

Introduction

Watermelon, a popular summer vegetable crop worldwide (Wu *et al*., 2014) is an important crop and provides phytochemicals. However, watermelon is susceptible to numerous diseases and pests, such as *Tetranychus urticae* Koch. (Acari: Tetranychidae), *Aphis gossypii* Glover (Hemiptera: Aphididae), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Spodoptera* sp. (Lepidoptera: Noctuidae) larvae (Wu *et al*., 2012). The sweet potato whitefly, *B. tabaci* attacks watermelon and a wide range of other plant species and on a global scale. In addition to injuries from direct feeding, problems from this pest are intensified because its vectors

over 100 plant viruses (Jones, 2003 and Simmons *et al*., 2010). As it is farmed primarily by protected and successive cultivation techniques, many pesticides are required for the control of pests (Park *et al.*, 2010).

Neonicotinoid insecticides represent the fastest-growing class of insecticides introduced to the market since the launch of pyrethroids (Nauen and Bretschneider, 2002) and are the most used class of insecticides for controlling sucking insects (Jiang *et al.*, 2019). Neonicotinoids interfere with the nicotinic acetylcholine receptor and therefore have specific activity against the insect nervous system (Maienfisch *et al*., 2001). It is considered an important group of insecticides being used against sucking insects for several years (Muhammad *et al.*, 2011), especially active on hemipteran pest species such as aphids, whiteflies, thrips, leaf miners and plant hoppers, but also commercialized to control many coleopteran and some lepidopteran pest species (Elbert *et al*., 1998 and Nauen *et al.*, 2003). Due to the potent systemic characteristics, they can be absorbed via the roots and transferred to almost all parts of targeted crops (Jeschke and Nauen, 2008). But this irreversible uniting effect may not vary much between target and non-target species, inducing similar detrimental impacts on the biocontrol agents (predators) (Cloyd and Bethke, 2011). Currently, global concerns about the negative influence of neonicotinoids on non-target organisms (particularly bees) and human have led to the regulation by the European Union (EU) since 2013, to date, the use of three typical neonicotinoids i.e. imidacloprid, thiamethoxam and clothianidin has been totally banned on field crops by EU (Jiang *et al.*, 2019).

The sulfoximines are a new class of insecticides targeting sap-feeding insects including the aphids, whiteflies, hoppers, and lygus (Nawaz *et al*., 2018; Babcock *et al*., 2011 and Zhu *et al*., 2011), that are resistant to other classes of insecticides, and that are resistant to the neonicotinoids (Sparks *et al*., 2013). Sulfoxaflor is the initial compound in this class selected for commercial development and is an agonist at insect nicotinic acetylcholine receptors (nAChRs) (Liao *et al*.,2017; Watson *et al*., 2017 and Sparks *et al*., 2013). Yet, sulfoxaflor is also reported as being slightly harmful to biological control agents, including *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) (Sparks *et al.* ,2013; Wanumen *et al*., 2016 and Nawaz *et al*., 2018).

Therefore, two field experiments were carried out during 2018 and 2019 summer seasons, at Nubarya district, El-Beheira Governorate to evaluate the side effect of sulfoxaflor and some neonicotinoids treatment against sucking pests infesting watermelon and their associated predators at recommended rates.

Materials and methods

1.Tested compounds:

Sulfoxaflor (Closer 24% SC) was provided by Dow Agro Sciences Co., Ltd. Imidacloprid (Gaucho® 70%WS) was provided by Bayer Crop Science. Clothianidin (Supertox-1® 48%SC) was provided by Jiangs Jiag chemical industry Co. Ltd China. Thiamethoxam (Actara 25% WG) provided by Syngenta Company. Acetamiprid (Mospilan 20% SP) provided by Nippon Soda Chemical Industry Co. Ltd.

2. Field trials:

Field experiments were carried out throughout two successive seasons (2018 and 2019) during summer plantation in Nubarya district, El-Beheira Governorate. These experiments were cultivated with watermelon. The experimental site was divided into 24 plots, each plot 1/100 feddan (42m²). Randomized complete blocks design was used with four replicates for each treatment with the control plots. Field concentrations were 40ml, 60gm, 1000ml, 60gm and 50gm/200 liter per feddan for sulfoxaflor, imidacloprid, clothianidin, thiamethoxam, and acetamiprid, respectively. The insecticides were sprayed by Knapsack sprayer equipment (CP3). For counting the numbers of whiteflies, *B. tabaci* (immature stages), and *T. urticae*, samples of 25 leaves (from three different levels of the plants) were collected at random in the morning for both diagonals of the inner square area of each experimental plot. Pre-treatment counts were done in the early morning just before application while post-treatment counts were done on 1, 4, 7and 10 days after treatment. In the same time, sample of 25 watermelon plants were examined and the number of the aphid lion, *C. carnea*, the rove beetle, *Paederus alfierii* and the lady birds, *Coccinella* spp. were counted. Counts were done by the lenses in the early morning when flight activity is minimal according to **Butler *et al.* (1988)**. Percentage of pest reduction numbers were calculated according to **Henderson and Tilton equation (1955)** and subjected to analysis of variance (ANOVA) **(CoStat Statistical software,1998).**

Results and discussion

In this study, field evaluation of some insecticides treatments against *B. tabaci* immature stages on watermelon plantation at 2018 and 2019 seasons was carried out. The % reductions of *B. tabaci* caused by sulfoxaflor, imidacloprid, clothianidin, thiamethoxam, and acetamiprid formulation were summarized in Table (1). Mean of % reduction was 95.47, 95.35, 91.25, 92.87 and 85.25%, respectively at 2018, while were 95.62, 91.17, 93.27, 92.97 and 79.72%, respectively at 2019 season. In both seasons, the highest reduction percentages were achieved sulfoxaflor where the least reduction percentages were recorded by acetamiprid.

Table (1): Efficacy of certain treatments against *Bemisia tabaci* immature stages on watermelon plantations.

Season	Tested compounds	Rate / feddan	%Reduction After				
			1-day	4-days	7-days	10-days	Mean
2018	Sulfoxaflor	40ml	85.4	96.5	100.0	100.0	95.47a
	Imidacloprid	60g	88.5	94.5	98.4	100.0	95.35a
	Clothianidin	1000ml	82.2	92.3	96.5	94.0	91.25b
	Thiamethoxam	60g	81.5	94.2	97.4	98.4	92.87b
	Acetamiprid	50ml	77.4	85.1	88.7	89.8	85.25c
2019	Sulfoxaflor	40ml	88.1	94.4	100.0	100.0	95.62a
	Imidacloprid	60g	78.2	90.2	96.3	100.0	91.17a
	Clothianidin	1000ml	80.5	96.3	100.0	96.3	93.27a
	Thiamethoxam	60g	81.3	100.0	96.3	94.3	92.97a
	Acetamiprid	50ml	72.3	80.0	84.3	82.3	79.72b

Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$ for the same season.

This result indicates that, neonicotinoids provides excellent control *B. tabaci* (Kuhar *et al*., 2002). Muhammad *et al*. (2011 and 2013) reported that, *B. tabaci* has developed resistance to some of neonicotinoids. Sulfoxaflor is also effective against a wide range of sap-feeding insect pests that are resistant to other classes of insecticides, including many that are resistant to the neonicotinoids (Zhu *et al*., 2011; Sparks *et al*., 2013; Jeschke *et al*., 2015; Liao *et al*., 2017 and Wang *et al*., 2017).

In this study, field evaluation of the side effect of certain treatments against some predators (*C. carnea, P. alfierii and Coccinella* spp.*)* and spider mites (*T. urticae*) on watermelon plantations at 2018 and 2019 seasons.

Data from Tables (2, 3 and 4) indicated that, reduction percentages of *C. carnea*, *P. alfierii* and *Coccinella* spp. caused by sulfoxaflor, imidacloprid, clothianidin, thiamethoxam, and acetamiprid. For *C. carnea* were 31.47, 49.55, 47.67, 44.55 and 37.50%, respectively at 2018 and 30.15, 48.85, 54.52, 43.10and 33.90%, respectively at 2019, and for *P. alfierii* were 28.35, 28.90, 43.00, 42.75 and 38.17, respectively at 2018 and 35.85, 49.37, 51.15, 47.27 and 45.47%, respectively at 2019. While reduction percentages of *Coccinella* spp. caused by sulfoxaflor, flupyradifurone, clothianidin, thiamethoxam, and acetamiprid were 29.60, 34.50, 47.77, 45.80 and 31.77%, respectively at 2018 and 39.20, 51.55, 53.55, 52.02 and 46.85%, respectively at 2019. Concerning data, all treatments have toxic effect on natural enemies except sulfoxaflor have slightly toxic effect.

Table (2): Side effect of certain treatments against *Chysoperla carnea* on watermelon plantations.

Season	Tested compounds	Rate / feddan	%Reduction After				
			1-day	4-days	7-days	10-days	Mean
2018	Sulfoxaflor	40ml	32.4	34.3	30.4	28.8	31.47c
	Imidacloprid	60g	44.4	52.6	52.6	48.6	49.55a
	Clothianidin	1000ml	42.5	55.3	48.2	44.7	47.67ab
	Thiamethoxam	60g	45.5	45.5	54.5	32.7	44.55ab
	Acetamiprid	50ml	34.4	40.2	36.3	39.1	37.50bc
2019	Sulfoxaflor	40ml	28.2	33.6	32.4	26.4	30.15d
	Imidacloprid	60g	44.6	52.6	50.0	48.2	48.85b
	Clothianidin	1000ml	55.3	60.2	52.6	50.0	54.52a
	Thiamethoxam	60g	39.1	42.4	46.5	44.4	43.10c
	Acetamiprid	50ml	32.2	44.6	28.4	30.4	33.90d

Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$ for the same season.

Table (3): Side effect of certain treatments against *Paederus alfierii* on watermelon plantations.

Season	Tested compounds	Rate / feddan	%Reduction After				
			1-day	4-days	7-days	10-days	Mean
2018	Sulfoxaflor	40ml	26.3	34.4	28.2	24.5	28.35c
	Imidacloprid	60g	30.2	34.4	26.6	24.4	28.90c
	Clothianidin	1000ml	44.5	46.5	42.4	38.6	43.00a
	Thiamethoxam	60g	40.0	46.4	44.4	40.2	42.75a
	Acetamiprid	50ml	33.3	42.5	40.5	36.4	38.17b
2019	Sulfoxaflor	40ml	32.2	34.4	40.2	36.6	35.85c
	Imidacloprid	60g	42.5	50.0	50.0	55.0	49.37ab
	Clothianidin	1000ml	50.0	56.4	50.0	48.2	51.15a
	Thiamethoxam	60g	46.5	50.0	48.2	44.4	47.27ab
	Acetamiprid	50ml	40.2	45.3	50.0	46.4	45.47b

Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$ for the same season.

Insecticides can be disruptive to natural and biological control by reducing natural enemy populations (Johnson and Tabashnik, 1999 and Nasr and Keratum, 2009). Our results were comparable with Rizk *et al*. (1999) and Omar and El-Kholy (2001), where they reported that, the possibility of controlling sucking pests by a combination of biological and chemical methods had proved to be less costly, safe on the environmental constituents. Neonicotinoid insecticides are considered an important group of insecticides being used against sucking, but also commercialized to control many coleopteran and some lepidopteran pest species. But this irreversible uniting effect may not vary much between target and non-target species (predators) (Cloyd and Bethke, 2011).

Table (4): Side effect of certain treatments against *Coccinella* spp. on watermelon plantations.

Season	Tested compounds	Rate / feddan	%Reduction After				
			1-day	4-days	7-days	10-days	Mean
2018	Sulfoxaflor	40ml	22.2	28.4	34.4	33.4	29.60c
	Imidacloprid	60g	32.5	35.4	36.6	33.5	34.50b
	Clothianidin	1000ml	44.5	48.2	50.0	48.4	47.77a
	Thiamethoxam	60g	40.2	50.0	48.6	44.4	45.80a
	Acetamiprid	50ml	28.4	32.5	34.0	32.2	31.77bc
2019	Sulfoxaflor	40ml	38.4	42.4	40.0	36.0	39.20c
	Imidacloprid	60g	50.0	55.3	52.5	48.4	51.55a
	Clothianidin	1000ml	52.5	55.4	56.3	50.0	53.55a
	Thiamethoxam	60g	46.5	52.2	57.1	52.3	52.02a
	Acetamiprid	50ml	44.6	48.2	48.2	46.4	46.85b

Means within the same column followed by the same letters are not significantly different according to the $LSD_{0.05}$ for the same season.

Predators are very effective and practical in biological control programs against sucking insect pests such as, *C. carnea* and *Coccinella* spp. (Brook and Barnard, 1990). Sparks *et al.* (2013); Wanumen *et al*. (2016) and Nawaz *et al*. (2018) reported that, sulfoxaflor is slightly harmful to biological control agents, including, *C. carnea* and *Clitemnestra bipunctata* (Say) (Hymenoptera: Crabronidae).

Neonicotinoids have negative impact coccinellids through several routes of entry, including: topical contact, residual contact, inhalation of volatiles, ingestion of toxified plant products and ingestion

of toxified prey tissues (Ruberson *et al*., 1998; Johnson and Tabashnik, 1999 and Moser and Obrycki, 2009).

The field evaluation of the side effect of certain treatments against *T. urticae* on watermelon plantations at 2018 and 2019 seasons was carried out (Figures, 1 and 2). All treatments have not toxic effect on spider mites at a long time, except sulfoxaflor have slightly toxic effect at short time period. In both seasons, the highest mean numbers of *T. urticae* on watermelon plantations achieved by clothianidin where 91.1 and 96.5/25 leaves at were 2018 and 2019, respectively. The least mean numbers recorded at untreated plants followed by sulfoxaflor and acetamiprid at the both of seasons.

Biological control approach is considered as a main component of the integrated pest management programs (IPM). Natural enemies are usually efficient in regulating population of pests, especially in balanced ecosystem. Pesticides alone will not solve the problem for controlling pests. Insecticides can be disruptive to natural and biological control by reducing natural enemy populations (Johnson and Tabashnik, 1999), so the population of *T. urticae* on watermelon will be increased. *C. carnea, P. alfierii and Coccinella* spp. are known that aphidophagous, consume different food types because aphids are abundant only during a restricted time period. Besides this there are other arthropod prey items documented in the literature, e.g. Acari, Thysanoptera, and larvae of Diptera, Coleoptera, and Lepidoptera (Hodek, 1967, 1970 and Singh *et al*., 1991).

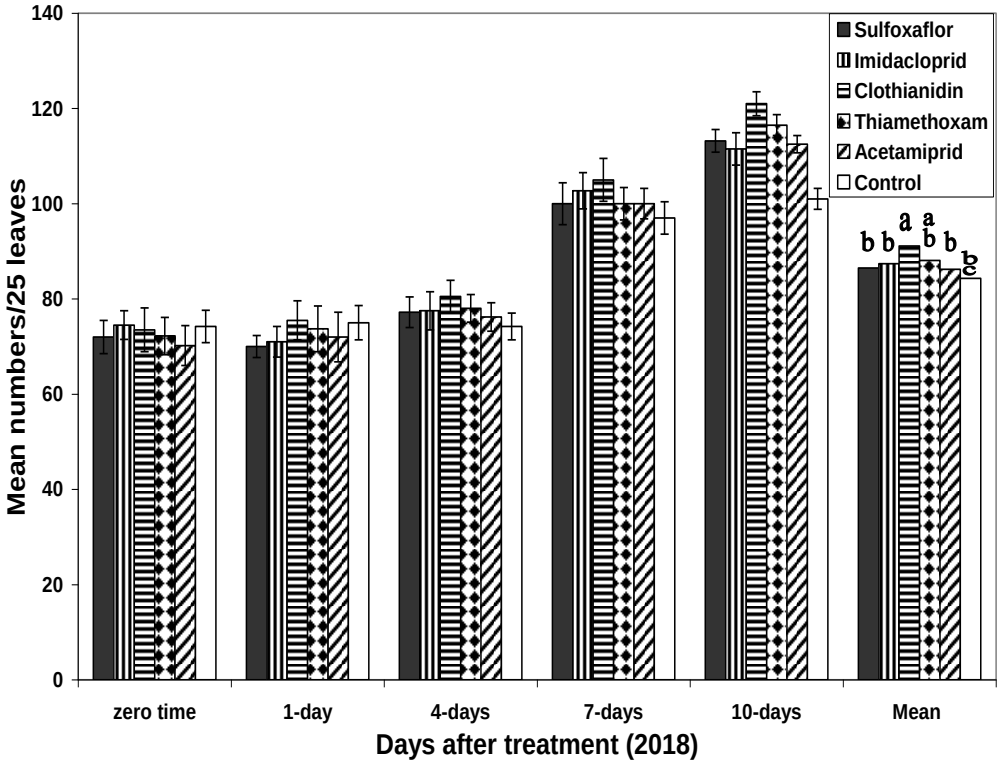

Figure (1): Side effect of certain treatments against *Tetranychus urticae* on watermelon plantations during 2018 season.

Error bars represent standard deviation of four replications. Columns within a group with the same letter are not significantly different according to (LSD at P<0.05).

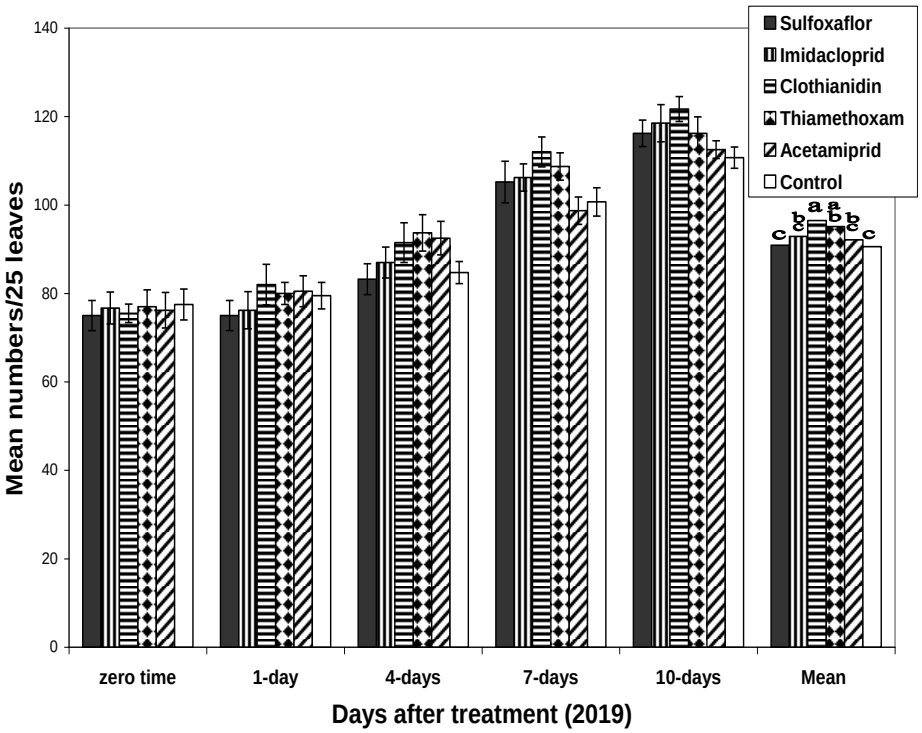

Figure (2): Side effect of certain treatments against *Tetranychus urticae* on watermelon plantations during 2019 season.

Error bars represent standard deviation of four replications. Columns within a group with the same letter are not significantly different according to (LSD at P<0.05).

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Monthly and seasonal fluctuations study of some harmful birds in old lands at Sohag Governorate

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Abstract:

The present work was carried out the effect of habitat types and daytime of the population density of house sparrow, *Passer domesticus niloticus* (L.) (Passeriformes: Passeridae) , hooded crow, *Corvus corone sardonius* (L) (Passeriformes:Corvidae) and palm dove, *Streptopelia senegalensis egyptica* (L.) (Columbiformes: Columbidae) at Tahta district, Sohag Governorate were studies in four major habitat nearby (buildings, field crops, trees and water canals). The results revealed that the highest value of population density of *P. domesticus niloticus*; *C. corone sardonius* and *S. senegalensis egyptica* was recorded in fields nearby trees (54.88, 5.25 and 3.21 birds). Followed by buildings (48.13, 4.50 and 2.42 birds). Then the lowest value was recorded in fields nearby field crops of *P. domesticus niloticus* and *S. senegalensis egyptica* (23.13 and 0.75 birds) and fields near by water canals of *C. corone sardonius* (1.46 birds). The yearly population trend *P. domesticus niloticus* indicated the presence of three major peak of abundance. The first peak was recorded in March, April and May (58.25, 43.38 and 47.25 birds). The second beak was recorded in September (100.38 birds) and third beak was recorded in December (94.50 birds). The highest value of *P. domesticus niloticus* was recorded during autumn (48.29 birds). The yearly population trend of *C. corone sardonius* indicated the presence of one major peak of abundance. Peak was recorded in December, October and November (12.38, 9.25 and 9.75 birds). The highest value of population *C. corone sardonius* was recorded during autumn (10.46 birds). The highest values were recorded in two months October and December (4.38 and 5.00 birds), followed by September and November (3.50 and 2.63 birds). The highest value of population was recorded during autumn (4.29 birds).

Introduction

Agricultural ornithology aims to obtain scientific information on birds in relation to agriculture and use this information for their management. Most of bird species play a useful role in agriculture by having a potent check on insect and rodent pests. Birds are a group of Animals following to Subkingdom

Metazoa, Phylum Chordata, Class Aves and Subclass Newornithes. Class Aves divided into 19 orders of which the order Passeriformes, which consists of 56 family and 5000 species, this order contain different species existing in different habitats in Egypt. Among them, some birds beneficial and harmful birds. Also, it was divided to resident and migratory birds. In Egypt, the number of bird species were 515 the resident birds are 186 bird species, 12 are extinct and 17 are endemic. The rest of bird species and subspecies 300 bird species are migratory (Tharwat, 1997). Birds dominated the air and managed to invade a lot of different environments, whether land or water due to their unique anatomical and morphological structure. These make the existence of factions in the movement of permanent and continuous environment, to others and from country to country. For example, house sparrow, *Passer domesticus niloticus* (L.) (Passeriformes: Passeridae) , hooded crow, *Corvus corone sardonius* (L) (Passeriformes:Corvidae) and palm dove, *Streptopelia senegalensis egyptica* (L.) (Columbiformes: Columbidae) were the resident birds in Egypt during all seasons of year (Metwally *et al*., 2009 and Omar , 2010). The present work was done in the fields of old lands at Tahta district at Sohag Governorate in order to study the monthly and seasonal fluctuation of some harmful bird species.

Materials and methods

These studies were carried out under the field conditions in old lands at Tahta districts at Sohag Governorate. The work it has been conducted at four different habitats representing different environmental and ecological areas. These habitats were nearby each of (buildings, field crops, trees and water canals). The field trails started from April 2014 to March 2015. The resident bird species were surveyed two feddans inside the chosen cultivated habitat. Number of the different bird species was counted in each habitat, by using the method of Redinger and Libay (1979) as a plot equivalent two feddans from the determined cultivated area in each habitat. The identification and counts of bird species were achieved by using field glass (binoculars) from rising position, which gave clear sighted vision of the plots. This work has been accomplished twice daily, the first at sunrise and second at sunset during one hour for four days monthly. Bird classification were carried out according to Sibley and Monoroe (1990) under review by the checklist committee of the American Ornithologists Union (A. O. U.) were followed in bird classification. The population fluctuations of bird species were studied monthly daytime (Sunrise and Sunset) at four different habitats which were mentioned above to find the relationship between population of bird species and different seasons of year. The population fluctuation and daily activities of dominant harmful bird species (i.e. house sparrow, *P. domesticus niloticus* (L.), hooded crow, *C. corone sardonius* (L.) and palm dove, *S. senegalensis egyptica* (L.).

Data obtained were statistically analyzed using a randomized complete block design. Means were compared according to Duncan's Multiple Range test, at 0.05 level of probability.

Results and discussion

The effect of habitat types and daytime of the population density of house sparrow, *P.domesticus niloticus* , hooded crow, *C. corone sardonius* and palm dove, *S. senegalensis egyptica* in Tahta district at Sohag Governorate were studies in four major habitat nearby (buildings, field crops, trees and water canals) from April 2014 to March 2015.

1. Population fluctuation of house sparrow, *Passer domesticus niloticus*:

Data in Table (1) showed the highest value was recorded in fields nearby trees (54.88 birds). Followed by buildings (48.13 birds). Then the lowest value was recorded in fields nearby field crops and fields near by water canals with means (23.13 and 28.08 birds). The statistically analysis for means indicated that there were highly significant differences between the fields nearby trees and fields nearby field crops and water canals. The allow level of abundance during June with mean (3.50 birds). While there were no individuals recorded during July. Through the next months, August and September, the population trend of increase gradually and reached to relatively high levels of abundance with means (15.50 birds) and (100.38 birds), respectively. After wards, the population density trends to decrease gradually for two months October and November with means (37.50 and 12.88 birds). Regarding to the general means, the highest value was recorded during December with mean (94.50 birds). The statistically analysis for September and December the results indicated that there were highly significant differences between other months of the study period. Throw, the population of sparrows decreased during January (7.50 birds). Then the population started to grow up slightly for four months February and March with means (42.00 and 58.25 birds), respectively. Generally, the yearly population trend of house sparrow indicated the presence of three major peak of abundance. The first peak was recorded in March, April and May. This may be due to the adult sparrows starting in nesting and reproduction season. Or the appearance of wheat ears and broad bean horns till the harvest during this period. On the other hand, the second beak was recorded in September; this may be due to the appearance of the head sorghum crops in the studied areas. Faunally, peak was recorded in December; this may be due to the planting of wheat crops during this month and the sparrows starting of stay nests and reproduction season. With aspects, the seasonal fluctuation of house sparrow birds in Tahta district. Data in Table (2) showed the highest value of population abundance of house sparrow birds were in autumn with mean (48.29 birds). Moderate numbers of house sparrow were recorded in summer and winter (38.63 and ٣٥,٩٢ birds). While, the low level of population was recorded in spring (31.38 birds). **Mosallm (2017)**

reported that seasonal fluctuation of the highest values of house sparrow birds during summer with following by spring and autumn seasons. While the lowest value was recorded during winter.

Table (1): Monthly of population fluctuation of house sparrow, *Passer domesticus niloticus* at Tahta district, Sohag Governorate during 2014/2015.

Month	Different habitats				Mean*
	Buildings	Field crops	Trees	Water canals	
Apr.	68.00	19.50	56.50	29.50	**43.38bcde**
May	67.00	22.00	76.00	24.00	**47.25bc**
Jun.	0.00	0.00	0.00	14.00	**3.50 ef**
Jul.	0.00	0.00	0.00	0.00	**0.00f**
Aug.	29.50	0.00	32.50	0.00	**15.50cdef**
Sep.	135.50	51.50	175.00	39.50	**100.38a**
oct.	0.00	47.50	69.50	33.00	**37.50bcdef**
Nov.	0.00	0.00	0.00	51.50	**12.88cdef**
Dec.	137.50	71.50	122.50	46.50	**94.50a**
Jan.	0.00	0.00	0.00	30.00	**7.50cdef**
Feb.	61.50	23.00	47.50	36.00	**42.00bcdef**
Mar.	78.50	42.5	79.00	33.00	**58.25bcd**
Mean	**48.13ab**	**23.13b**	**54.88a**	**28.08b**	

* **Means have the same are not significantly differed by using Duncan's analysis**.

Table (2): Seasonal fluctuation of house sparrow, *Passer domesticus niloticus* (L.) at Tahta district, Sohag Governorate from 2014 to 2015.

Season	Different habitats				Mean*
	Buildings	Field crops	Trees	Water canals	
Spring	45.00	13.83	44.17	22.50	**31.38b**
Summer	55.00	17.17	69.17	13.17	**38.63ab**
Autumn	45.83	39.67	64.00	43.67	**48,29a**
Winter	46.67	21.83	42.17	33.00	**35.92ab**
Mean	**48.13a**	**23.13b**	**54.88a**	**29.09b**	

* **Means have the same are not significantly differed by using Duncan's analysis.**

2. Population fluctuation of hooded crow, *Corvus corone sardonius*:

Data in Table (3) showed the highest value was recorded in fields nearby trees (5.25 birds) followed by fields nearby buildings and field crops (4.50 and 3.42 birds). While, the lowest value of population was recorded in fields nearby water canals with mean (1.46 birds). The highest value of population abundance was recorded during December with (12.38 birds). Followed by October and November (9.25 and 9.75 birds). Moderate value of population abundance of hooded crow was recorded during September with (5.25 birds) followed by January and February (2.13 and 1.50 birds). The lowest value of population abundance of hooded crow was recorded during months, April, May, June, July, August and March (0.75, 0.75, 0.88, 0.38, 0.13 and 0.75 birds), respectively. Generally, the yearly population trend of hooded crow indicated the presence of one major peak of abundance. Peak was recorded in December, November and October. This may be due to the appearance of maize and sunflower crops horns, till the harvest during this period.

With respect to seasonal fluctuation of hooded crow birds in Tahta district at Sohag Governorate. Data in Table (4) showed the highest value of population Hooded crow was recorded during autumn with mean (10.46 birds).

Moderate numbers were during summer and winter (1.92 and 1.46 birds). While the lowest value was recorded during spring (0.79 birds). Bonnah (2007) studied the population density of hooded crow, *C. corone sardonius* occurred all-over the year in Sohag Governorate. The population density of each month expressed in terms of percent of individual numbers from their overall year grand total. El-Danasory (2006) stated that the population fluctuation of dominant bird species viz., house sparrow, *P. domesticus niloticus* and hooded crow, *C. corone cornix* at El-Behira Governorate and El-Menofia Governorate were studied in five major locations (near buildings, near orchards, near trees and near water canals).

Table (3): Monthly of Population fluctuation of hooded crow, *Corvus corone sardonius* (L.) at Tahta district, Sohag Governorate during 2014/2015.

Month	Different habitats				Mean*
	Buildings	Field crops	Trees	Water canals	
Apr.	0.50	0.00	1.50	1.00	**0.75e**
May	0.00	0.00	3.00	0.00	**0.75e**
Jun.	0.50	0.00	3.00	0.00	**0.88e**
Jul.	0.00	0.50	0.00	1.00	**0.38e**
Aug.	0.00	0.00	0.00	0.50	**0.13e**
Sep.	7.00	6.00	6.50	1.50	**5.25cd**
oct.	12.00	9.50	1200	3.50	**9.25ab**
Nov.	12.50	11.00	11.50	4.00	**9.75ab**
Dec.	17.00	14.00	1500	3.50	**12.38a**
Jan	2.50	0.00	3.50	2.50	**2.13de**
Feb.	1.50	0.00	4.50	0.00	**1.50de**
Mar.	0.50	0.00	2.50	0.00	**0.75e**
Mean	**4.50ab**	**3.42ab**	**5.25a**	**1.46b**	

*** Means have the same are not significantly differed by using Duncan's analysis.**

Table (4): Seasonal fluctuation of hooded crow, *Corvus corone sardonius* (L.) at Tahta district, Sohag Governorate from 2014 to 2016.

Season	Different habitats				Mean*
	Buildings	Field crops	Trees	Water canals	
Spring	0.33	0.00	2.50	0.33	**0.79b**
Summer	2.33	2.17	2.17	1.00	**1.92b**
Autumn	13.83	11.50	12.83	3.67	**10.46a**
Winter	1.50	0.00	3.50	0.83	**1.46b**
Mean	**4.50ab**	**3.42b**	**5.25a**	**1.47b**	

*** Means have the same are not significantly differed by using Duncan's analysis**

3. Population fluctuation of palm dove, *Streptopelia senegalensis egyptica*:

Data in Table (5) showed the highest value of palm dove were in fields nearby trees with mean (3.21 birds). Followed by in fields nearby building (2.42 birds). The moderate number was recorded in fields nearby water canals (1.38 birds). While, the lowest value of palm dove was in fields nearby field crops (0.75 birds). The monthly population abundance of palm dove. The lowest values were recorded in May, July, February and March (0.63, 0.75, 0.75 and 0.88 birds), respectively. The moderate values were in April, June and January (1.63, 1.50 and 1.63 birds), with insignificant differences between them. While, the highest values were recorded in two months October and December (4.38 and 5.00 birds), followed by September and November (3.50 and 2.63

birds).

The highest value of population was recorded during autumn (4.29 birds). While, the lowest values were during winter, summer and spring, (1.09, 1.13 and 1.25 birds) (Table,6).

Omar (2010) stated that the population of palm dove birds was more abundant during summer season. Spring ranked the second order regarding the population for the studied year. The minimum numbers were recorded during winter season. Noura-Barakat (2016) studied that the highest values of palm dove, *S. senegalensis* were in field nearby buildings (2.437). While the lowest values were in field nearby field crops (0.562). El-Sawy (2017) revealed that the high average number of population fluctuation of palm dove, *S. senegalensis* in different habitats during (December 2013 and November 2015) recorded with (24.00 and 22.50 birds), during (June and May 2014) nearby water canals and trees habitats respectively, while the low level of population was recorded during (April 2014) nearby trees and water canals, as well as with mean number (0.5 birds) during (November 2014 and March 2015) nearby buildings and field crops respectively.

Table (5): The monthly population abundance of palm dove *Streptopelia senegalensis egyptica* at Tahta district, Sohag Governorate during 2014/2015

Month	Different habitats				Mean*
	Buildings	Field crops	Trees	Water canals	
Apr.	2.00	0.50	2.50	1.50	**1.63 bcde**
May	0.00	0.50	0.00	2.00	**0.63 cde**
Jun.	2.00	0.50	3.50	0.00	**1.50 bcde**
Jul.	0.00	0.00	0.00	3.00	**0.75 cde**
Aug.	0.00	0.00	0.00	0.00	**0e**
Sep.	4.00	2.00	4.50	0.00	**2.63abcde**
Oct.	5.00	2.50	7.50	2.50	**4.38 ab**
Nov.	6.00	1.00	7.00	0.00	**3.50 abcd**
Dec.	7.00	1.50	8.50	3.00	**5 .00a**
Jan	2.00	0.00	2.00	2.50	**1.63 bcde**
Feb.	0.00	0.00	1.00	2.00	**0.75 cde**
Mar.	1.00	0.50	2.00	0.00	**0.88 cde**
Mean	**2.42ab**	**0.75b**	**3.21a**	**1.38b**	

* Means have the same are not significantly differed by using Duncan's analysis

Table (6): The seasonal population abundance of palm dove *Streptopelia senegalensis egyptica* at Tahta district, Sohag Governorate during 2014/2015

Season	Different habitats				Mean*
	Buildings	Field crops	Trees	Water canals	
Spring	1.33	0.50	2.00	1.17	**1.25b**
Summer	1.33	0.67	1.50	1.00	**1.13b**
Autumn	6.00	1.67	7.67	1.83	**4.29a**
Winter	1.00	0.17	1.67	1.50	**1.09b**
Mean	**2.42ab**	**0.75b**	**3.21a**	**1.38b**	

*** Means have the same are not significantly differed by using Duncan's analysis**

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Effects of fungicides use in wheat fields on the damage caused by house sparrow *Passer domesticus niloticus* (Passeriformes: Passeridae) at Assiut Governorate

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Control, repellency, house sparrow, wheat and fungicides.

Abstract:

This experiment was carried out during the ripening stages of wheat crop. The trial was aimed to evaluate the effectiveness of different application rates of punch 40% EC., recommended for controlling some wheat pests, as repellent compounds. Two schedules of spray programme were performed to protect wheat spikes from birds, e.g. house sparrow *Passer domesticus niloticus* (L.) (Passeriformes: Passeridae), attack and reduce birds damage. The results revealed that there were significant differences among concentrations of punch. The highest protection performance was exhibited by using the recommendation rate of pesticide for controlling pests. The protection indices (PI%) of tested fungicides were when applied punch (18.75 cm^3/100L.water). The following highest protection indices was attained by applying the second concentration for punch (12.50cm^3/100L.water). The lowest values of protection indices were when applied (6.25 cm^3/100L.water). The results showed that increasing the rate of punch application, resulted to increasing of repellency and protection performance to the wheat spikes during ripening stage.

Introduction

The house sparrow *Passer domesticus niloticus* (L.) (Passeriformes: Passeridae) enjoys a world-wide distribution and affects a variety of habitat types under a wide range of climatic conditions. Now, the house sparrow is thought to be one of the important vertebrate pests for cereal crops, human habitations and wildlife in Egypt. During certain seasons of the year it forages in the cropland in large numbers. Such foraging flocks damage the standing crops to a great extent. Damage caused by house sparrow birds is one of the problems facing many farmers in Egypt. However, the amount of crop lost, and the economic damage sustained is largely unquantified. As the house

sparrow has great predilection for maturing seeds, it inflicts great damage on the maturing crops of wheat. Infect, sparrows damage to wheat crop represents a serious problem as the losses reach up to 14% of the yield (Soliman, 1993.; Wilson *et al*., 1995 and Omar, 2005).

On the other side, it is considered as natural enemies to harmful insects when they feed on them in considerable amounts. In this study, our aim was to reduce the damage caused by house sparrow birds attack for the wheat plants. Due to the nature of these birds and the speed of movement, they are struggling in various ways from the rest of agricultural pests, such as: fishing net, or by noise forced to flee, the destruction of nests, the cultivation of crops on non-economic important crops to feed them, Lack of grain storage in the open, and the use of pesticides is the most effective in the rapid elimination of the lesion, but the wrong use with increase in the number of times of use, and high concentrations of some has led to increased pollution levels, and the accumulation of harmful pesticide residues in human food in the soil and the environment surrounding it

The present work was done in the farm of Faculty of Agriculture, Al-Azhar University in Assiut Governorate in order to study the punch (fungicides) was tested to evaluate it repellency effects on house sparrow birds during of the ripening stages of wheat under field conditions.

Materials and methods

This study was executed at the farm of the Faculty of Agriculture, Al-Azhar University at Assiut Governorate during 2016. The experiment included the chemical control of the house sparrow birds during of the ripening stages. Punch was tested to evaluate it repellency effects on house sparrow birds. The chemical compound was tested at three rates of concentration. Table (1) indicated that the trade, common, chemical names and rate of application of tested compound.

Table (1): Tested compound.

Trade name	Common name	Chemical name	Rate of application
Trade name	**Common name**	**Chemical name**	**Rate of application**
'Punch' (40%EC)	Flusilazole	Bis(4-fluorophenyl) (methyl)(1H-1,2,4triazol-1-ylmethyl) sailane (IUPAC).	1.18.75cm³/100 L. Water * 2. 12.50 cm³/100 L. Water 3. 6.25 cm³/ 100 L. Water

* **According to the technical recommendation of the Ministry of Agriculture and Land Reclamation, Egypt.**

Punch fungicide used and their rates of application were designed here under each of levels as well as the recommended rate, according to the technical recommendations of the Ministry of Agriculture and Land Reclamation, Egypt. Full coverage of the wheat crops with Punch was secured using a knapsack sprayer fitted with one nozzle. The five replicates of the check treatment were sprayed with water only.

An area of about half feddans was divided into plots, each of (1/400feddan). The experiment was arranged in split-split plot design with planting method as a main plot treatment, chemical treatment as a subplot and spray program as a sub-sub plot treatment.The field experiment

included 4 treatments (3 rates of concentration + control) each treatment was replicated five times and tested in two programmes of pesticide applications at wheat plants against house sparrow birds. Samples of fifty plants were taken from the studied field crops of each chemical method in order to estimate the efficacy of various chemical methods .

The damage assessment and protection index (PI) in all experimental plots (treated and untreated spikes), were used as criteria to evaluate the effectiveness of the tested fungicides and application programmes on repellency potential and protection of wheat spikes against house sparrows attack. Protection index (PI) was also calculated by the equation adopted by **(Inglis and Isscson., 1987)** as follows:

$$\text{Protection Index (PI)} = \frac{A - B}{A} \times 100$$

Where: (A) = mean damage percentage in untreated plots.

(B)= mean damage percentage in treated plots.

Statistical analysis :

Data obtained were statistically analyzed using a randomized complete block design. Means were compared according to Duncan's Multiple Range test, at 0.05 level of probability.

Results and discussion

1.Field experiments :

Bird repellents to protect seeds are a potentially impotent of integrated vertebrate pest management strategies (Avery *et al*., 1993). This experiment was carried out during the ripening stages of wheat. The trial was aimed to evaluate the effectiveness of different application rates of punch 40%EC., recommended (According to the technical recommendation of the Ministry of Agriculture and Land Reclamation, Egypt.) for controlling some wheat pests, as repellent compounds. Two schedules of spray programme were performed to protect wheat spikes from birds attack and reduce birds damage.

1.1. One- spray programme schedules:

The protection indices (PI%) were calculated after different post-treatment intervals from fungicide applications in one- spray programme Protection indices after 3-day represents the initial, after 7-day the actual and after the rest intervals the residual effects of Punch as bird repellents. Data in Table (2) and Figure (1) indicated different patterns of persistence/ degradation behavior with each of the three tested concentrations of Punch on the ripening stages of wheat against the house sparrow birds. Generally, the results revealed significant differences among concentrations of punch. The highest protection performance was exhibited by using the recommendation rate of Punch for controlling pests. The protection indices (PI%) of tested fungicide was (96.25%) when applied Punch (18.75 cm^3/100L.water). The following highest protection indices (84.5%) were attained by applying the second concentration for Punch (12.50 cm^3/100L.water). The lowest values of protection indices were (33.41%) when applied Punch (6.25 cm^3/100L.water).

Generally, the results revealed that no significant differences between the first and second concentration while the significant difference were found between the concentrations pervious and the third concentration of punch. The highest protection performance was exhibited by using the recommended rate of Punch for controlling pests.

1.2. Two- spray programme schedules:

The statistical analysis of data representing the protection indices (PI%) resulted from applying two- spray

programme schedules, are presented in Table (2) and Figure (1). The obtained results supported the former data regarding the tested rates of the different fungicide applications. Field repellency and subsequently protection performance of wheat spikes from house sparrows attack were noticeably differed according to the chemical structures, rates of application and post-treatment intervals.

The protection indices after applications of two-spray programme schedules may be demonstrate the importance of the second application of Punch for enhancing the protection potential and expanding the protection period to wheat spikes against attack of house sparrows. In respect to the protection indices achieved by the tested Punch of post-treatment intervals, data in Table (2) and Figure (1) indicated that the highest protection indices for wheat spike (97.50%) when applied (18.75 cm^3/100 L. water) concentration. The following highest protection indices (87.50%) were attained by applying the second concentration for punch (12.50cm^3/100 L. water). The lowest values of protection indices were (47.41%) when applied (6.25 cm^3/100L.water) concentration. Generally, the results showed that increasing the rate of Punch application, resulted to increasing of repellency and protection performance to the wheat spikes during ripening stage.

Rizvi *et al*. (2002) in Pakistan showed that mithiocarb grain bait at 0.1% proved to be highly effective in repelling sparrows and may function as an ideal crop protection against bird invasion. Gabr (2005) used laboratory and field experiments for conducting the repellent and toxic effect of five pesticide compounds against the house sparrow, *Passer domesticus niloticus*, in Beni-Suef Governorate, Egypt. Both no-choice and free choice feeding tests in the laboratory showed that pirimicarb (carbamate compound) was the most repellent pesticide, followed by chloropyrifos, diazinon (organophosphorus compounds) and cyphenothrin (Pyrethroid), while propineb compound (carbamate) was the least repellent one. Eman-Tolba (2006) studied the effectiveness of different application rates of certain pesticides, recommended for controlling some wheat pests, as repellent compounds against the house sparrow, *P. domesticus niloticus* (L.). Kennedy and Connery (2008) evaluated seed treatments for the control of crow damage to seed and seedling in winter and spring wheat in field trials from 2004 to 2007. Treatments included six fungicides, three insecticides, a product marketed as a bird repellent and three potential repellents. Various rates of selected compounds were investigated. Winter wheat was sown in December and spring wheat in late-January to mid-February. Omar (2010) revealed that spraying on wheat plants during ripening stages by sumi-eight with a rate of (35 cm^3/100 L. water) resulted in significant high protection indices. But the middle protection indices were obtained by using the one insecticide compounds (Malathion) with a relatively high rate of applications (150 cm^3/100 L. water), comparing with the control during 2007 and 2008. He found that, the repellency effects enhanced with increasing of pesticide concentrations were studied. The protection indices were exhibited by using high, middle and low rates of applications, 50 cm^3, 100 cm^3 & 150 cm^3/ 100 L. Water and 11.6 cm^3, 23.3 cm^3 and 35 cm^3 / 100 L. water, for malathion and sumi-eight.

Table (2): Average protection indices (PI %) after different post-treatment intervals, induced from application of punch (40%EC) with different in one and two programme during wheat ripening stages, Assiut Governorate.

Concentrations	Avg. (PI%) at post-treatment intervals		Mean
	One- spray programme	Two- spray programme	
18.75 cm^3/100 L. Water	96.25%a	97.50%a	96.88%a
12.50 cm^3/100 L. Water	84.50%a	87.50%a	86.00%a
6.25 cm^3/100 L. Water	33.41%b	47.41%b	40.41 %b
Mean	71.39%a	77.47%a	

*** Means with each examined week for treatments followed by the same letter are not significant differences at the 0.05 level probability.**

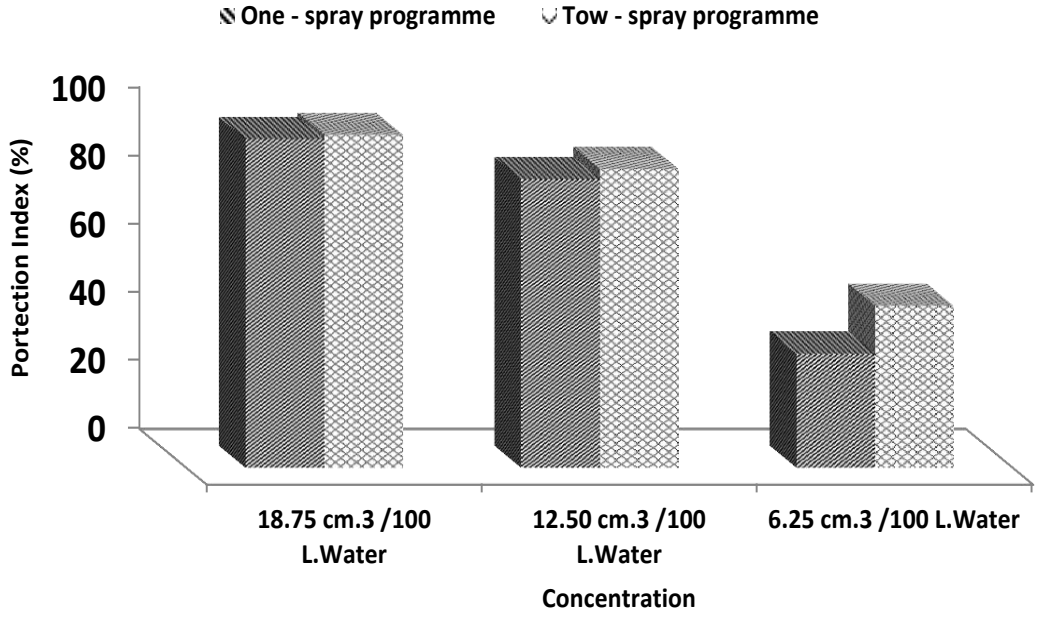

Figure (1): Average protection indices (PI %) after different post-treatment intervals, induced from application of punch (40%EC) with different in one and two programme during wheat ripening stages, Assiut Governorate.

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Responses of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) to treatment with chemically synthesized zinc and copper oxides nanoparticles

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Desert locust , *Schistocerca gregaria* , nanoparticles, Zinc oxide (ZnO) , copper oxide (CuO) and alternative insecticides.

Abstract:

The desert locust *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) is harmful insect which cause huge economic losses in agricultural sector all over the world. Nanotechnology is a promising field of interdisciplinary research. Moreover, it provides as ecofriendly and efficient alternatives for the management of insect pests in agriculture. The current work strives for obtaining safe, effective and economic insecticides; Zinc oxide (ZnO) and copper oxide (CuO) as alternative insecticides through the assessment of biological effect on *S. gregaria*. The nanoparticles were chemically synthesized in laboratory scale and Transmission Electron Microscopy (TEM) confirmed the nanostructure. The resulted LT50s were recorded after 10.994, 21.148 days respectively while latent biological responses of desert locusts were recorded in ZnO more than CuO NPs. Also, treatments revealed a notable inhibition in activity of Acetylcholinesterase **(**AChE.) enzyme.

Introduction

The desert locust *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) is polyphagous insect with much diversified food mode, including a high number of vegetable plants (up to 400 species) belonging to numerous families.) (Hahn, 2005). It affects vast area of about 28 million km2 that extends from the Atlantic coast of Africa to eastern India and from northern Turkey to Tanzania in the south (Symmons, 2009). Chemical pesticides were an emergency solution to these challenges. Consequently, managing the pathogens and pests need about 2 million metric tons pesticides worldwide per year (worth US $35 billion) (Stephenson, 2003). Given the ambition of scientists and agronomists for protecting agricultural crops from pests, overuse of chemical pesticides created pest resistance problems, and serious Impacts on environment and human (Safi *et al.*, 1993). Sequently, scientists turned to

search for alternative solutions; nanoparticles are organic, inorganic or hybrid materials with at least one of their dimensions ranging from 1 to 100 nm (at the nanoscale) (NPs) it represents a new generation in agricultural technology which could be considered new agrochemicals (Owolade *et al.,* 2008). The unique physical and chemical properties make nanocompounds are more effective compared with conventional pesticides and fertilizers, (Chinnamuthu and Murugesa, 2009 and Torney *et al.,* 2007). Finally, it is clear that increasing nanocompounds applications in agriculture take into consideration diversity impacts of using different size of nanoparticls, encouraged scientists to study the changes in eco-system and its biological effects on cells and their components (Nowack and Bucheli, 2007 and Ramesh *et al.,* 2013).

It is worth mentioning that there are no complete studies on use nano compounds in control desert locust. Further, the current study suggests novel strategies for desert locust control, using Zinc oxide (ZnO) and copper oxide (CuO) NPs besides its common usage as fertilizers.

Material and methods

1.Preparation in nanoparticles:

Solvolthermal method was used to synthesize ZnO nanostructures according to Sangari (2015). by reacting of NaOH with Zn (CH3COO) 2.2H2O under continuous stirring to adjust PH value of mixture to (pH.8). A standard procedure was followed to synthesis of CuO-NPs, via chemical precipitation method, described by (Luna *et al.,* 2015) using copper chloride dehydrate as precursor. Morphology (size and shape) of the nanoparticles was obtained by Transmission electron microscope (TEM). The concentration 0.25% (w/v) of ZnO and CuO nanoparticles were used to study the latent effects on tested insect.

2.Experimental insect:

Adult (males and females) of the desert locust, *S. gregaria* were obtained from Plant Protection Research Institute, Dokki, Giza, Egypt. Adults were breed in the laboratory of Pest's Physiology Department, Plant Protection Research Institute, Sharkia branch, under crowded conditions as described by **Hunter-Jones (1961) and Hassanein (1965).**

3. Nymphal treatments:

The newly moulted 4th instar nymphs were isolated from the stock colony and divided into groups. Each group consisted of three replicates of 10 nymphs. the isolated groups were treated with different concentrations previously prepared ZnO and CuO nanoparticles, besides, group of untreated nymphs (negative control) and another group treated with 0.1% tween solution (positive control). Individuals full-spray technique described by **(Simon, 2014**) was applied, where nymphs were placed in 250 ml glass beaker with filter paper at the bottom and sprayed directly by 1ml of treatment suspensions then transferred in 1kg perforated plastic jars covered with gauze to insure high ventilation with maintaining the conditions and feeding that has been followed through the rearing system. Mortality was recorded daily for 14 days. The lethal/time median (LT_{50}) were determined by probit analysis based on the method by **Finney (١٩٧١).**

The same technique that applied in toxicological investigation with same experimental design of replicates. The observations of the mortality and deformities were recorded daily and photographed besides following up vitality and development's period of tested nymphs to adult.

4.Determination of Acetyl-cholinesterase (AchE):

Insect samples were collected after 48 h. after treatments of ZnO and CuO nanoparticles. Samples were homogenized in 5 - 10 ml cold buffer (50 mM potassium phosphate, pH 7.5., 1 mM EDTA) per gram sample weight using glass mortar. Homogenates were perfused in refreeze for a night then were centrifuged at 4,000 rpm for 15 minutes at 4 ° C. The supernatant was subjected to biochemical assay and store on ice. AchE (Acetylcholinesterase) activity was measured according to the method described by **Simpson *et al.* (1964),** using acetylcholine bromide (AchBr) as substrate. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

Results and discussion

Resulted Zinc oxide (ZnO) was white amorphous powder while copper oxide (CuO) was a dark brown powder. Both Nano-powders showed low solubility in water and were semi-soluble in methanol. Transmission electron microscope showed micrograph of zinc oxide nanoparticles denoted by revealed that they were spherical with little agglomeration. Most of particles were present in the range 10-15 nm in size and possess an average size of 12 nm (Figure,1A) Moreover, TEM micrograph of CuO NPs revealed that the particles were rods in shape and the size came in the average between 11.5 and 13.5 nm which confirmed the formation of Nano structure (Figure, 1 B).

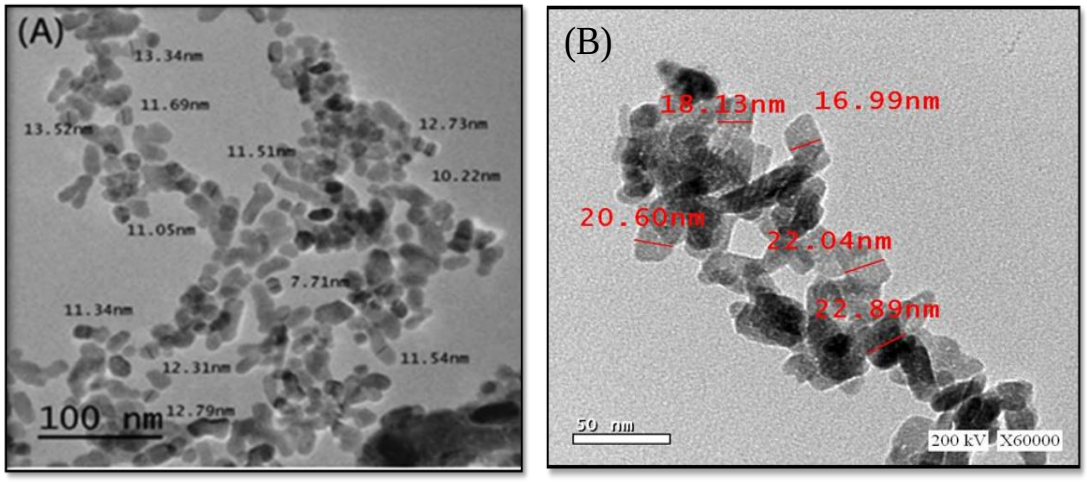

Figure (1): Transmission electron microscope (TEM) morphology; size and shape of ZnO (A) and CuO (B) nanoparticles.

Table (1) represented the LT50 of (4th) instar nymphs treated with the concentration of sub lethal concentration (0.25%) of CuO and ZnO NPS. the resulted LT50s were recorded after 10.994, 21.148 days respectively Furthermore, CuO clued an acute toxicity while ZnO maybe has chronic toxicity. these results lead to that ZnO nanoparticles may resulted in a residual effect more than CuO. results compatible with recent study by (Tuncsoy *et al.*, 2019) who described the Insecticidal effect for nanoparticles against insects models by inhalation or ingestion and attributed that the physiological changes, occurring in the larvae were returned to accumulation of NPs in the larval midgut and fat body . On the other hand, biocontrol by microorganisms-based pesticides and plant extracts showed nearly the same latent mortality in *S. gregaria.* (Danyk *et al.,* 2005; Bravo *et al.*, 2011 and Sharma, 2014).

Table (1): LT_{50} of CuO and ZnO NPs 0.025% against 4th instar of *Schistocerca gregaria.*

Treatments	LT_{50} (Day)	Confidence limits		LT_{90} (Day)	Confidence limits		Slope
		lower	Upper		lower	upper	
CuO	10.994	9.287	14.103	33.192	22.568	70.991	2.671
ZnO	21.148	14.978	52.58	79.122	37.39	683.301	2.236

Effect of sub lethal concentration of NPs on Nymphal mortality was initially observed during 4th instar where both ZnO and CuO NPs resulted in a considerable mortality compared to untreated controls. Mortality rates attained 10.34%, 16.67% respectively, whereas the residual mortality recorded during the 5th instar didn't exceed 12% for CuO and 4.4% for ZnO compared to untreated controls (Table, 2). Furthermore, paralysis and decreased locomotion, trembling of abdominal segments were noted in treated nymphs, demonstrating the effect of nanoparticles on the nervous system. This phenomenon is often noted in insects treated with neurotoxic insecticides (Proux *et al*., 1993).

Furthermore, the effect of NPs on Nymphal duration was verified. At ZnO treatment duration of nymphal instars was longer as insects developed into a more advanced stage. While exposure to cuo resulted in reversed findings on the duration of nymphal instars which was shorter compared with untreated controls. Nymphs attained the longest duration (7.85 and 8.45 days) in 4th and 5th instar that respectively with ZnO treatments. Moreover, nymphes treated with CuO recorded 5.3 and 7.09 days in 4th and 5th instar respectively. Additional findings were reported in Table (2) and Figure (2). Nymphal development was affected by treatments with ZnO NPs which revealed a notable deficient molting during development from 4th instar to 5th instar reached 12.4% of survival nymphs likewise, 18.5% deficient molting recorded in development from 5th to adult stage. No deficient development was recorded with CuO treatment. Controversial reports regarding the toxicity of the ZnO in the living cells, particularly in mammalian cells. Some of the reports have shown that ZnO is biocompatible and nontoxic (Zvyagin *et al*., 2008), while some studies have recently reported both in vivo and in vitro toxicity of the ZnO on mammalian cells (Tian *et al*., 2015). It can be elucidated from such studies that the toxicity of ZnO depends upon the concentration and its nano size. On the other hand, there were no studies that can be considered to understand the CuO NPs effects on living organisms.

Table (2): Biological effects of CuO and ZnO NPs (0.025%) on nymphalid stage of *Schistocerca gregaria.*

	4th instar			5th instar		
	Mortality%	Duration (Day)	Deficient-molting %	Mortality%	Duration (Day)	Deficient-molting%
Control	0.0c	6.50b	0.0a	0.0b	7.72b	0.0b
Control-tween (0.01%)	0.0c	6.75b	00.0a	0.0b	7.63b	0.0b
ZnO	10.344b	7.85a	11.489a	4.412b	8.45a	18.793a
CuO	16.670a	5.3c	0.0a	12.00a	7.09c	0.0b
$LSD_{0.05}$	2.996	0.219	9.477	2.145	0.149	5.438
P	0.0000 ***	0.0000 ***	0.0943 ns	0.0000 ***	0.0000 ***	0.0002 ***

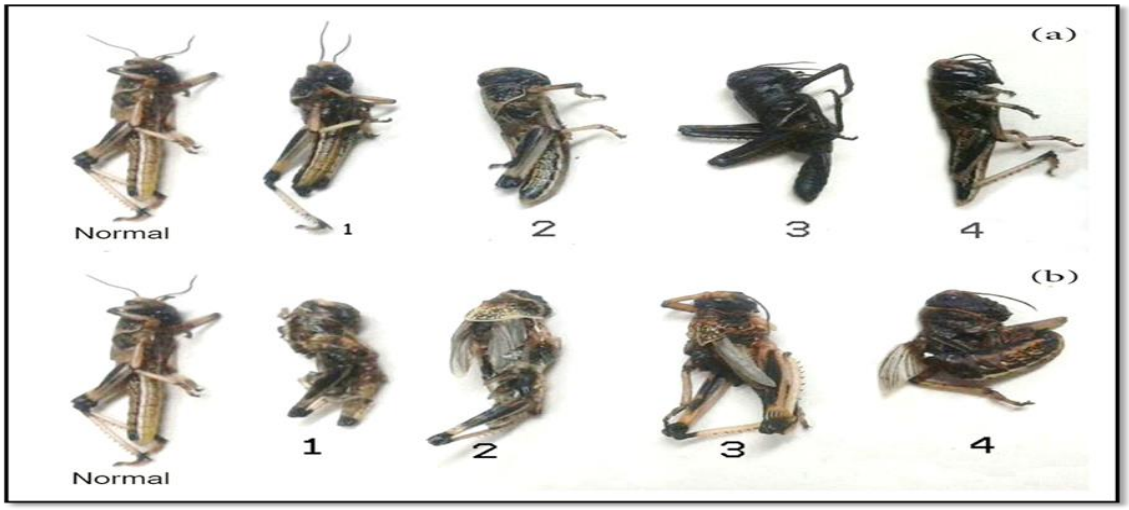

Figure (2): Death symptoms and incomplete molting of *Schistocerca gregaria* treated with CuO (a) and ZnO (b) nanoparticles during nymphal stage.

The activity of AChE. enzyme in Figure (3) showed a marked decrease in both tested nanopartecle where the enzyme activity with ZnO reached (4.781 U/gm), and (9.41 U/gm) with CuO treatment. Comparing to the negative and positive control that recorded (22.48, 15.06 U/gm.) respectively, these obtained results cleared that ZnO was more effective than CuO in inhibition of AChE with a statistically significant difference (LSD=4.566, P=0.0001). this reduction attributed to nanoparticles crossed the blood-brain barrier and gained access to the central nervous system, this led to binding with acetylcholinesterase (AChE) and affected its activity and hydrolyzing the neurotransmitter Acetylecholine into choline and acetic acid in cholinergic synapses as discussed by Hu and Gao (2010).

Our results enhanced by other studies detected that TiO_2 NPs reduced AChE. activity by Yixi *et al.* (2014). Although disagreed with Milivojević *et al.* (2015) that stated increasing in AChE. by exposer bees to ZnO NPs. Where there are many parameters can affect such as chemical, physical and geometrical properties

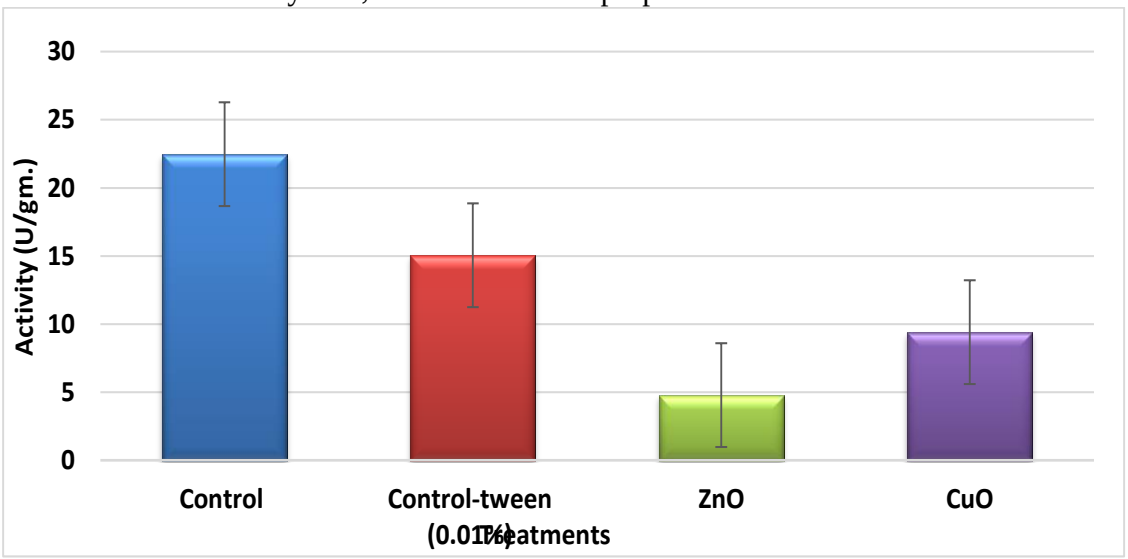

Figure (3): Effect of CuO, ZnO NPs on activity of AChE. enzyme in 4th instar of *Shistocereca gregaria* .

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Biodiversity and population dynamics of mites inhabiting date palm trees in Qalyubia and New Valley Governorates, Egypt

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Abstract

Incidence and population dynamics of mites inhabiting date palm trees were studied at two localities (Tanan village in Qalyubia and Paris oasis in the New Valley Governorate) from March to November during two seasons 2017-2018. Obtained results indicated that 22 mite species belonging to 21 genera under 15 families. These mites were classified according to their feeding habits into three categories: eight species are phytophagous mites (Tetranychidae, Tenuipalpidae, Eriophyidae and Phytoptidae), nine species are predacious mites (Bdellidae, Cheyletidae, Cunaxidae, Eupalopsellidae, Hemisarcoptidae, Phytoseiidae and Stigmaeidae), while the remaining five species are miscellaneous feeding behaviors (Acaridae, Tarsonemidae, Tydeidae and Oribatulidae). The date palm dust mite, *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae) has become an important pest of immature date palm fruits on Sewi variety in the New Valley Governorate. Whereas, *Raoiella indica* Hirst and *Phyllotetranychus aegypticus* (Sayed) (Acari: Tenuipalpidae) are an important pest on fronds on Zaghlol variety in Qalyubia Governorate. Results indicated that, the population dynamics of *O. afrasiaticus* started with attacks fruits at second week of April and reached its peak in mid June in the first year and in late of June in the second year on Sewi date palm variety. After that the mites migrate from fruits to fronds and weeds. The population density of phytophagous and predaceous mites as well as weather factors was studied at the two governorates. The dust mite, *O. afrasiaticus* and tenuipalpid mites and their relatives, were more dangerous mites; therefore, more studies were carried out. Recognize the time of the annual peaks of seasonal abundance for each phytophagous mite species, concerned with the time of starting the application of the suggested control program.

Introduction

Palms are one of the most important treasures in the Arab Republic of Egypt, which have been famous for their nutritional value in the oases and many agricultural areas of Egypt throughout the ages. Accordingly, Egypt is ranked in the first place among the date-producing countries in the world. Despite of Egypt's high rank in terms of date production that amounts to more than 1.7 million tons,

almost 21% of the world production estimated at eight million tons, its export contribution to the international Dates market is low. The strategy aims to raise date exports from 38 000 tons in 2016 to 120 thousand tons over the next five years (El-Sharabasy and Rizk, 2019).

Diseases and pathogen pests are causing great economic loss to the growers, reducing about 52% of the total yield (Sanad *et al.,* 2017). Date palm trees were observed to be severely affected by different injurious mites, which cause considerable damage and lead to economic losses (Taha *et al.,* 2019). In Iraq, *Oligonychus afrasiaticus* (McGregor) caused 50–80% yield loss of dates in years of dry, dusty and stormy weather (Al-Jboory and Al-Suaide, 2010). Many researchers were studied the population density of mites on date palm trees, the mites can be affected by different environmental condition and biotic factors (El-Halawany *et al.,* 2001; Idder and Pintureau, 2008; Aldosari, 2009; Palevsky *et al.* , 2010; Latifian, 2012; El-Sanady and Mohamed, 2013; Mesbah (2014) and Roshdy *et al.* 2018).

The present work aims to study the incidence and population dynamics of mites inhabiting date palm trees were studied at Qalyubia and New Valley Governorates, from March to November during two seasons 2017-2018.

Materials and methods

Two separate areas for date palm were selected for the study. The area for Sewi (semi dry) date palm variety at Paris Oasis in the New Valley Governorate, and Zaghlol (soft variety) at Tanan Village in Qalyubia Governorate. In each survey area, 20 palms (15 years old) were selected were chosen to survey the mites on palms. Samples were biweekly collected from March to November during two successive years 2017-2018. The sampling included random collection of 40 fronds and 100 fruits from each cultivar. The collected leaves of each cultivar were placed in individual paper bags. Mite specimens were collected from plants by direct examination under stereo–microscope and cleared in Nesbitt solution for about one hour after that, mounted on microscope slides in Hoyer's medium was used to set most mites on the slides (Jeppson *et al.*, 1975). For Eriophyoidea specimens were mounted on microscope slides in Keifer's F–medium (Amrine and Manson, 1996). Mounted slides were kept for 24hrs in electric oven at 45-60°C. The mites were identified with the help of a phase-contrast (Carl Zeiss, Germany); identification, using the world taxonomic literature. Mite specimens are deposited in the mite reference collection of Fruit Trees Acarology Research Department, Plant Protection Research Institute, Agricultural Research Center, Egypt.

Statistical analysis: Data were subjected to the statistical analysis Daily recorded minimum and maximum temperatures (°C) and average relative humidity (R.H%) prevailing in the area during the study were obtained from site http://www.wunderground.com. Simple correlation was used to correlate between weather factors and average monthly number of mites, using SAS statistical software (SAS Institute, 2003).

Results and discussion

1. Ecological studies:

1.1. Incidence:

Incidence of mites inhabiting date palm trees were carried out at two localities (Tanan village in Qalyubia and Paris oasis in New Valley Governorates)

from March to November during two seasons 2017-2018.

1.1.1. Phytophagous mites:

The phytophagous mites cause severe harmful of leaves and fruits. Mites feeding produce variable symptoms such as rusting, leaf chlorosis and malformation of flowering, severe infestations cause fruit distortion and orchard deterioration. The phytophagous mites included eight species representing by four families Eriophyidae, Phytoptidae, Tenuipalpidae and Tetranychidae. These families were recorded in Table (1).

Family Eriophyidae Nalepa, 1898.

This family was represented by one mite species, *Epitrimerus saudiarabis.* Wang *et al.* (2014) was collected vagrant on inner and outer fronds surfaces in moderate number from Qalyubia and New Valley. No damage to the host plant was observed. Similar results were obtained by Elhalawany *et al.* (2014).

Family Phytoptidae Murray, 1877.

This family was represented by one mite species, *Mackiella phoenicis* Keifer, 1939, was collected from inner fronds with moderate number of the two Governorates. This mite infesting inner fronds and buds, preferring folds of unopened central fronds of date palm, causing leaf-folds and rust similar results were obtained by Wang *et al.* (2014).

Family Tenuipalpidae Berlese, 1913.

Members of this family comprise four species, which were mostly infesting date palm trees, causing serious damage. Date palm red flat mite *Brevipalpus pheonicis* (Geijskes), infests fronds, bunches and fruits. It prefers the lower surface around the midrib as well as the places which are protected. By sucking the plant sap, the injured areas become pale, then change to rusty brown. This mite was recorded with low population in Qalyubia Governorate. The tenuipalpid mite, *Phyllotetranychus aegyptiacus* Sayed was recorded with high numbers on upper and lower surface of the fronds of the two Governorates. The incidence of red date palm mite, *Raoiella indica* Hirst was recorded with high numbers on upper and lower surface of the fronds of the two Governorates associated with *P. aegyptiacus* This mite appears as a reddening of the upper surface of the leaf, the reddened area may be either a small blotch or many such blotches that often encompass the entire leaf surface, eventually resulting in complete defoliation of affected trees these results agreement with finding by El-Halawany *et al.* (2001) . The flat mite, *Tenuipalpus eriophyoides* Baker was recorded with a high number at the New Valley Governorate. This mite species was found infesting date palm trees on the lower sides of leaves near the veins, causing a great damage and losses such findings coincide with that was found by Taha *et al.* (2019).

Family Tetranychidae Donnadieu, 1875.

Two species of tetranychid mites were recorded inhabiting date palm trees, the citrus brown mite *Eutetranychus orientalis* (Klein) was recorded with moderate number of upper fronds at two Governorates. This species feeding on the upper leaf surface produces a multitude of gray spots, which gives leaves a chlorotic appearance. The second species is the date palm dust mite, *Oligonychus afrasiaticus* (McGregor) was recorded with a high number in New Valley governorate on fruits and fronds. It attacks the dates from its early stages of development (kamry and khelal fruit stages), spinning its web around the date bunches and multiplies in large numbers, especially on Sewi and

Barhi date palm varieties. The first report of *Oligonychus* spp. as date palm common pests was in Kharga Oasis on Sewi semi dry variety (Saleh and Hosny, 1979); in North and South Sinai Peninsula (El-Kady, 1997); in allover governorate (El-Halawany *et al.,* 2001), in Sharq El-Owainat province from New Valley (Elhalawany *et al.,* 2017) and in Giza, Assiut, Matruh and the New Valley Governorates (Sanad *et al.* , 2017).

1.1.2. Predaceous mites:

The predaceous mites considered the most important agents of biological control of different phytophagous mites and insects. These mites helped to decrease the population of phytophagous mites and increase the yield of date palm orchard trees by limiting the infestation of injuries mites. Nine predacious mites species belonging to seven families and eight genera were registered (Table,2).

Family Bdellidae Dugès, 1834.

Spinibdella bifurcate Atyeo was recorded with moderate numbers on upper fronds at the two Governorates associated with scale insects and phytophagous mites.

Family Cheyletidae Leach, 1815.

One species, *Cheletogens ornatus* (Canestrini &Fanzago) was collected from fronds in the Qalyubia Governorate with moderate number associated with phytophagous mites and scale insect infestation.

Family Cunaxidae Thor, 1902.

Only one species, *Cunaxa capreolus* (Berlese) was detected in this family. It was found on fronds with rear numbers in the two Governorates associated with phytophagous mites and scale insects infestation.

Family Eupalopsellidae Willmann, 1952.

A single predator, *Saniosulus nudus* Summers, 1960 was recorded on fronds with moderate numbers in the two Governorates associated with scale insect**.**

Family Hemisarcoptidae Oudemans, 1904.

One mite species, *Hemisarcoptes malus* Shimer, were usually recorded in moderate numbers in association with scale insects infesting date palm trees in Qalyubia and New Valley Governorates.

Family Phytoseiidae Berlese, 1916.

Many species of the phytoseiid mites are, possibly ranked among the most effective predators of different phytophagous mites, including several serious pests of agricultural crops. The predator mite, *Amblyseius swirskii* (Athias–Henriot) was recorded with high numbers on fronds and fruits in Qalyubia and New Valley Governorates. While *Amblyseius cydnodactylon* (Shehata and Zaher) and *Euseius scutalis* (Athias–Henriot) were recorded with moderate numbers on fronds and fruits in Qalyubia and New Valley Governorates associated with phytophagous mites and scale insects on date palm trees.

Family Stigmaeidae Oudemans, 1931.

Members of this family are potential predators of various phytophagous mite species. *Agistemus exsertus* (Gonzalez) seemed to be the most important stigmaeid mite on date palm trees occurring in Qalyubia and New Valley Governorates. It was recorded in high numbers on the fronds.

1.1.3. Miscellaneous feeding habits:

During this study, five species belonging to five genera and five families were recorded in Table (3).

Family Acaridae Latreille, 1802.

The family Acaridae was represented by a single species *Tyrophagous putresentiae* (Schrank), which was moderate numbers found feeding on fungi from fruits and

fronds in Qalyubia and New Valley Governorates.

Family Tarsonemidae Kramer, 1877.

Tarsonemus stiffer (Ewing) was recorded in moderate numbers from fruits and fronds in Qalyubia and New Valley Governorates. This species was usually found in association with fungal growth.

Family Tydeidae Kramer, 1877.

Two mite species were found on palm trees belonging to family Tydeidae, *Pronematus ubiquitus* (McGregor) was recorded in high numbers of fronds and moderate numbers of fruits in the two Governorates. *Tydeus californicus* (Banks) was recorded with moderate numbers on fronds in the two Governorates. Individuals of this species were seen moving quickly on both sides of leaves and branches, usually in association with the tetranychid and tenuipapid mites.

Family Oribatulidae Thor, 1929.

A single species from this family, *Oribatula sayedi* (El Badry and Nasr) was recorded with moderate numbers on fronds and rear numbers on fruits in the two Governorates. Similar results were obtained by, El-Halawany *et al.* (2001) who collected 16 species of mites belonging to eleven families and classified according to their feeding habits to seven species plant feeders, six species predacious and three species of miscellaneous feeding habits. El-Sanady and Mohamed, 2013 recorded 37 mite species representing 31 genera, 17 families on date palm at Giza and Sohag Governorates. Mesbah (2014) recorded twenty-six mite species in 22 genera and 17 families were collected from date palm trees in the two Governorates, Giza and Sharkia. Roshdy *et al.* (2018) who recorded thirteen species in eleven genera belonging to nine families in Dakahleya and New Valley Governorates.

1.2. Population dynamics:

1.2.1. Population dynamics of phytophagous and predaceous mites on Sewi date palm variety in New Valley Governorate during 2017-2018.

1.2.1.1. Phytophagous mites:

The date palm dust mite *Oligonychus afrasiaticus* population:

The dust mite, *O. afrasiaticus* has become an important pest of immature date palm fruits on Sewi variety in the New Valley Governorate. This pest mite affects the fruits of palm trees during the growth and ripening stages as they suck the fruit juice, leading to stop fruit growth and destroying the crop (Figure,1). The population dynamics of *O. afrasiaticus* started with attacks fruits at second week of April during Kamry stages (characterized by the green color of fruits), and reached its peak at mid of June in the first year 2017 recorded 11450 individuals/100 fruits when maximum, minimum temperatures and averaged relative humidity were 40.9, 23.8°C and 17.2 %, and in late of June in the second year 2018 recorded 11330 individuals/100 fruits when maximum, minimum temperatures and averaged relative humidity were 43.1, 27.0°C and 13.8 %, respectively on Sewi date palm variety. After that the mites migrate from fruits to fronds during khelal stage (characterized by the yellow color of fruits). Whereas, *O. afrasiaticus* started with attacks fronds at the third week of May and gradually increased in number and reached its peak in mid August recorded 120 individuals/40 fronds when temperatures ranged 26.07-41.33°C and relative humidity averaged 21.0 % in the first year 2017, and in late of August 2018 in the second year recorded 133 individuals/40 fronds when the temperature ranged 40.14- 26.6°C and relative humidity averaged 20.8 % (Figure, 2).

Table (1): Incidence of phytophagous mites associated with date palm trees at Qalyubia and New valley Governorates.

Families	Species	Localities	Habitat and abundance
Tetranychidae	*Eutetranychus orientalis* (Klein, 1936)	Qalyubia & New valley	Fronds ++
	Oligonychus afrasiaticus (McGregor, 1939)	New valley	Fronds +++& fruits ++++
Tenuipalpidae	*Brevipalpus pheonicis* (Geijskes, 1936)	Qalyubia	Fronds +
	Phyllotetranychus aegyptiacus Sayed, 1938	Qalyubia & New valley	Fronds +++
	Raoiella indica (Hirst, 1924)	Qalyubia & New valley	Fronds +++
	Tenuipalpus eriophyoides Baker, 1948	New valley	Fronds +++
Eriophyidae	*Epitrimerus saudiarabis* Wang & Elhalawany, 2014	Qalyubia & New valley	Fronds ++
Phytoptidae	*Mackiella phoenicis* Keifer, 1939	Qalyubia & New valley	Inner fronds +++

+ = Low (1-4 individuals/fronds) ++ = Moderate (5-10 individuals/fronds +++ = High (more than 10 individuals fronds)

Table (2): Incidence of predaceous mites associated with date palm trees at Qalyubia and New valley Governorates.

Families	Species	Localities	Habitat and abundance
Bdellidae	*Spinibdella bifurcate* Atyeo, 1960	Qalyubia & New valley	Fronds ++
Cheyletidae	*Cheletogens ornatus* (Can. & Fan., 1876)	Qalyubia	Fronds ++
Cunaxidae	*Cunaxa capreolus* (Berlese, 1889)	Qalyubia & New valley	Fronds +
Eupalopsellidae	*Saniosulus nudus* Summers, 1960	Qalyubia & New valley	Fronds date ++
Hemisarcoptidae	*Hemisarcoptes malus* (Shimer, 1868)	Qalyubia & New valley	Fronds ++
Phytoseiidae	*Amblyseius swirskii* (Athias–Henriot, 1962)	Qalyubia & New valley	Fronds +++ & Fruits +++
	A. cydnodactylon (Shehata and Zaher, 1969)	Qalyubia & New valley	Fronds ++ & Fruits ++
	Euseius scutalis (Athias–Henriot, 1958))	Qalyubia & New valley	Fronds ++
Stigmaeidae	*Agistemus exsertus* (Gonzalez)	Qalyubia & New valley	Fronds +++

+ = Low (1-4 individuals/fronds) ++ = Moderate (5-10 individuals/fronds +++ = High (more than 10 individuals fronds)

Table (3): Incidence of mites of miscellaneous feeding habits associated with date palm trees.

Family	Species	Area	Habitat and abundance
Acaridae	*Tyrophagous putrescentiae* (Schrank, 1781)	Qalyubia & New valley	Fronds ++ & fruit +
Tarsonemidae	*Tarsonemus stiffer* (Ewing)	Qalyubia & New valley	Fronds ++ & fruit +
Tydeidae	*Pronematus ubiquitus* (McGregor, 1932)	Qalyubia & New valley	Fronds +++ & fruit ++
	Tydeus californicus (Banks, 1904)	Qalyubia & New valley	Fronds ++ & fruit +
Oribatulidae	*Oribatula sayedi* (El Badry & Nasr, 1974)	Qalyubia & New valley	Fronds ++ & fruit +

+ = Low (1-4 individuals/fronds) ++ = Moderate (5-10 individuals/fronds +++ = High (more than 10 individuals fronds)

Figure(1): Date palm dust mite *Oligonychus afrasiaticus* infestation symptoms.

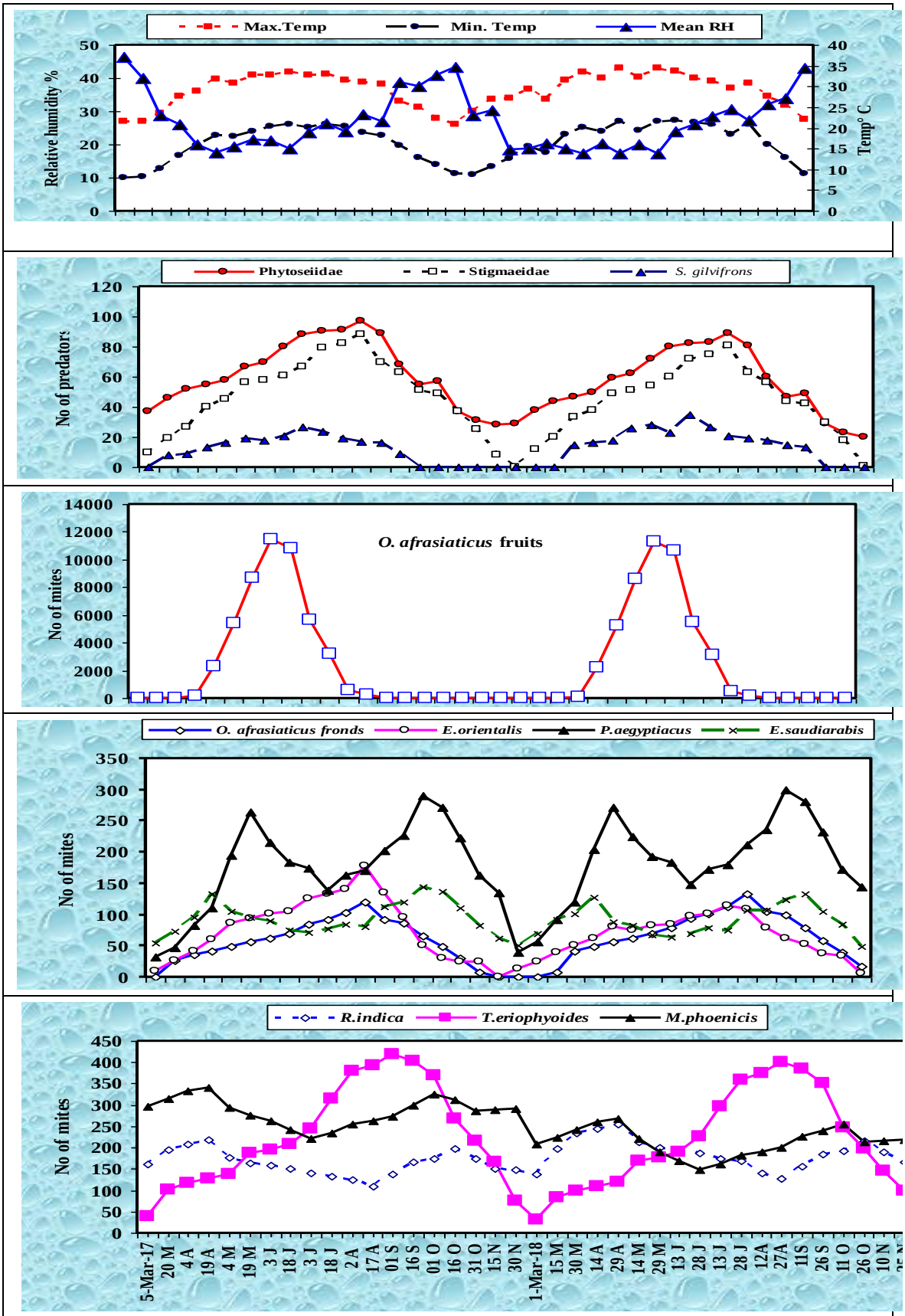

Figure (2): Population dynamics of phytophagous mites and predaceous on sewi date palm variety in New Valley Governorate during 2017-2018.

Statistical analysis present in Table (4) showed that, the temperature was non-significant positive correlation with the density of the population of *O. afrasiaticus* on fruits, during the two successive years, while on fronds it had a high positive correlation between the mite population and temperature. However, Relative humidity had non-significant negative correlation with the mite population during the two successive years of fruits, but on fronds in the first year this relation significant negative. These results indicated that the date palm dust mite prefers high temperature and low humidity.

The obtained results are in harmony with that detected by (Saleh and Hosny, 1979) who indicated that, the dust mite attacks the dates from their early stages of development, and dust collected in the webs plus the remnant of different developmental stages of the date dust mite affect the date bunches, giving them a dusty appearance. (El-Halawany *et al.* , 2001) who reported that the population date palm dust mite on dates begins to increase in June and Peak in July and August. (Negm *et al.,* 2015) who reported that *O. afrasiaticus* infests fronds and feeds on date palm on both sides, mainly along the midrib and at high infestation levels. In Saudi Arabia, *O. afrasiaticus* active from early March until mid october. Infestation usually starts around mid-May to June. Numbers of mites per 100 date fruits may reach it's maximum with 9095.5 mites at the end of July; the population then gradually decreases until the mid of october to an average of 124 mites/ 100 date fruits (Aldosari, 2009). Palevsky *et al.* (2010) indicated that, *O. afrasiaticus* was rarely found during winter and spring; it occurs on fronds only from late April until late August and maintains small populations in the summer, whereas very large numbers occur on the fruit strands, with a rapid increase during June–August, in most cases averaging much more than the 1000 mites per fruit strand. El-Sanady and Mohamed (2013) was collected *O. afrasiaticus* in moderate numbers on both Zaghloul and Sewi varieties in Giza and with high number in Sohag Governorate; the population of mites increasing during July and August. Roshdy *et al.* (2018) who collected *O. afrasiaticus* from date fruits in April and May, the highest peak was observed in June at Gamassa village from Dakahleya Governorate.

The citrus brown mite *Eutetranychus orientalis* population:

E. orientalis was recorded with moderate number of upper fronds on Sewi date palm at the New valley Governorate. Data illustrated from (Figure, 2) clearly showed that *E. orientalis* has one annual peak of seasonal abundance in mid August recorded 177 and 114 individuals/40 fronds when temperatures ranged 26.07-42.0 °C and relative humidity averaged 18.9 -19.25%, during the two successive years. After that, the population gradually decreased in number and the mite disappeared in late November in the first year.

Table (4): the correlation coefficient between temperatures, relative humidity and mite populations on sewi date palm variety in New Valley Governorate during 2017-2018.

Season	Parameters	Correlation coefficient values								Max. Temp	Min. Temp	Mean RH
		Eutetranychus orientalis	***Oligonychus afrasiaticus***	***O. afrasiaticus* fronds**	***Phyllotetranychus aegyptiacus***	***Raoiella indica***	***Tenuipalpus eriophyoides***	***Epitrimerus saudiarabis***	***Mackiella phoenicis***			
2017	Phytoseiidae	0.96***	0.52	0.95***	0.23	-0.55	0.71*	-0.03	-0.65*	0.91***	0.89***	-0.77*
	Stigmaeidae	0.93***	0.4	0.97***	0.47	-0.56	0.83**	0.18	-0.61	0.94***	0.96***	-0.76*
	S. gilvifrons	0.84**	0.75**	0.70*	0.03	-0.41	0.30	-0.23	-0.70*	0.78**	0.70*	-0.85***
	Max.Temp	0.90***	0.58	0.91***	0.51	-0.48	0.73*	0.19	-0.60	-	-	-
	Min. Temp	0.89***	0.51	0.92***	0.60	-0.54	0.82**	0.23	-0.62	-	-	-
	Mean RH	-0.79**	-0.63	-0.72*	-0.38	0.23	-0.44	-0.17	0.46	-	-	-
2018	Phytoseiidae	0.95***	0.68*	0.76**	0.63	-0.30	0.69*	-0.01	-0.58	0.90***	0.82**	-0.67*
	Stigmaeidae	0.97***	0.59	0.88***	0.64	-0.24	0.82**	0.17	-0.49	0.92***	0.93***	-0.58
	S. gilvifrons	0.86***	0.75**	0.71*	0.53	0.13	0.59	0.04	-0.52	0.85***	0.80**	-0.66*
	Max.Temp	0.90***	0.64	0.76*	0.63	-0.16	0.69*	0.14	-0.51	-	-	-
	Min. Temp	0.89***	0.50	0.90***	0.60	-0.22	0.82**	0.32	-0.40	-	-	-
	Mean RH	-0.62	-0.55	-0.24	-0.57	-0.32	-0.12	-0.08	0.25	-	-	-

Statistical data obtained from Table (4) showed that the mite population of *E. orientalis* had highly significant positive correlation with temperature (0.90*** and 0.89***) in the two successive years. The relative humidity was highly significant negative correlated in the first year (-0.79**) and non-significantly correlated (-0.62) in the second year. These results agree with El-Sanady and Mohamed, 2013 and Roshdy *et al.* 2018.

The tenuipalpid mite *Phyllotetranychus aegyptiacus* population:

The tenuipalpid mite, *P. aegyptiacus* Sayed was recorded with high numbers on upper and lower surface of the fronds of Sewi date palm at the New valley Governorates. The obtained data from Fig.(2) indicated that, it has two peaks, which were recorded in mid May and early october recorded 263 and 291 ind./40 fronds in the first year 2017, while, in the second year reached its peaks in mid May and in late october recorded 272 and 300 ind./40 fronds. The tenuipalpid mite appeared in few numbers on fronds in March and increased gradually to may but it had to decrease in number from June to August during the two successive years. Population density of *P. aegyptiacus* exhibited a positive correlation with temperature (0.51& 0.60, 0.63& 0.60), while relative humidity was non-significant negative correlated with the mite population (-0.38& -0.57), during the two successive years on Sewi date palm variety Table (4). These results are in accordance with that of El-Halawany *et al.* 2001; El-Sanady and Mohamed, 2013 and Roshdy *et al.,* 2018.

The red palm mite *Raoiella indica indica* population:

The red palm mite, *R. indica* appeared in low numbers in the summer months, it has two annual peaks were recorded in mid April and october 2017 (218 and 198 ind./40 fronds) when the temperature ranged between 16.7-34.6℃, and in late April and october 2018 (255 and 215 ind./40 fronds) when the temperature ranged between 17.7-35.5℃ Fig.(2). Statistical data obtained from Table (4) showed that, the population of *R. indica* had non-significant negative correlation with temperature (-0.48& -0.54 and -0.16& -0.22) in the two successive years. The relative humidity was non-significant positive correlated in the first year (0.23) and non-significant negative correlated (-0.32) in the second year. These results are in agreement with the finding by Mesbah (2014) who proved the abundant of this pest in the spring months and Roshdy *et al.* (2018) indicated that the red palm mite recorded the highest peak was recorded in May.

The flat mite *Tenuipalpus eriophyoides* population:

The flat mite *T. eriophyoides* mite appeared in few numbers on fronds in March and increased gradually to reached its peak in early September 2017 recorded 419 ind./40 fronds when the temperature ranged between 25.4-39.47℃, and relative humidity 19.07%, but it had to decrease in number from late october to November. While, in the second year 2018 it has one annual peak in late August recorded 400 ind./40 fronds when the temperature ranged between 26.6-40.1℃, and relative humidity 20.8% (Fig. 2). The flat mite, *T. eriophyoides* population was a highly significant positively correlated with temperature (0.73* and 0.82** and 0.69* and 0.82**), whereas, non-significant negative correlation between mite population and relative humidity(-0.44 and -0.12) during the two successive years, respectively (Table, 4) . Similar results were obtained by Roshdy *et al.* (2018) indicated that *T. eriophyoides* was recorded all over the year on the two surfaces of the frond but preferring the upper surface.

The eriophyid mite *Epitrimerus saudiarabis* population:

The eriophyid mite was recorded for the first time in Egypt by Elhalawany *et al.* (2014) in Giza Governorate and this study the first report the population of this mite. The eriophyid mite, *E. saudiarabis* mite appeared in few numbers on fronds in March. The obtained data from (Figure, 2) indicated that, it has two peaks, which were recorded in mid April and early october recorded 133 and 145 ind./40 fronds in the first year 2017 when the temperature ranged between 16.7-34.6℃ in April, and 22.7-38.1℃ in october, while, in the second year reached its peaks in the third week of April and second week of october recorded 128 and 132 ind./40 fronds. Population density of *E. saudiarabis* exhibited non-positive correlation with temperature (0.19& 0.23 and 0.14& 0.32), while relative humidity was non-significant negative correlated with the mite population (-0.17 and -0.08) during the two successive years, respectively on Sewi date palm variety Table (4).

Date palm bud mite *Mackiella phoenicis* population:

The eriophyid mite *M. phoenicis* was appeared on a base of inner fronds in March then increased in number. Two annual peaks of seasonal abundance on Sewi date palm variety were recorded (Figure, 2). The first peak of *M. phoenicis* in the third and fourth week of April recorded (340 and 267 ind./40 fronds) when the temperature ranged between 16.73-34.6℃ and 17.73-33.55℃, and relative humidity 20.8 and 16.36%, during the two successive years, respectively. The second peak was noted in early october in the first year and in the second week of october in the second year were (326 and 254 ind./40 fronds) when the temperature ranged between 22.7-38.1℃ and 26.0-38.5℃, and relative humidity 21.5 and 21.7%, during the two successive years, respectively. Statistical analysis of data showed that non-significant negative correlation occurred between mite population and temperature (-0.60 and -0.62 and -0.51 and -0.40), while non-significant positive between mite population and the relative humidity (0.46 and 0.25) during the two successive years, respectively. These findings are less similar to that detected by El-Halawany *et al.* (2001) who mentioned that *M. phoenicis* was recorded in high number on old fronds and buds in Behera, Alexandria, Kafre El-Shiech Governorates.

1.2.1.2. Predacious mite and insect population:

The phytoseiid mites *A. swirskii*, *A. cydnodactylon* and *E. scutalis* appeared on Sewi date palm variety trees in March during the two successive years. The predators have one annual peak in mid August recorded (97 and 89 ind./ 40 fronds), during two successive years, respectively. After that, the population gradually decreased till November during the two successive years (Figure, 2). Statistical analysis of the obtained data from (Table, 4), clearly demonstrated that the relationship between the predator mites population and density of the tetranychid mite *E. orientalis* was highly positively correlated (0.96***and 0.95***) during two successive years, respectively. However, the relationship between the phytoseiid mite population and the *O. afrasiaticus* on fronds was highly significant positively affected (0.95*** and 0.76**) during two successive years, respectively, while these relations in fruit non-significant positive (0.52) in the first year and significantly affected (0.68*) in the second year. The relationship between the phytoseiid mite population and the tenuipalpid mite, *T. eriophyoides* were significant positively affected (0.71* and 0.69*) during two successive years, respectively. Whereas, the relationship between the phytoseiid mite population and the two eriophyid mites *E. saudiarabis* and *M. phoenicis* had a negative effect during two years, As shown by correlation values, Correlation coefficient values (-

0.03 and - 0.01) and (- 0.65* and - 0.58) in the first and the second year, respectively. These results indicated that, the phytoseiid mites seemed to be important predators to suppress the population density of tetranychid and tenuipalpid mite population. These facts indicate that tetranychid and tenuipalpid mite prey probably plays an important part of the predator diet.

Statistical analysis present in Table (4) showed that, the temperature was a significant positive correlation with the density of the population of the phytoseiid mites (0.91***& 0.89*** and 0.90*** and 0.82**), while relative humidity had a significant negative correlation with the mite population (-0.77 and -0.67*) during the two successive years, respectively.

Data as shown in Table (4) and illustrated in Figure (2) showed that, the population density of the stigmatid mite, *A. exsertus* was recorded with low numbers in May and gradually increased in number and reached the maximum number in mid August during recorded (88 and 81 ind./40 fronds) the two successive years, respectively. Statistical analysis present in Table (4) showed that, temperature was a highly significant positive correlation with density of the population of the stigmaid mite, while relative humidity had significant negative correlation with the mites population in the first year but, non-significant negative correlation in the second year. These results agree with those of Elhalawany and Abou-Setta (2013) who found this predator had one peak in spring and decreased in November then disappeared in winter months; and they reported that it was widely distributed on guava associated with tenuipalpid mites. In addition, El-Sanady and Mohamed (2013) found this predatory mite in associated with pests infesting date palm varieties in Giza and Suhag governorates of zaghloul and sewi varieties as well as, it was recorded by Mesbah (2014) in Giza and Sharkeya.

The results in Table (4) and Figure (2) Clarified that, the ladybirds coccinellid predator, *Stethorus gilvifrons* (Mulsant) was recorded in mid March with a few numbers on Sewi date palm during seasons 2017 and in mid April in 2018. After that, the population gradually increased in mid May in both seasons 2017-2018. Then reach its peak during early July and mid July were 27 and 35 individuals/ 40 fronds at the temperature ranged between 25.6-40.93℃ and 24.29-40.36℃ and relative humidity 16.93& and16.06%, during the first and second year, respectively after that the population gradually decreased in number and disappeared in october. A highly significant positive correlation between the predacious insect density and temperature (0.78** and 0.90***), while, relative humidity had a significant negative correlation with the coccinelid predator population (-0.85*** and -0.66*) during the two successive years, respectively.

Statistical analysis of Table (4) data revealed that, highly significant positive correlation between the predacious insect density and the population fluctuation of tetranychid mites, *E. orientalis* (0.84** and 0.86***) and *O. afrasiaticus* (0.75** and 0.70*) in two seasons (0.74* to 0.95**); but non-significant positive between the predatory insect and both tenuipalpid mites, *P. aegyptiacus* and *T. eriophyoides* during two seasons (0.03 to 0.59), while these relation non-significant with predator *S. gilvifrons* and the two eriophyid mites during two seasons. These facts indicate that tetranychid prey probably play an important part of the predator insect diet.

These results are agreement with finding by Idder and Pintureau (2008) and Latifian (2012) showed that, natural enemies of *O. afrasiaticus* include predatory insects, such as the coccinellids *Stethorus punctillum* Weise, *S. gilvifrons* and phytoseiid mites.

1.2.2. Population dynamics of phytophagous mites and predaceous on zaghlol date palm variety in Qalyubia Governorate during 2017-2018.

The citrus brown mite *Eutetranychus orientalis orientalis* population:

The tetranychid mite, *E. orientalis* was recorded with moderate number of upper fronds on Zaghlol date palm at the Qalyubia Governorate. Data illustrated from (Figure, 3) clearly indicated that, *E. orientalis* has one annual peak of seasonal abundance in early August in the first year recorded 208 individuals/40 fronds and 175 ind./40 fronds in the second year. After that, the population gradually decreased in number till November. Statistical analysis of Table (5) showed that the mite population of *E. orientalis* had highly significant positive correlation with temperature (0.88*** 0.86***) in the two successive years. The relative humidity was non-significant negative correlated in the first year (-0.17) and non-significantly positive correlated (0.11) in the second year.

These results are in accordance with that of Singla (2001) who showed that fluctuations in the populations of *E. orientalis* on guava, in relation to climatic factors and the population of a predatory mite, *E. scutalis*, were investigated in Punjab.

The tenuipalpid mite *Phyllotetranychus aegyptiacus* population:

The flat mite, *P. aegyptiacus* Sayed was recorded with high numbers fronds of Zaghlol date palm at the Qalyubia Governorate. As shown in Figure (3) indicated that, it has two peaks, which were recorded in early June and early october recorded 268 and 296 ind./40 fronds in the first year 2017, while, in the second year reached its peaks in late May and late September recorded 301 and 329 ind./40 fronds. Population density of *P. aegyptiacus* exhibited a positive correlation with temperature (0.55 and 0.58, 0.59 and 0.0.67*), while relative humidity was non-significant negative correlated with the mite population (-0.25) in 2017 season and non-significant positive (0.23) in 2018 season Table (5).

The red palm mite *Raoiella indica* population:

The red palm mite, *R. indica* has two annual peaks were recorded in early May and late october 2017 (199 and 179 ind./40 fronds), and in late April and the second week of october 2018 (232 and 212 ind./40 fronds) Figure (3). Statistical data obtained from Table (5) showed that, the population of *R. indica* had non-significant negative correlation with temperature and relative humidity during two seasons.

The eriophyid mite *Epitrimerus saudiarabis* population:

The data as shown in (Figure, 3) showed that, the eriophyid mite it has two peaks, which were recorded in third week of April and early october recorded 126 and 138 ind./40 fronds in season 2017, while, in the second year 2018 reached its peaks in mid April and fourth week of September recorded 143 and 155 ind./40 fronds. Population density of *E. saudiarabis* exhibited non-positive correlation with temperature and while relative humidity during the two successive years Table (5).

Date palm bud mite *Mackiella phoenicis* population:

The eriophyid mite *M. phoenicis* has two peaks, which were recorded in third week of April and early october recorded 377 and 363 ind./40 fronds in season 2017, while, in the second year 2018 reached its peaks in mid April and fourth week of September recorded 340 and 326 ind./40 fronds. Statistical analysis of data proved that non-significant positive correlation occurred between the eriophyid mite population and both temperature and relative humidity during the two seasons Table (5).

Table (5): the correlation coefficient between temperatures, relative humidity and mite populations on zaghlol date palm variety in Qalyubia Governorate during 2017-2018.

Season	Parameters	Correlation coefficient values					Max. Temp	Min. Temp	Mean RH
		Eutetranychus orientalis	***Phyllotetranychus aegyptiacus***	***Raoiella indica***	***Epitrimerus saudiarabis***	***Mackiella phoenicis***			
2017	Phytoseiidae	0.15	0.79**	0.31	0.73*	0.12	0.39	0.33	-0.62
	Cheyletidae	-0.16	0.76*	0.24	0.82**	0.34	0.11	0.21	0.04
	Stigmaeidae	-0.55	-0.2	0.97***	0.46	0.57	-0.48	-0.58	-0.50
	S. gilvifrons	0.97***	0.25	-0.57	-0.29	-0.86***	0.88***	0.85***	-0.17
	Max.Temp	0.88***	0.55	-0.5	0.16	-0.64	-	-	-
	Min. Temp	0.84**	0.58	-0.061	0.22	-0.62	-	-	-
	Mean RH	-0.17	-0.25	-0.42	0.20	-0.07	-	-	-
2018	Phytoseiidae	0.14	0.82**	0.45	0.73*	0.12	0.43	0.38	-0.09
	Cheyletidae	-0.16	0.76**	0.30	0.80**	0.34	0.13	0.26	0.39
	Stigmaeidae	-0.39	0.12	0.82**	0.46	0.57	-0.38	-0.40	0.48
	S. gilvifrons	0.84**	0.23	-0.44	-0.29	-0.86***	0.78**	0.67	-0.10
	Max.Temp	0.86***	0.59	-0.34	0.16	-0.64	-	-	-
	Min. Temp	0.78**	0.67*	-0.44	0.22	-0.62	-	-	-
	Mean RH	0.11	0.23	-0.46	0.20	-0.07	-	-	-

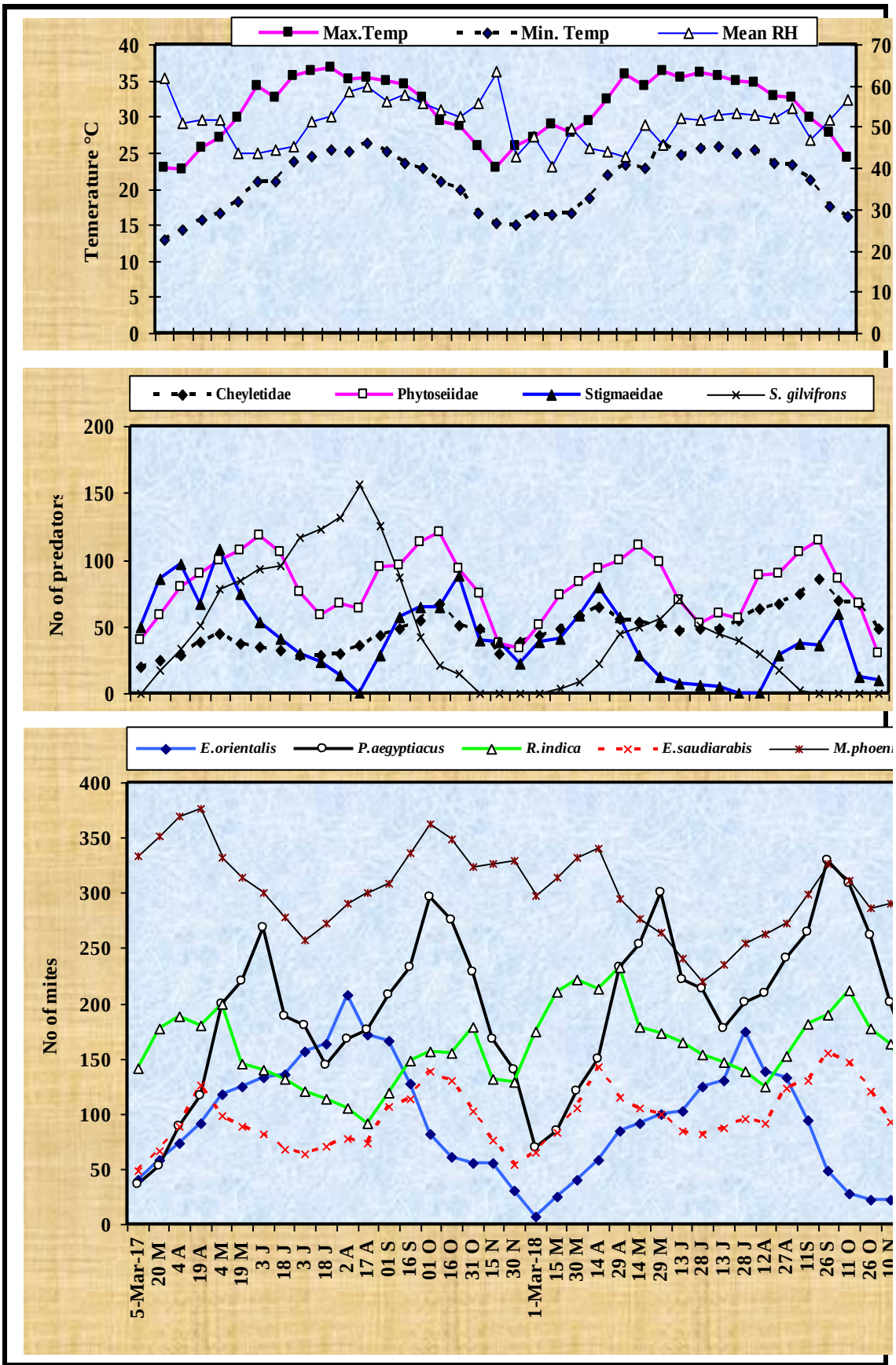

Figure (3): Population dynamics of phytophagous mites and predaceous on zaghlol date palm variety in Qalyubia Governorate during 2017-2018.

2.Predacious mite and insect population:

The predacious cheyletid mite population was appeared on fronds with low number early march and reached it's the first peak in early May 45 ind., and in late April 64 ind., and the second peak in mid october recorded 67 and 86 ind./40 fronds in the first and second season, respectively Figure (3). Statistical analysis of data indicated that, non-significant positive correlation occurred between the cheyletid mite population and both temperature and relative humidity during the two seasons Table (5). Also, the relationship between density of the population of the predator mite and *P. aegyptiacus* and *E. saudiarabis* was positive affected during two successive years.

The phytoseiid mites have two annual peaks in early June and mid october recorded 118 and 121 ind. In the first year, and in late May and mid october in the second year recorded (97 and 89 ind./ 40 fronds), respectively Figure (3). Statistical analysis in (Table, 5), indicated that, the relationship between the predator mites population and density of *P. aegyptiacus* and *E. saudiarabis* was a significant positive affected during two successive years. However, the relationship between the phytoseiid mite population and the *E. orientalis*, *R. indica* and *M. phoenicis* was positive affected during two successive years. These results indicated that, the phytoseiid mites seemed to be important predators to suppress the population density of phytophagous mite population. These results are in accordance with that of (Mukherjee and Singh, 1993) who showed that, the populations of *E. orientalis* were positively correlated with those of *E. scutalis*. *E. orientalis* was observed from July to September, and had a population peak in August at a median temperature of 28.18°C, RH of 81.39%, when the population of *E. scutalis* was also at its peak.

Data as shown in (Figure, 3) and Table (5), showed that, the stigmatid mite, *A. exsertus* has two peaks in early May and late october (108 and 121, 80 and 60 ind./40 fronds) during two seasons, respectively. Statistical analysis proved that, the relation between the stigmatid mite population and *R. indica* was a highly significant positive affected during two successive years (Table, 5).

The results in Table (5) and Figure (3) Showed that, the coccinellid predator, *S. gilvifrons* was recorded in spring with a few numbers on Zaghlol date palm during two seasons. After that, the population gradually increased and reached its peak in mid August 157 ind./40 fronds in the first season, and in late June recorded 71 ind./ 40 fronds in the second year, after that the population gradually decreased in number and disappeared in November. A highly significant positive correlation between the predacious insect density and temperature, while relative humidity had a non-significant negative correlation with the coccinelid predator population during the two seasons.

These results are agreement with finding by Payandeh *et al.* (2013) who found that, the population densities and spatial distribution pattern of *S. gilvifrons* were investigated in date palm orchards in Iran, and the highest population density of this predator on fruits and pinnae has been observed in the first half of August and the spatial distribution pattern of *S. gilvifronsis* a random distribution.

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Functional response of the predatory bug *Orius sauteri* (Hemiptera: Anthocoridae) to chilli thrips *Scirtothrips dorsalis* (Thysanoptera: Thripidae) infested mango trees in Hainan province, China

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Abstract:

Scirtothrips dorsalis Hood. (Thysanoptera: Thripidae) is a very important pests in southern China, it can infest vegetable, ornamental plants and fruit crops as well as, transmission various tospoviruses pathogen to host plants. Also, *Oruis sauteri* is very widespread predator in China, it can be consumed different preys like, Aphids, thrips and spider mites. We measured the feeding consumption of *O. sauteri* different stages to 2nd larvae and adult female of *S. dorsalis* under three constant temperatures and different densities of prey. As well as, measured the functional response of *O. sauteri* different stages to 2nd larvae and adult female of *S. dorsalis*.The functional response of the*O. sauteri* different stages were fitted to II type of functional response (Holling, 1965).The feeding consumption of different *O. sauteri* stages increased with the temperatures and *O. sauteri*different stages. However, the female of *O. sauteri* consumed more 2nd larvae of *S. dorsalis* than 4th and 5th nymph of *O. sauteri.* Also, the females of *O. sauteri* consumed more females of *S. dorsalis* than 4th and 5th nymph of *O. sauteri.*AS well as, the increasing in temperatures lead to increase in attack rate and decreased the handling time as well as, increasing the limit of predation of *O. sauteri* different stages.

Introduction

Mango trees *Mangifera indica* Linnaeus (Anacardiaceae) is familiar cultivated in over 100 countries in the world, especially in tropical and subtropical regions as well as, it is one of the most important tropical fruits in China in terms of production, marketing and consumption due to their exotic flavor and delicious taste, as well as high

nutritional value and give high yield. AS well as, China the second largest mango producer in the world with 5.1 million ton produced in 2018. Most of mango orchards cultivated in southern of china. Hainan Province is the largest mango producer in china, followed by Guangxi Autonomous Region, Guangdong Province, Yunnan Province, Sichuan Provinces and Fujian Province, (Chen, 2013 and NBSPRC, 2015). Mango orchards are attacked by several groups of pests i.e. floral malformation, powder mildew, anthracnose, fruit fly, stem borer and acarina (Amer *et al.*, 2017). The most important mango's pests in Hainan are thrips (Chen *et al.*, 2017 and Gao *et al.*, 2006) belonging to the Order: Thysanoptera which includes families of Thripidae, Aeolothripidae and Phlaeothripidae. Both immatures and adult females and males cause severe damages. Chili thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) is an important pest of various vegetable, ornamental and fruit crops in southern and eastern Asia, Africa, and oceania (Ananthakrishnan, 1993; CABI/EPPO, 1997; CAB, 2003 and Sandeep *et al.*, 2017). In Hainan, the dominant thrips species during the shoot period and young fruit stage was chili- thrips, S. *dorsalis* . Thrips are become primary and very dangerous pest on mango orchards in China (Huang, 2010 and Han *et al.*, 2015). It had wide range of host plants Over 100 host plants including 40 families (i.e., banana, beans, chrysanthemum, citrus, corn, cotton, cocoa, eggplant, ficus, grape, grasses, holly, jasmine, kiwi, litchi, longan, mango, onion, peach, peanut, pepper, rose, soybean, strawberry, tea, tobacco, tomato (Mound and Palmer 1981 and Mound , 2007). As well as, *S. dorsalis* also can transmit many viruses (i.e., chilli leaf curl (CLC) virus, and peanut necrosis virus (PBNV) (Mound and Palmer, 1981 and Ananthakrishnan, 1993) and tobacco streak virus (TSV) in groundnut crops in India (Rao *et al.* , 2003). Also, in Thailand AS well as, can transmit three viruses (i.e., melon yellow spot virus (MYSV), watermelon silver mottle virus (WsMoV), and capsicum chlorosis virus (CaCV) field crops was confirmed (Chiemsombat *et al.* , 2008). Chilli thrips attacks all young leaves, buds and fruits. Heavy infestation of these pests cause defoliation and drought branches on the plants due to turns tender leaves, buds, and fruits bronze to black in color as well as, causing considerable damages and consequently reduce mango production and bring down the marketing value by decreasing quantity and quality of fruits(Vivek Kumar *et al.* , 2014).

Oruis species is well known to be one of the important natural enemies of thripsin China (Tan *et al*., 2013). As well as, it is a polyphagous natural enemies which that had a lot of preys such as Aphids (Zhou *et al*., 2006 and Ahmadi *et al*., 2007) spider mites (Zhou *et al*., 2006) and the eggs of moths (Bullter and O'neil, 2007; Zhou *et al.*, 2006 and Zhou and Lei, 2002). As well as, its role in controlling many species of thrips (i.e. *Orius insidiosus* (Say) on *Thrips tabaci* Lindeman and *Orius laevigatus* (Fieber) on *Frankliniella occidentalis* (Pergande). Also, *Orius sauteri* (Poppius) has been studied its effective in decreasing the population density of *T. palmi* (NAGAI, 1993 and 1999 and Xux and Enkegaard, 2009). AS well as, there were most of insect pest can be efficiently controlled by *O. sauteri* (Zhang *et al*., 2007) and recorded as an important predator of many pests of thrips, spider mites, aphids and eggs of lepidopteran insects in fields and orchards in Japan, China, Korea, and Russian Far East (Yano, 1996 and Yang *et al*., 2018). Functional response is refers to the number of prey successfully attacked by predator during unit of time (Solomon, 1949) as well as, it described the relationship between the

predator rate of consumption and prey density. There were many factors that affected in the functional response of a predator; the most important is temperature which both predators and predators are to be found, (McCaffrey and Horsburgh, 1986).

Functional responses according to prey consumption by the predator is very important studies which using natural enemies in biological control programs to decrease the pest densities on different crops (Lia and Yano, 2010; Ganjisaffar and Thomas, 2015 and (Wang *et al*., 2018). This study will provide us the basic information for optimal use of *O. sauteri*in biological control programs to control *S. dorsalis* on mango trees.This work was carried out to study the functional response of *O. sauteri* on different densities of the 2nd larvae and adult female of chilli- thrips, *S.dorsalis*.

Materials and methods.

1. Predator rearing:

O. sauteri were obtained from Beijing Kuo Ye Bio-Tec co., Ltd Company (China) and had been reared for several generations under laboratory conditions at 26 ±2 °C, 60 ± 10% RH and L18:D6 photoperiod. The predators were reared using the methods described by (Isenhor and Yeargan, 1981). Adults and nymphs of *O. sauteri* were collected to be kept in plastic jars of 10 cm (diameter) ×20 cm (height) covered with muslin and held in place by means of rubber bands. Each jar was provided with both small balls of white foam to reduce cannibalism behavior and sufficient quantities of *Corcyra cephalonica* eggs as food supply for the enclosed predators. A piece of bean pod (*Phaseolus vulgaris*) was provided in each jar as an ovipostional substrate (Isenhor and Yeargan, 1981). Eggs are inserted into the tissue of bean pods. Bean pods with newly deposited eggs inside were kept in plastic jars previously described. Jars were examined daily until hatching. Soon after hatching; newly-hatched nymphs on bean pods were carefully transferred to plastic jars and provided with eggs of *Corcyra cephalonica.*

2. Prey colony:

Chilli- thrips, *S. dorsalis* was collected from mango orchards (*Mangifera indiaca*: Anacardiaceae) in Hainan province, China during 2018 from. The colony was maintained fresh mango leaves, which collected from the symptomatic plants and place them in a ziplock bag to prevent adults from escaping. As well as, put the infested mango leaves in metalic cages (100 x 135 x 135 cm) with nylon gauze sides under laboratory conditions at 26 ±2 °C, 60 ± 10% RH and L18:D6 photoperiod and we used the 2nd larvae and adult female directly as a prey.

3. Predation consumption by different developmental stages of *Orius* ***sauteri*:**

Newly individual hatched nymphs of *O. sauteri (*0-12 hrs) were collected from the stock colony which reared in laboratory and were put in separate plastic petri dishes (7 cm diameter) and provided with 2nd instar of larvae of *S.dorsalis*. We checked the number of prey which had been consumed by *O. sauteri* different nymphal stages by using a stereomicroscope (20×). All petri dishes were checked for predation consumsion after 24 h, and the number of second instar larvae of *S. dorsalis* which consumed by different developmental stages of *Oruissauteri* (4th ,5th) during 24 h period.

Couples of newly emerged adults (male and female) were placed separately in plastic petri dish (6cm diameter) without preys to stimulate mating occurrence. Twelve hrs later, males were removed to another petri dish. Then, these dishes were supplied with different densities of prey (20, 40, 60, 80 and 100 individuals 2nd instars larvae of *S. dorsalis*). The tests were conducted for a 24 h period. We checked the number of prey which had been consumed by adults of *O. sauteri* by using a stereomicroscope (20×).

There were 20 replicates per each thrips density. A predator was tested only once. A control experiment consisted of a similar setup but without the predator.

4. *Orius sauteri* functional response to *Scirtothrips dorsalis* different densities at three constant temperatures:

The predation ability of the developmental stages (4^{th}, 5^{th} and females) of *O. sauteri* to feeding on 2^{nd} larvae and adult of *S. dorsalis* at three constant temperatures (22, 26 and 30oc) and various densities were compared by analysis of variance (ANOVA) using SPSS for (Windows version 18). Also, we used analysis of variance to compare differences in the numbers of 2^{nd} larvae and adult *S. dorsalis* which consumed by *O. sauteri* between different densities and temperatures. The mean values were compared using Tukey test at the P= 0.05 level of significance. The functional response data were fitted to type-II responses (Holling, 1959). Parameters of a type-II model were estimated by the random predator equation:

$$N_a = N_t[\,1 - \exp(aTP_t/1+aT_hN_t)]$$

where $N_a$is the rate consumption of predator on prey during selected time period (24 h); a' is the instantaneous attack rate; N is the density of prey ; T is the selected predation period (1 day); T_h is the duration of one prey consumption by predator, i.e., the handling time. The potential maximum *Na* (*Na*-max) was estimated by dividing instantaneous attack rate by the handling time (Holling, 1959).

Results and discussion

1.Functional response of (4^{th}, 5^{th} and females) of *Orius sauteri* to 2^{nd} larvae of *Scirtothrips dorsalis* at three constant temperatures :

With regard to Data on Table (1) and graphically illustrated in Figure (1) showed that the functional response of (4^{th}, 5^{th} and females) of *O. sauteri* to 2^{nd} larvae of *S. dorsalis* at three constant temperatures (22, 26 and 30°C)were typed II response (Holling, 1965) and they approximated by the functional reaction disk equation. As well as, Data on Table (1) showed the different parameters of functional response of different stages of *O. sauteri* to 2^{nd} larvae of chili-thrips. There was a general increase in thrips consumption with increasing temperature. There was also an increase in thrips consumption with a corresponding increase in prey density, indicating that *O. sauteri* functionally responded to this prey species. The predation rate (1/b) on *S. dorsalis* 2^{nd} larvae was increased with predator stages. We found that the predation rate increased on females and 5^{th} of *O. sauteri*. The increasing in temperatures lead to increase in predation rate(1/b). Whereas, the rate of successful search (a) was also, increased with predator stage and increasing with increasing temperatures. The handling time(b)was decreased with increased in and increasing of prey densities and the shorter handling time and searching time and at higher densities of prey. As well as, the *O. sauteri* can kill more thrips at high densities also in high temperatures as well as, females and 5^{th} of *O. sauteri* can kill more thrips than 4^{th} of temperatures thrips. The Predation efficiency (a/b) was increased with increasing temperatures and predator stages. The 5^{th} nymph and females of chili-thrips consumed more thrips than 4^{th} nymph at three temperatures.

2.Functional response of (4^{th}, 5^{th} and females) of *O. sauteri*to females of *S. dorsalis* at three constant temperatures :

The obtained results in Table (2) showed that, the functional response of (4^{th}, 5^{th} and females) of *O. sauteri* to females of *S. dorsalis* was also, fitted by type II model of response. There was increasing in the numbers of the adult females of *S. dorsalis* killed by the predator, *O. sauteri* with an increase in temperature and thrips density, the functional responses of *O. sauteri*

preying on adults female of *S. dorsalis* at various densities (Figure, 2), show that the functional response curves were affected by differences in the predation rates over 24-h period of *O.sauteri* at all the densities and temperature ranges. The functional response parameters showed that, The attack rates against females of thrips were significantly increased at each of the three constant temperatures (Table 2). The handling times for females of thrips was decreased with an increase in temperature and predator stages while for the adults. the rate of successful search (a) was also, increased with predator stage and increasing with increasing temperatures.

There were many researchers have mentioned that, *Oruis sp*. show a II type of functional response (Holling, 1965).All type of functional response has been indicated in the western flower thrips, *Frankliniellaoccidentalis* (Pergande) (Coll and Ridgway ,1995), *S. dorsalis* (Wang *et al*., 2014), *Aphis gossypii* Glover and *Thrips palmi* (Nagi, 1993). Also, McCaffrey and Horsburgh (1986) reported that functional response of *O. insidiosus* to *Panonychus ulmi* (Koch) were (Holling's type I and II) as well as, Lia and Yano (2010) also, founded the functional response of predatory bug *Orius sauteri* (Poppius) to Thrips palmi Karny on eggplant leaves were type I and Type II curves. This study also, showed the type II functional response of *O. sauteri* to *S. dorsalis* (Tables 1 and 2) and (Figures 1 and 2).

McCaffrey and Horsburgh (1986) studied the effect of different temperatures on the rate of successful search and the handling time of functional response of *O. insidiosus* to *P. ulmi* at, he found that the increasing in temperatures lead to increase in attack rate and decreased the handling time as well as, in this study also, showed that the handling time of 4^{th}, 5^{th} and adult females of *O. sauteri* decreased with increasing in temperatures at 26 and 30℃ as well as, the Predation efficiency and the predation rate increased with each increase in temperatures and increase of prey densities. (Isenhor and Yeargan, 1981a) mentioned that, the predation rate of *O. insidiosus* was increased with increase of prey densities.

This study was aimed to know the effects of temperatures , different densities of prey and predators stages on parameters of functional response of *O. sauteri.* This study was used for evaluating the biological control of *S. dorsalis* on mango trees. Generally, we found that, the different developmental stages (4^{th}, 5^{th} and females) of *O. sauteri* to 2^{nd} larvae and females of *S. dorsalis* were fitted by type II model of response. The (4^{th}, 5^{th} and females) of *O. sauteri* was consumed more 2^{nd} larvae of *S. dorsalis* than females because adult female of chili- thrips are more able to move and fly than larvae, and the *Oruis* may escape attack by the predators, thereby resulting in more larvae being killed at all temperatures and different densities. The feeding consumption increased at 30°C than other temperatures. The functional response parameters was affected by temperatures, various densities and predator stages, the increasing in temperatures lead to increase in attack rate and decreased the handling time as well as, increasing the limit of predation of *O. sauteri* different stages.

Table (1): Effect of different densities 2nd larvae of *Scirtothrips dorsalis* on the attack rate (a), handling time and maximum consumption rate on 4th, 5th and females of *Orius sauteri* derived from random predator equation at three constant temperatures.

Predator stages	Temperatures	Coefficient of correlation (r)	Functional reaction disk equation	The rate of successful search(a)	Handling time (b)	Predation efficiency (a/b)	The expected maximum consumption/ day (1/b)	χ^2 Chi-square	P
4th	22°C	0.9893	Na=0.3880N/(1+0.04148N)	0.38809	0.1069	3.63	9.35453695	0.09074	0.999
	26°C	0.9674	Na=0.5887N/(1+0.04333N)	0.58878	0.0736	7.99	13.58695652	0.25302	0.992
	30°C	0.91	Na=0.68723N/(1+0.02810N)	0.68723	0.0409	16.80	24.44987775	1.02898	0.905
5th	22°C	0.968	Na=0.38800.80 /(1+0.0630N)	0.6351	0.0782	10.36	12.82051282	0.03497	0.999
	26°C	0.9231	Na=0.7336N/(1+0.0528N)	0.7336	0.0528	13.89	18.93939394	0.91053	0.923
	30°C	0.9747	Na=0.7384N/(1+0.0270N)	0.7384	0.0366	20.17	27.32240437	0.50680	0.972
Female	22°C	0.9684	Na=0.7722N/(1+0.0517N)	0.7722	0.0672	11.52	14.92537313	0.22187	0.994
	26°C	0.9184	Na=0.8306N/(1+0.0359N)	0.8306	0.0433	19.18	23.09468822	1.36996	0.849
	30°C	0.9796	Na=1.0259N/(1+0.0292N)	1.02595	0.0285	35.99	35.0877193	0.36673	0.985

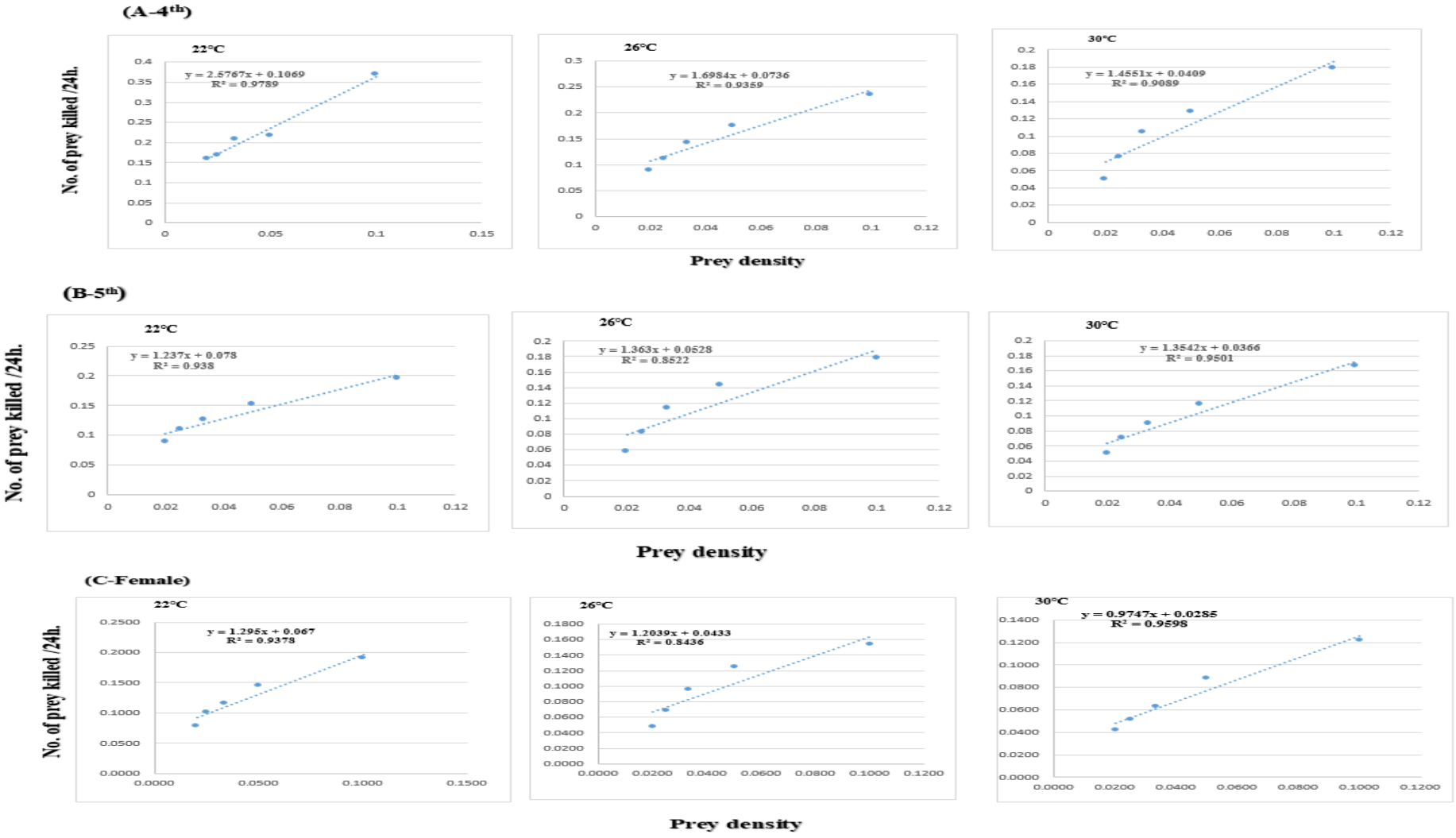

Figure 1 (A, B and C): Observed functional response of 4th, 5th and female of *Orius sauteri* to 2nd larvae of *Scirtothrips dorsalis* at three constant temperatures.

Table (2): Effect of different densities of *Scirtothrips dorsalis* females on the attack rate (a), handling time and maximum consumption rate on 4th, 5th and females of *Orius sauteri* derived from random predator equation at three constant temperatures.

Predator stages	Temperatures	Coefficient of correlation (r)	Functional reaction disk equation	The rate of successful search(a)	Handling time (Th)	Predation efficiency (a/Th)	The expected maximum consumption/ day (1/Th)	χ^2 Chi-square	P
4th	**22°C**	0.9423	Na=0.533N/(1+0.1104N)	0.533447	0.207	2.577	4.83	0.17157	0.99652
	26°C	0.9104	Na=0.60088N/(1+0.1017N)	0.600889	0.1694	3.547	5.90	0.25397	0.99259
	30°C	0.9723	Na=0.6101N/(1+0.05674N)	0.610165	0.093	6.560	10.75	0.29332	0.99024
5th	22°C	0.9074	Na=0.6090N/(1+0.09325N)	0.609087	0.1531	3.978	6.53	0.20202	0.99523
	26°C	0.9377	Na=0.7304/(1+0.0901N)	0.730460	0.1234	5.919	8.10	0.05654	0.99648
	30°C	0.8872	Na=0.7910N/(1+0.0711N)	0.791014	0.09	8.789	11.1	0.34516	0.98672
Female	22°C	0.9455	Na=0.5648/N/(1+0.05055N)	0.531688	0.1085	4.900	9.216	0.41154	0.98152
	26°C	0.9576	Na=0.5648/N/(1+0.05055N)	0.564844	0.0895	6.311	11.17	0.44144	0.97895
	30°C	0.988	Na=0.6088/N/(1+0.0371N)	0.608828	0.0611	9.964	16.36	0.10230	0.99874

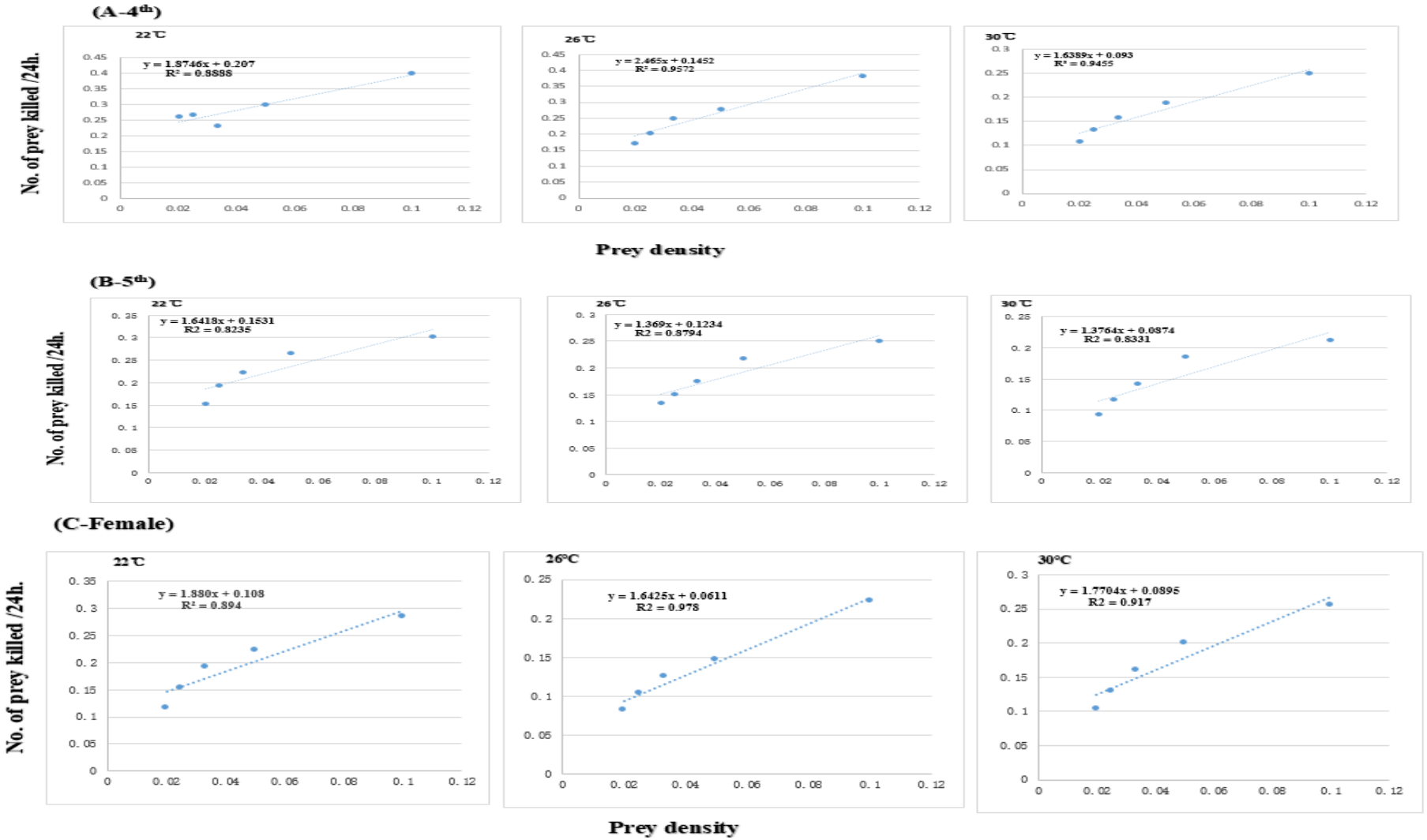

Figure 2 (A, B and C): Observed functional response of 4th, 5th and female of *Orius sauteri* to females of *Scirtothrips dorsalis* at three constant temperatures.

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Cotton mealybug *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) population density in eggplant and okra plantations and effect of some insecticides

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Abstract:

The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a polyphagous sap sucking insect with a wide geographical and host range causing serious losses in several economically important crops. Thus, laboratory and field experiments were conducted at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate during the seasons 2018 and 2019 to study the population density of *P. solenopsis* on eggplant and okra as affected by weather factors and to determine its, efficiency to certain synthetic insecticides. In both seasons, the infestation of *P. solenopsis* started during the early July. The highest population densities were recorded in the third week of August and first of September. The infestation of cotton mealybug was high significantly and positively correlated with the maximum and minimum temperature, while populations had insignificant negative correlation with relative humidity. Based on the multiple regression analysis and the coefficient of determination values (R2), the maximum and minimum temperature and the relative humidity were responsible for the changes in the insect population by 47.5 to 65.3%. Thiamethoxam was the most effective insecticide (LC_{50}= 3.19 and 3.46 mg AI L-1) against the third instar nymphs of *P. solenopsis* using the leaf-dip method, while buprofezine was the least toxic one with LC_{50} value of 121.79 and 146.14 mg AI L-1 on eggplant and okra. In an attempt to control this pest, seven toxic materials viz., imidacloprid, acetamiprid, spirotetramat, buprofezin, dinotefuran, thiamethoxam, and abamectin + thiamethoxam, belonging to different chemical groups, were tested for their influence against *P. solenopsis* on eggplant and okra under field conditions. Abamectin + thiamethoxam, imidacloprid, thiamethoxam, and acetamiprid showed the highest efficacy against *P. solenopsis* recording 91.05 to 81.50% reduction of the insect population. Spirotetramat was the least in this pest control.

Introduction

Vegetable crops are economic agricultural products in Egypt and allover the world. Both eggplant (*Solanum melongena* L.), family Solanaceae and

okra (*Abelmoschus esculentus* L.) (Moench), family Malvaceae are an economically important vegetable crop grown in tropical and sub-tropical parts of the world (Nwangburuka *et al.,* 2011). Eggplant is a major fruit vegetable with world production exceeding 31 million tonnes (Mt),Egypt (1Mt) (Daunay *et al.*, 2007).

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a polyphagous pest, feeding on a wide variety of host plants including such as Malvaceae, and Solanaceae. It attacks more than 166 plant species including field crops, vegetables, ornamentals, weeds, bushes and trees (Nagrare *et al*., 2012; Fallahzadeh *et al.*, 2014 and Abdel-Razzik *et al.*, 2015). The order of importance of hosts of *P. solenopsis* from the documented families was Malvaceae> Solanaceae > Astaracea > Euphorbiaceae > Amaranthaceae > Portulaceae (Harde *et al.*, 2018). It causes economic damage mainly to cotton, brinjal, okra, tomato, sesame, sunflower and china rose (Arif *et al.,* 2009 and Fallahzadeh *et al.*, 2014). Most *P. solenopsis* hosts belonging to families Solanaceae, Malvaceae and Cucurbitaceae, accounting for 48 % of the reported host plants (Fallahzadeh *et al.*, 2014 and Abdel-Razzik *et al.*, 2015).

P. solenopsis infestations on different hosts could be effectively controlled using synthetic insecticides, plant extracts, mineral oils and biological control agents (El-Zahi *et al.,* 2016; Seni and Naik, 2017; Mostafa *et al.,* 2018 and Rezk *et al.,* 2019). The present investigations were planned to study the population density of *P. solenopsis* on eggplant and okra as affected by weather factors and to determine its, efficiency to certain synthetic insecticides under laboratory and field conditions.

Material and methods

Field experiments were conducted during 2018 and 2019 seasons at the experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate and laboratory of Vegetable Pest Research Department, Sakha Agricultural Research Station.

1. Population density of *Phenacoccus solenopsis* in eggplant and okra:

The survey for *P. solenopsis* on infestation eggplant (*Solanum melongena* L. var. Black Beauty) and okra (*Abelmoschus esculentus* L. var. white velvet) crops were conducted at the farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, during 2018 and 2019 seasons. An area of 1000 m^2 was divided into four equal plots and considered as four replicates arranged in a complete randomized block design. On May 5, okra seeds and eggplant seedlings were sown or transplanted in both seasons. Inspection started 30 days after sowing or transplanting and continued weekly till harvesting. At each examination, 40 apical twigs of the same age were randomly chosen from this area (5 twigs from each corner plus 5 from the center/replicate) to count the adult females and nymphs of the mealybug.

2. Climatic factors:

Daily mean temperature and relative humidity were obtained from the Meteorological Department of Sakha Agricultural Research Station. The correlation (r) values were calculated between the climatic factors and *P. solenopsis* on using the SPSS statistical software package 16.0 (SPSS Inc., Chicago, IL, USA).

3. The tested compounds were:

The current study was carried out to evaluate the laboratory and field

performance of seven insecticides in their respective commercial formulations available on the market. Imidacloprid (Magknock 70% WG, Jiangsu, Aijin, Agrochemical Co., Ltd, China), acetamiprid (Mosprid 20%SP,Indogulf Crop Sciences Ltd), spirotetramat (Movento 10% SC, Bayer Crop Science, Germany), buprofezine (Hank 25%SC, Shandong, Sino- Agri United Biotechnology Co., Ltd), dinotefuran (Oshin 20% SG, Mitsui Chemicals Agro Inc. Japan), thiamethoxam (Medal 25%WG, Barigat and Estries Pvt. Ltd. India) and abamectin 3.32% + thiamethoxam 15.24% (Agri-Flex 18.56% SC, Syngenta Agroswissra). The insecticide generic and chemical information is given in Table (1) . The concentrations used were based on the recommendations of the Egyptian Ministry of Agriculture for each insecticide to control sucking pest insects under field conditions.

Table (1): Common and trade names of tested insecticides, their chemical classes and application.

Common name	Trade name	Chemical classes	Application rate/100L
Imidacloprid	Magknock	Neonicotinoid	70 g
Acetamiprid	Mosprid	Neonicotinoid	25 g
Spirotetramat	Movento	Tetramic acid derivative (ketoenole)	75 ml
Buprofezin	Hank	Buprofezin	150 ml
Dinotefuran	Oshin	Neonicotinoid	125 g
Thiamethoxam	Medal	Neonicotinoid	30 g
Abamectin 3.32%+thiamethoxam15.24%	Agri-Flex	Avermectin + neonicotinoid	120 ml

4. Laboratory assessments:

4.1. Insect colony:

To establish a culture of *P. solenopsis*, infested eggplant and okra plants were collected from plants in fields those do not have any previous exposure to pesticides. Adult females were separated and inoculated on eggplant and okra plants , potted under laboratory conditions of 30±2^{oc}, 65±5 RH. Two days later, the females settled on plant leaves and stems and started egg laying. The newly moulted third instar nymphs were used in the laboratory experiments

4.2. Toxicity of tested compounds to *Phenacoccus solenopsis:*

Leaf-dip method: A serial of concentrations was prepared from each insecticide using tap water for dilutions. Fresh eggplant and okra leaves with petioles were washed thoroughly with tap water and shad dried. Five leaves were dipped in each concentration for 20 seconds and in the tap water only for the control, and then shade dried. Circular openings were created in nine cm diameter plastic Petri dishes. A filter paper was put underneath each leaf to absorb any water vapor. Twenty newly moulted third instar nymphs of *P. solenopsis* were transferred to each Petri dish using a fine camel hair brush, representing one replication. Five replications were made for each concentration and the control. A binocular microscope was used to distinguish dead insects from live ones. Number of dead insects and the percentages of mortality were recorded after 24, 48 and 72 h of the treatment. Mortality was corrected according to Abbott's Formula (1925). Data were plotted on log dosage-probit papers and statistically analyzed according to Finney (1971).

5. Field assessments:

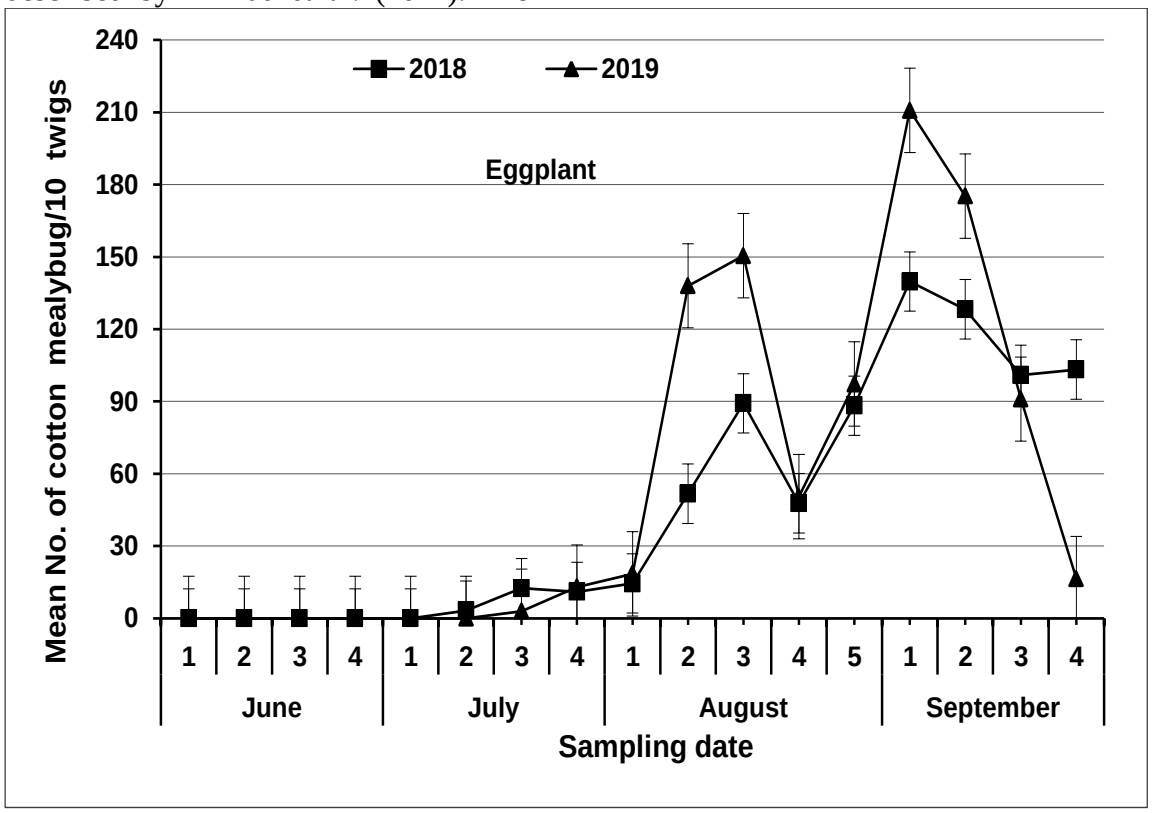
Field experiments were conducted

during September, 2018 and 2019 seasons at the experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh governorate. An area of 1000 m^2 planted with eggplant (*Solanum melongena* L. var. Black Beauty) and okra (*Abelmoschus esculentus* L. var. White velvet) was divided into plots 42 m^2 each treatment and infested with *P. solenopsis* on. This area did not receive any insecticidal treatments before the start of the experiment. Seven insecticides + control were tested in a arranged in a complete randomized block design with four replicates. The tested compounds were applied at recommended rates using a motor knapsack sprayer, tap water was used for dilutions. Ten eggplant and okra plants were randomly chosen from each replication to count the cotton mealybug population. According to the method described by Ahmad *et al.* (2011). The chosen plants were examined before spraying and 3, 7,10, 14 and 21 days post spray. The mean number of *P. solenopsisper* per eggplant and okra plant was recorded. Percentage of infestation reductions in mealybug population among treatments in relation to control was calculated according to Fleming and Retnakaran (1985) equation.

Results and discussion

1. Population density of *Phenacoccus solenopsis:*

During the seasons, 2018 and 2019, the infestation of *P. solenopsis* on eggplant and okra plants at Sakha Agricultural Research Station, Kafr El-Sheikh governorate started at low density in July (Figures, 1 and 2), the population increased gradually and the highest peaks of *P. solenopsis* were recorded in the third week of August and first week of September.



Figure(1): Population fluctuations of *Phenacoccus solenopsis* on eggplant plants at Sakha Agricultural Research Station farm in 2018 and 2019 seasons.

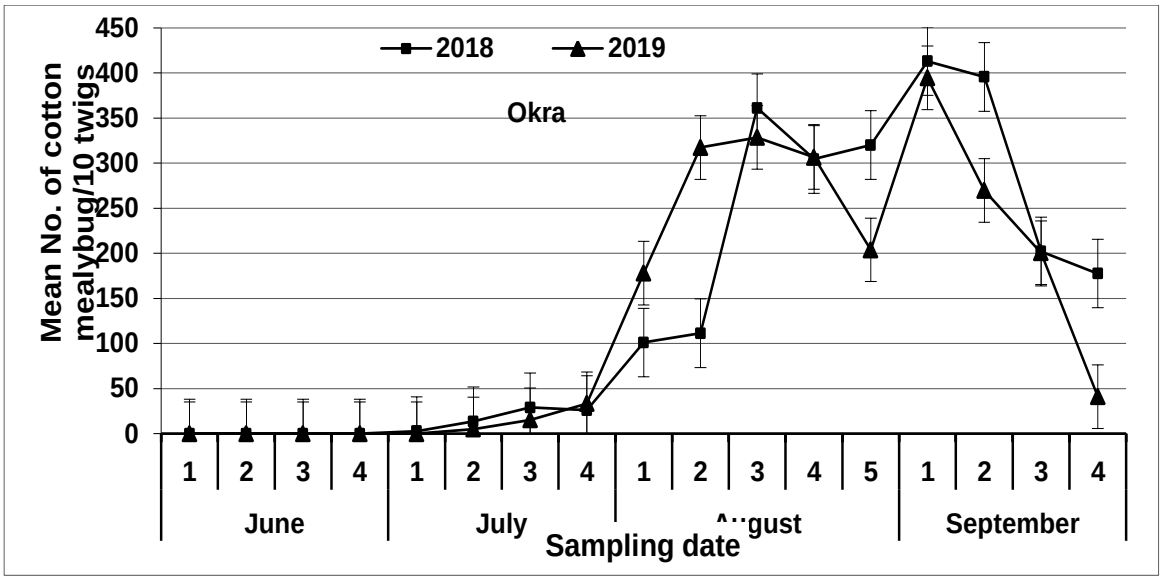


Figure (2): Population fluctuations of *Phenacoccus solenopsis* on okra plants at Sakha Agricultural Research Station farm in 2018 and 2019 seasons.

The present results are in parallel with Sahito *et al*. (2011) who observed the highest infestation of *P. solenopsis* on cotton during September and october, while Shahid *et al.* (2012) recorded the peak of the mealybug population in August and September. The highest this pest population was observed during the second half of September (Shah *et al.*, 2015). Singh and Kumar (2012) showed that *P. solenopsis* population is higher in october on cotton and okra whereas maximum population of mealybug were seen in February on tomato and potato host plants. *P. solenopsis* was recorded on eggplant during growing summer plantation 2016 from July till September, at Fayoum Governorate, infected with a few numbers of this pest and its was recorded one peak in the second week of August (Abd El-Wareth, 2016). The highest peaks the infestation this pest on eggplant plants were observed in June, July, August and September (Nabil, 2017). Also, El-Zahi and Abd-Elsalam (2017) found that the infestation of *P. solenopsison* cotton plants started at low density in June, the population increased gradually and its highest peak was observed in September. Nabil and Hegab (2019) found the infestation with *P. solenopsis* females started on the fourth week of July and the first week of August during 2017 and 2018 seasons, respectively. The population of females had two peaks, the first one occurred in August and the second peak recorded in September on okra plant.

2. Relationship between temperature, relative humidity and cotton mealybug populations

The population of *P. solenopsis* was high significantly and positively correlated (Table, 2) with the maximum (r = 0.624, 0.735, 0.797 and 0.663) and minimum (r = 0.658,0.735, 0.630 and 0.608) temperatures in 2018 and 2019 on eggplant and okra, respectively. Cotton mealybug populations had insignificant negative correlation with relative humidity on eggplant and okra all two growing seasons (Table, 2).

Table (2): Correlation (r) and regression (b) coefficients between some weather factors and *Phenacoccus solenopsis* per population on eggplant and okra twigs.

Season	Variable	Eggplant		Okra	
		r	% R^2	R	% R^2
2018	Maximum temperature (c^o)	0.624**	51.7	0.797**	64.1
	Minimum temperature (c^o)	0.658**		0.630**	
	Mean R.H (%)	-0.356NS		-0.212 NS	
2019	Maximum temperature (c^o)	0.735**	65.3	0.663**	47.5
	Minimum temperature (c^o)	0.735**		0.608**	
	Mean R.H (%)	-0.288 NS		-0.095 NS	

** Highly significant, P ≤ 0.01 —* Significant, P ≤ 0.05 —NS Not significant

According to the coefficient of determination values (R^2) of this study, the maximum and minimum temperatures and the relative humidity were responsible for the change in the population density of *P. solenopsis* by 51.7, 65.3, 64.1 and 47.5% during 2018 and 2019 on eggplant and okra, respectively. The obtained results of correlation and regression analysis clearly showed that weather factors play important role in the development of *P. solenopsis* population.

Singh and Kumar (2012) found that *P. solenopsis* population was showing positive correlation with higher temperature, whereas negative correlation with lower temperature and humidity. Also, the infestation of *P. solenopsison* cotton was positively correlated with the maximum and minimum temperature (Babu and Meghwal, 2014), and showed a positive correlation with the relative humidity (Hameed *et al*., 2014). El-Zahi and Abd-Elsalam (2017) found that the population of *P. solenopsis* was significantly and positively correlated with the maximum and minimum temperatures. The correlation between the relative humidity and the population was positive and insignificant. Nabil (2017) found that maximum and minimum temperature (˚C) and relative humidity showed positive significant relationship with the cotton mealybug population. Nabil and Hegab (2019) found that significantly positive correlation between maximum temperature and the population females of this insect whereas, a significant negative correlation was found with mean of relative humidity.

3. Toxicity of tested compounds to *Phenacoccus solenopsis* in laboratory:

Thiamethoxam was the most toxic compound to *P. solenopsis* nymphs (3.19 and 3.46) followed by imidacloprid with LC_{50} values of 12.67 and 14.98 mg AI L^{-1} after 72 hours, on eggplant and okra respectively. The LC_{50} was 14.97 and 15.76 mg AI L^{-1} for abamectin + thiamethoxam followed by dinotefuran with LC_{50} value of 27.92 and 27.34 mg AI L^{-1} on eggplant and okra, respectively (Table, 3). Buprofezine was the least toxic compound to *P. solenopsis* third instar nymphs.

The field rates of thiamethoxam and imidacloprid applied in the laboratory resulted in 95.2 and 81.6% mortality, respectively in the 2nd instar nymphs of *P. solenopsis* (Rashid *et al.*, 2011). Ujjan *et al.* (2015) found that lambda-cyhalothrin was highly effective with 50% lethal concentration followed by acetamiprid and Imidacloprid. Seni

and Naik (2017) found that lamda cyhalothrin was the most toxic followed by chlorpyriphos , imidacloprid and thiacloprid. El-Zahi and Abd-Elsalam (2017) found that thiamethoxam was the most effective insecticide against the third instar nymphs of *P. solenopsis* using the leaf-dip method, while lufenuron was the least toxic. Methomyl, acetamiprid and imidacloprid showed insignificant differences among them.

Table (3): Laboratory evaluation of tested compounds against *Phenacoccus solenopsis* (Third-instar).

Compound	LC_{50}[a] 95%CL[b]	LC_{90}[a] 95%CL[b]	Slope	LC_{50}[a] 95%CL[b]	LC_{90}[a] 95%CL[b]	Slope
	Eggplant			Okra		
Imidacloprid	12.67 10.92-14.52	48.65 39.79-63.12	2.19	14.98 8.82-23.42	72.12 54.36-234.26	1.88
Acetamiprid	38.95 33.61-44.83	163.20 130.57-218.54	2.06	42.15 36.32-48.67	184.92 146.11-252.28	1.99
Spirotetramat	88.72 76.98-103.94	352.88 264.27-507.99	2.14	96.94 83.91-114.19	383.27 290.02-559.63	2.15
Buprofezine	121.79 107.27-139.59	409.47 327.58-548.75	2.43	146.14 125.22-174.59	649.83 476.79-995.29	1.98
Dinotefuran	27.92 17.39-47.76	97.58 81.04-330.18	2.36	27.34 24.13-31.02	92.27 75.61-119.10	2.43
Thiamethoxam	3.19 2.76-3.64	11.71 9.64-15.04	2.27	3.46 3.01-3.94	12.59 10.36-16.20	2.28
Abamectin + thiamethoxam	14.97 13.52-16.54	34.03 29.51-10.86	3.59	15.76 14.28-17.37	34.68 30.19-41.41	3.74

[a]LC_{50} and LC_{90} are expressed in mg AI L^{-1}--[b]95%CL Confidence limits

4. Field assessments:

The insecticide efficacy of seven compounds, from different chemical groups presented in Table (4) and (5) were evaluated under field conditions for their efficacy against *P. solenopsis* infesting eggplant and okra plants at Sakha, Kafr El-Sheikh Governorate during two growing seasons, 2018 and 2019. The mealybug populations per eggplant and okra plant were not the same before application of the tested compounds. In fact this is a common problem where the crops are grown under natural field conditions and infested plants are randomly chosen and sampled (Ahmad *et al.*, 2011). Hence, the formula of Fleming and Retnakaran (1985) was used to calculate the percentage of mealybug population change using the mean population pre and post sprays in treated and control plots.

It is obvious that abamectin +thiamethoxam (90.38 and 90.78%), imidacloprid (89.16 and 89.52%), thiamethoxam (86.69 and 87.75%) and acetamiprid (83.28 and 84.63%) were the most potent compounds in reducing the population density of cotton mealybug in eggplant plants. It was followed by dinotefuran, buprofezine and spirotetramat (Table,4).

Table (4): Potency of tested compounds in reducing *Phenacoccus solenopsis* populations on eggplant plants at Sakha Agricultural Research Station farm, Kafr El-Sheikh Governorate

Compound	Used* conc. [mg a.i.l^{-1}]	Aver. No. pre- treat./5 plants	% Reduction					
			Initial effect %	Residual effect after indicated days				Grand average
				7	10	14	21	
2018								
Imidacloprid	489.51	150.5	75.66	86.29	91.56	95.33	96.98	89.16
Acetamiprid	50.0	140.75	61.44	82.57	88.34	92.05	92.07	83.28
Spirotetramat	75.0	133.0	55.93	76.20	85.57	86.31	34.45	67.69
Buprofezine	375.0	155.0	56.39	75.79	79.25	77.84	88.54	75.56
Dinotefuran	250.0	183.0	59.60	80.27	85.25	84.36	92.61	80.42
Thiamethoxam	75.0	210.25	73.0	84.27	89.93	92.58	94.69	86.69
Abamectin+thiamethoxam	222.63	233.0	76.85	88.12	92.50	96.37	98.05	90.38
Control(No.)	-	83.25	125.0	177.5	157.25	160.0	201.5	-
2019								
Imidacloprid	489.51	85.0	76.96	86.56	92.10	95.84	96.13	89.52
Acetamiprid	50	111.5	63.76	83.75	90.43	92.07	93.16	84.63
Spirotetramat	75	91.5	57.02	78.87	89.53	87.85	30.71	68.80
Buprofezine	375	123.0	57.03	76.25	80.73	75.35	89.12	75.70
Dinotefuran	250	109.75	59.78	79.99	84.16	82.04	93.69	79.93
Thiamethoxam	75	181.0	75.27	84.95	91.49	91.90	95.14	87.75
Abamectin + thiamethoxam	222.63	199.25	77.69	89.14	93.46	96.20	97.41	90.78
Control(No.)	-	71.5	115.0	162.75	181.0	141.5	152.25	-

*** the used concentrations were determined based on the recommendations of Egyptian Ministry of Agriculture**

Table (5): Potency of tested compounds in reducing *Phenacoccus solenopsis* populations on okra plants at Sakha Agricultural Research Station farm, Kafr El-Sheikh Governorate

Compound	Used* conc. [mg a.i.l-1]	Aver. No. pre- treat./ 5 plants	% Reduction					
			Initial effect %	Residual effect after indicated days				Grand Average
				7	10	14	21	
2018								
Imidacloprid	489.51	75.5	70.30	83.13	92.73	94.36	95.19	87.14
Acetamiprid	50.0	105	50.87	81.07	90.44	92.70	92.44	81.50
Spirotetramat	75.0	99.75	58.57	79.88	89.78	85.27	26.79	68.06
Buprofezine	375.0	115.5	55.62	74.52	79.91	77.51	90.77	75.67
Dinotefuran	250.0	111.0	65.22	77.75	83.48	81.78	95.39	80.72
Thiamethoxam	75.0	133.0	70.97	82.59	91.75	90.40	96.59	86.46
Abamectin+thiamethoxam	222.63	141.0	75.01	88.64	93.77	95.17	97.83	90.08
Control(No.)	-	66.0	103.0	171.0	105.25	77.5	145.5	-
2019								
Imidacloprid	489.51	212.0	68.32	81.30	95.15	97.28	97.77	87.96
Acetamiprid	50.0	92.5	58.46	79.87	91.83	92.69	92.79	83.13
Spirotetramat	75.0	140.0	66.62	78.27	92.19	86.92	24.79	69.76
Buprofezine	375.0	227.25	60.09	73.22	80.34	71.91	91.40	75.39
Dinotefuran	250.0	347.0	66.88	76.92	85.18	86.50	98.35	82.77
Thiamethoxam	75.0	227.25	69.46	80.44	90.10	89.38	98.53	85.58
Abamectin+thiamethoxam	222.63	336.0	74.76	87.79	96.17	97.15	99.38	91.05
Control(No.)	-	56.25	81.25	140.5	175.0	85.25	210.75	-

*** The used concentrations were determined based on the recommendations of Egyptian Ministry of Agriculture**

Abamectin +thiamethoxam and imidacloprid were the most effective compounds in reducing cotton mealybug in okra plants, with reduction of (90.08 and 91.05%) and (87.14 and 87.96 %) respectively in 2018 and 2019. It was followed by thiamethoxam and acetamiprid with reductions of (86.46 and 85.58 %) and (81.50 and 83.13 %), respectively (Table 5). It was followed by dinotefuran, buprofezine, while spirotetramat was the least in this pest control.

Rizvi *et al.* (2015) found that spirotetramat proved significantly superior in controlling *P. solenopsis*. El-Zahi *et al.* (2016) found that imidacloprid and thiamethoxam showed the highest efficacy against *P. solenopsis* recording 89.2 and 84.6% reduction of the insect population while emamectin-benzoate failed to exhibit sufficient *P. solenopsis* control. Unfortunately, recent studies reported that *P. solenopsishas* developed resistance to spirotetramat (Ejaz and Ali Shad, 2017). Sulfoxaflor, abamectin+thiamethoxam, spirotetramat, thiamethoxam, imidacloprid, buprofezin, and pymetrozine were tested for their effect against nymphs and adult females of *P. solenopsis* on potato under field conditions. The obtained results indicated that sulfoxaflor, abamectin + thiamethoxam and spirotetramat had the highest efficacy against *P. solenopsis* recording 80.3–96.05% reduction of the insect population after 21 days of application. Thiamethoxam, imidacloprid, buprofezin and pymetrozine failed to exhibit sufficient *P. solenopsis* control (Rezk *et al.*, 2019)

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Repulsive effect of potassium tartrate against *Eobania vermiculata* and *Monacha obstructa* (Gastropoda: Helicidae: Hygromiidae) land snails under laboratory and field conditions

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Abstract

The effect of potassium tartrat (PT) was studied against Eobania vermiculata (Müller) and *Monacha obstructa* (Pfeiffer) (Gastropoda: Helicidae: Hygromiidae) land snails under laboratory and field conditions. Four concentrations of PT (2, 4, 6 and 8%) were used as dust discs of lettuce leaves using non and free choice feeding methods. The laboratory results revealed that the PT hasn't any toxic effect against both land snail species at non choice feeding test. While, it caused repellent effect against the two land snail species. The 8 % concentration was the most effective concentration which gave 93 % repellency for *E. vermculata* and 85 % for *M. obstrucata* at free choice feeding test. Under Field condition 8% of PT as dust application on plant leaves against *E. vermiculata* and *M. obstructa* land snails was evaluated. The PT achieved 53% reduction for *E. vermiculata* after 7 days of treatment, while it gave 6 % reduction for *M. obstructa*. It was observed the snails avoided feeding and climbing the plants. So, it can conclude that the PT can be using as a product to protect the foliage of plants from snails attack and prevent them to climb the plants. Also, it prevents the pollution with snail's mucus which reduces the economic value of plants. So, it can be using this substance in the organic fields for controlling the snails.

Introduction

Land snails and slugs are herbivorous pests. They are pests of cultivated plant species in many regions of the world (Feldkamp, 2002). The damage of land mollusca caused by different ways, direct way by feeding on several plants such as field crops, orchards and vegetables. While, indirect way as infection by bacteria, fungi and virus due to the feeding of snails by scratching the plants (Lindqvist *et al.*, 2006). The land snails *E. vermiculata* and *M. obstructa* are the most distributed land snails in Egypt. These snails caused severe damage to all parts of orchards trees, vegetables and field crops (El-Okda, 1979). Chemical control is useful, but it can be harmful to other organisms. The commonly used chemical control is in the form of snail's pellets containing the active ingredient metaldehyde (Plomi *et al*, 2009). Also,

methomyl is one of the pesticides use as a molluscicides in Egypt, is known to affect on non target organisms and ground water. Repellent effect is another method to reduce slug and snails herbivores on crops may be to divert the slugs from target plants by offering palatable alternative food plants (Frank and Barone, 1999). However, such an a approach seems to be unsuitable for lettuce as this crop may be suffer sever yield losses from plant competition. Lettuce appears to be an attractive food source to slugs due to its thin, soft leaves and low levels of secondary compounds (Hegnauer, 1964). Snails and slugs pests are usually controlled with bait pellets containing either methocarb or metaldehyde (Garthwaile and Thomas,1996 and Speiser, 2001). In organic farming, there is a need to use friendly bio-compound to control the pests. Potassium tartrate (PT) is a salt of tartaric very used in food additive. Potassium tartrate, as a bird repellent, was tested for protecting non-target bird species from the poisoning hazards of acute and anticoagulant rodenticides (Soliman *et al.* ,2009). PT, proved to be a good repellent for quails, but not toxic for honey bee colonies as well as to be environmentally safe and used as pest integrated control (El-Gohary and Eissa ,2015).

This study is a part of larger research program in which the effect of potassium tartrate as a repellent compound against (land snail as the current study). The repellent effect of potassium tartrate (PT) was studied using dust application method against two land snail species, *E. vermiculata* and *M. obstructa* under laboratory and field conditions.

Materials and methods

1. Chemical compounds:

1.1. Potassium tartrate (PT) :

The material has the following formula: $K_2C_4H_4O_6$ with a molecular weight of 262. The pure PT is in the form of white pure crystal.

1.2. Talc powder:

Clay mineral (used as carrier material) hydratedmagnesium silicate $Mg_3Si_4O_{10}(OH)_{12}$. Talcin powdered form, often in combination with corn starch is widely used as baby powder.

2.Tested animals:

Two land snail species were used in the experiments. Choclate band snail, *E. vermiculata* and clover snail, *M. obstructa*. *E. vermiculata* was collected from orchards of Abu- Roash district, Giza Governorate, and *M. obstructa* was collected from clover field of Kom- Hamada, Behira Governorate. Healthy adult individuals were kept in laboratory in separate glass terraria (70 × 40 × 35) cm containing mixture of clay, sand and peat (1: 1: 1) of about (10 cm) deep was wetted with water and covered by white muslin with rubber band. Snails were performed under controlled temperature and light conditions. Snails were fed on carrot, lettuce and acclimated under laboratory conditions for four weeks. Five replicates (each of 10 animals of each species) were used for each test

3. Laboratory experiments:

3.1.Non choice feeding method:

This method was described according to **Shefte *et al*. (1982).** Serious concentrations of Potassium Tartrate (PT) were used as dust application on foliage lettuce against two land snail species, *Eobania vermiculata* and *Monacha obstructa*i.e. 2, 4, 6 and 8% of PT. Animals were exposed to 25cm^2green lettuce foliage discs for four successive days before the treatment. The consumed area was daily estimated. This procedure was repeated daily for four days with lettuce foliage discs previously dusted by the tested concentrations of PT and other ten snails were exposed to 25 cm^2green foliage dusted by talc powder (as carrier). The repellency

potential was calculated using the following equation according to **Bullard and Shumake (1983).**

$$\text{Repellency\%} = 100 - \frac{\text{Average consumed treated food}}{\text{Average consumed (treated+ untreated food)}} \times 100$$

Value ≥ 60% considered repellent.

3.2. Free choice feeding method:

Free choice feeding method was used according to Russell *et al*. (1989). Animals were exposed to two green lettuce foliage discs treated with the concentrations of potassium tartrate i.e (2, 4, 6 and 8%) and others were untreated. Also, ten animals exposed individually to two green foliage of lettuce 25cm^2 area for each animal. The first leave treated with talc powder and others were untreated. The position of the two exposure leaves was altered daily to avoid any bias to certain location. The area of the treated and untreated leaves was daily estimated. The repellency potential was calculated according to the previous equation.

4.Field experiment:

4.1. Effect of potassium tartrate against *Eobania vermiculata*:

The infested citrus seedling with *E. vermiculata* which planted in Nursery of the Agricultural Ministry, EL-Dokki district Giza Governorat, into three group each (4mx 4m) and the number of snails were counted (5 replicates) pre-treatment in each group. group (1)) dusting by 8% Potasuim tartrate (gave highly repellent effect in Lab) Group (2) dusted by Talc powder (stander carrier), Group (3) untreated as a control The snails were counted after treatment in each group/ replicates after 1 day,3.7 15 and 21 days (post-treatment), according the protocol of agriculture ministry. Reduction percent of snails number on plant were calculated according to Henderson and Tilton (1955) as a following formula.

$$\text{Reduction \%} = 1 - \frac{C_1 \times T_2}{C_2 \times T_1} \times 100$$

C_1=population of snails in control before application.

C_2=population of snails in control after application.

T_1=population of snails in treatment before application.

T_2=population of snail in treatment after application.

4.2. Effect of potassium tartrate against *Monacha obstructa*:

Three kirat of infested clover field with *M. obstructa* which planted in Kom Hamada district, EL-Behira Governorat, Egypt May 2018. Three groups (each 5 replicates) were one kirat. The same method was done as mentioned above.

5. Stattiscal analysis:

The results were statistically analyzed using the standard statistical methods LSD- test was applied in the analyses by SAS (2006).

Results and discussion

1. Repellency tests under labortary conditions:

1.1.Non choice feeding method:

Results in Table (1) revealed that the snails consumed the treated slices of lettuce on first and fourth day of treatment at 2 and 4% of potassium tartrate (PT) while they avoided eating the treated slices of lettuce on second and third day. The consumption of treated lettuce was less than lettuce with standard treatment or control. Regarding of 6 and 8% of PT snails avoided to eat on all days of treatment. PT achieved 100% repellent effect against *Eobania vermiculata* at 8% and it hasn't toxic effect for snail. The effect of PT against *Monacha obstructa* using non choice test was shown in Table (2). Data recorded that the snails avoided tofeed on treated lettuce with 2% PT on the first and third day of treatment while they fed on the second and fourth day. At 4% PT snails fed on 1st and 2nd day while they avoided to eat on the 3rd and 4thday. At 6% and 8% concentrations of PT snails avoided

to eat on the first, third and fourth days. While, they ate on the third day. On the other hand, it was observed that eating of treated plant was little than the carrier material and control. Potassium tartrate achieved 88.3, 54.2, 85.03 and 95.0 repellency% at 2, 4, 6 and 8% concentrations, respectively comparing with 79.87repellencypercentage in case the carrier. On the other side, it was observed that PT has non toxic effect on *M. obstructa*. From the previous results, it means that PT was safety compound and it caused repellent effect against land snails *E. vermiculata* and *M. obstructa*. This effect may be the resulting from the non acceptable taste for snails. Also, data investigated that the higher concentrations of PT were more repellent. Frank *et al* (2002) studied that in non choice test of 0.25 0.75 ml/ L caravane extract reduced the slug feeding comparing with the un- treated. Also, Capinera and Dickens (2016) found that the high concentrations of copper were more effective against snails and slugs as feeding deterrents by four terrestrial mollusks, two slugs and two snails were more effective feeding deterrents. Lindavist *et al.* (2010) registered that Birch tar oil, mixed with vaselline serves as an excellent long-term repellent against *Ariantana arbistorum* land snail and *Arion luwitanicus* land slug.

Table (1): Repellent effect of potassium tartrate (PT) against *Eobania vermiculata* using non choice feeding test.

Concentrations	Average daily consumption of Lettuce cm^2/ snail.					Repellency %
	1 st day	2nd day	3rd day	4th day	Average	
2	4.76 ± 6.5^{BAC}	0 ± 0.0^{D}	0 ± 0.0^{D}	0.1 ± 0.22^{D}	1.22 ± 1.68^{B}	85
4	5.9 ± 8.5^{BAC}	0 ± 0.0^{D}	0 ± 0.0^{D}	$0.1\pm.22^{D}$	1.5 ± 2.10^{B}	82.74
6	0 ± 0.0^{D}	0 ± 0.0^{D}	0 ± 0.0^{D}	0 ± 0.0^{D}	0 ± 0.0^{B}	100
8	0 ± 0.0^{D}	0 ± 0.0^{D}	0 ± 0.0^{D}	0 ± 0.0^{D}	0 ± 0.0^{B}	100
Standard treatment (carrier material)	7.36 ± 8.5^{BA}	3.5 ± 4.35^{BDC}	1.66 ± 2.41^{DC}	8.1 ± 6.18^{A}	5.16 ± 1.43^{A}	58.22
Control	6.64 ± 2.9^{BA}	5.57 ± 2.84^{BAC}	8.1 ± 1.90^{A}	8.47 ± 6.71^{A}	7.193 ± 2.29^{A}	

The vertical columns marked with the same letters are not significantly different by SAS (2006).

Table(2) : Repellent effect of potassium tartrate (PT) against *Monaca obstructa* using non choice feeding test.

Concentrations %	Average daily consumption of Lettuce cm^2/ snail.					Repellency %
	1 st day	2nd day	3rd day	4th day	Average	
2	0.0 ± 0.0^{BA}	0.83 ± 1.44^{BA}	0.0 ± 0.0^{B}	0.5 ± 0.87^{B}	0.33 ± 0.58^{B}	88,34
4	5.83 ± 5.06^{A}	0.50 ± 0.87^{A}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	2.11 ± 1.84^{BA}	54.23
6	0.58 ± 1.01^{BA}	1.17 ± 2.02^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.44 ± 0.76^{B}	85.03
8	0.0 ± 0.0^{BA}	0.5 ± 0.87^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.13 ± 0.22^{B}	95,06
Standard treatment (carrier material)	0.17 ± 0.29^{B}	2.00 ± 3.46^{BA}	0.0 ± 0.0^{B}	0.33 ± 0.58^{BA}	$0.63+0.76^{B}$	79.87
Control	$1.28+0.48^{BZ}$	2.22 ± 0.41^{BA}	4.11 ± 2.21^{A}	2.39 ± 0.63^{BA}	2.50 ± 0.46^{A}	

The vertical columns marked with the same letters are not significantly different by SAS (2006).

1.2. Free choice feeding method.

Data in Table (3) response of *E. vermiculata* land snail to potassium tartrate as dust application using free choice feeding method. Results revealed that *E. vermiculata* snails consumed the un-treated lettuce discs in all concentrations on all days of treatment. While they ate the treated lettuce discs with 2, 4, 6 and 8% concentrations after one day of treatment. The consumed amount was 2.95, 0.0, 0.7 and 0.65 cm^2, respectively comparing with 14.2 cm^2/snails for control. The same trend occurred on the following days of treatment, whereas the consumed amount was little than control. While, the snails avoided to consume the treated lettuce discs after the second and third day of treatment at 6% and 8% concentrations. Results indicate that the repellency percent were 71.57, 91.75, 82.88 and 93.80% at the concentration of 2, 4, 6, and 8% of PT respectively. Concentration 8% of PT was the most effective as a repellent for *E. vermiculata* land snails. The repellent effect of different concentrations of PT as dusting application against *M. obstructa* land snails was shown in Table (4). The same trend was observed in case of *M. obstructa* whereas the consumed amount of treated lettuce discs was less than control. Snails avoided eating the treated lettuce after the second and third day of treatment. Animals began to eat again on the fourth day of treatment, but the consumption of treated lettuce discs was less than the consumption of untreated lettuce. Also, 8% concentration of the PT caused highly repellency percent for *M. obstructa* whereas it gave 85.45% repellency. The other concentrations of PT 2, 4% and 6% caused 75.45, 67.68, and 77.77% repellency, respectively.

Table (3): Repellent effect of potassium tartrate (PT) against *Eobania vermiculata* using free choice feeding test.

Concentrations%	Average daily consumption of Lettuce cm^2/ snail.										Repellency %
	1st Day		2nd Day		3rd Day		4th Day		Average		
	Un	T	Un	T	Un	T	Un	T	Un	T	
2	5.2±7.8^{CEBD}	2.95±4.9^{CEBD}	0.3±0.6^{E}	0.0±0.0^{E}	4.0±5.58^{CEBD}	0.45±1.01^{E}	6.65±6.8^{CBD}	3.0±3.02^{CEBD}	038±5.22^{B}	1.6±2.2^{CBD}	71.57
4	7.5±11,0^{B}	0.0.0±0.0^{E}	0.5±0.6^{E}	0.75±1.68^{E}	4.2±9.39^{CEBD}	0.35±0.78^{E}	0.1±0.2^{E}	0.0±0.0^{E}	3.08±5.3^{CBD}	0.28±0.6^{CD}	91.75
6	4.65±8.7^{CEBD}	0.7±1.5^{E}	2.0±3.6^{CEBD}	0.0±0.0^{E}	0.0±0.0^{E}	0.0±0.0^{E}	2.05±4.5^{CEBD}	1.10±1.4^{E}	2.18±4.2^{CBD}	0.45±6.7^{D}	82.88
8	17.34±9.^{A}	0.65±1.4^{E}	6.73±4.^{CBD}	0.0±0.0^{E}	2.3±3.22^{CEBD}	0.0±0.0^{E}	6.7±4.8^{CEBD}	1.4±3.1^{E}	8.27±5.0^{A}	5.13±1.1^{D}	93.80
St	4.25±5.1^{CEBD}	3.65±5.5^{CEBD}	1.4±3.1^{CEBD}	0.84±1.39^{CED}	1.2±2.17^{ED}	4.94±5.87^{A}	0.85±1.1^{E}	0.85±11.9^{E}	1.92±2.9^{CBD}	2.57±3.6^{CB}	42.55
Cont	14.2±8.3		18.4±6.68		16.7±10.59		13.12±1.0		15.61±6.65		

The vertical columns marked with the same letters are not significantly different by SAS (2006). St= Standard treatment (carrier material) . Cont= control .

Table (4): Repellent effect of potassium tartrate (PT) against *Monacha obstructa* using free choice feeding test.

Concentrations%	Average daily consumption of Lettuce cm^2/ snail.										Repellency %
	1st Day		2nd Day		3rd Day		4th Day		Average		
	Un	T	Un	T	Un	T	Un	T	Un	T	
2	1.3±1.79^{FECD}	0.4±0.89^{F}	5.15±1.79^{F}	0.2±0.45^{F}	0.0±0.0^{F}	0.7±1.57^{FE}	2.45±0.66^{FECD}	1.5±2.56^{FECD}	2.148±0.89^{BA}	0.7±0.87	75..45
4	0.7±0..97^{FE}	0.4±0.89^{FECD}	5.15±5.41^{BA}	2.25±3.18	0.35±0.78^{F}	0.4±0.89^{F}	2.3±2.36^{FECD}	1.01±1.14^{F}	2.125±2.00^{BA}	1.02±1.13^{B}	67.68
6	0.82±1.19^{FECD}	1.05±2.35^{FECD}	4.07±4.8^{FECD}	0.0±0.0^{F}	0.0±0.0^{F}	0.4±0.09^{F}	1.68±1.65^{FECD}	0.48±0.78^{F}	1.68±1.65^{BA}	0.48±0.77^{B}	77.77
8	0.6±0.8^{FE}	0.95±1.37^{FECD}	8.25±10.08^{A}	0.0±0.0^{F}	0.0±0.0^{F}	0.60±1.34^{FE}	2.92±3.58^{FEC}	0.52±0.74^{F}	2.94±3.60^{A}	0.52±0.74^{B}	85.45
St	4.5±10.06^{BC}	2.0±2.94^{FECD}	4.40±1.82^{FECD}	1.0±1.17^{F}	1.00±1.38^{FECD}	0.0±0.0^{F}	3.30±3.03^{FECD}	0.98±1.05^{FECD}	3.30±3.03^{A}	0.10±1.06^{B}	37.95
Con	3.7±2.34		1.23±0.78		1.60±0…91		1.44±0.98		2.01±1.14		

The vertical columns marked with the same letters are not significantly different by SAS (2006). St= Standard treatment (Carrier material). Cont= Control.

From the previous results of Tables (3 and 4) the results revealed that the two land snail species avoided eating the treated lettuce discs after the second and third day of treatment. Also, they fed again on the fourth day of treatment,

The results agreed with Ahmed (2005) who assayed 3% of Opoponax extract and 5% ocimen on *Helix aspersa*. Results showed to deter the snails from feeding on treated lettuce leaf discs. Also, the same results were found by Capinera and Dickens (2016) tested copper hydroxide on two land snails *Ileidyula floridanc* and *Deracera leave* using choice feeding method. Results revealed that the two species consumed the un-treated foliage more than the treated foliage. Capinera (2018) studied the repellent effect of Copper hydroxid on brown snails, *Zachrysia provisoria*. The result indicated that Copper hydroxiede reduced the number of snails crawling ever the treated area.

2. Field experiment.

Data in Table (5) showed the field performance of potassium tartrate (8%) using as dusting application for *E. vermiculata*. Results revealed that the number of snail individuals decreased to 90 and 91 snails/shrub after 1, 15 day of treatment comparing with 132 pretreatment while in the control number increased from 106 snail / shrub to 136, 183, 165 and 145 snails/ shrub after the same period. So, the reduction percentage of population density of snails was 46, 46.9, 53.09, and 50.39% at 1, 3, 7 and 15 days after treatment.

Table (6) showed the field performance of potassium tartrate (8%) using as dusting application for *M. obstructa*. Results cleared PT caused reduction percentages for *M. obstructa* whereas it was 28.4, 10, 6 and 5% after 1, 3, 7 and 15 days of treatment. And it was observed that snails can't climb the plant.

Table (5); Field performance of potassium tartrate against *Eobania vermiclata.*

Period / day	No. of snails in control	No. of snails in Standard	No. of snails after Treatment	Reduction% of snails.
Zero	106	105	132	-
1st day	136	83	90	46.9
3rd day	183	116	121	46.9
7th day	165	132	97	53,09
15th day	145	66	91	50.39

Table (6): Field performance of potassium tartrate against *Monacha obstructa.*

Period / day	No. of snails in control	No. of snails in Standard	No. of snails after Treatment	Reduction% of snails.
Zero	63	72	58	-
1st day	66	57	78	28.4
3rd day	74	59	62	10
7th day	61	57	53	6
15th day	56	56	49	5

From the previous results in Tables (5 and 6) the snails avoided to feed on the treated foliage of plants. So, the potassium tartrate (8%) caused feeding deterrent for two land snail species because it prevents snails from climbing the plants. It was indicated that the reduction of number of *E. vermiculata*

individuals were more than *M. obstructa* this is may be due to the repellent effect of PT differed according to snail species which attributed to differences in feeding patterns or feeding behavior among the snail species. So, the PT was more repellent effect for *E. vermiculata* than *M. obstructa*. Capinera and Dickens (2016) demonstrated that the Copper hydroxide functioned as a repellent and feeding deterrent for Mollusca species. Capinera (2018) found that effect of copper hydroxide prevents snails *Zachrysia provisoria* from climbing the side of pots to access plants in screen houses.

Finally, it concluded that potassium tartrate can be using in organic farmer or land escape for protect the plant from land snails attack.

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A faunistic study on Megachilidae (Hymenoptera: Apoidea) of Northern Iran

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Abstract:

In this faunistic research, totally 24 species of Megachilidae (Hymenoptera) from 8 genera *Anthidium* Fabricius, 1805, *Chelostoma* Latreille, 1809, *Coelioxys* Latreille, 1809, *Haetosmia* Popov, 1952, *Hoplitis* Klug, 1807, *Lithurgus* Berthold, 1827, *Megachile* Latreille, 1802, *Osmia* Panzer, 1806 were collected and identified from different regions of Iran. Two species are new records for the fauna of Iran: *Coelioxys* (*Coelioxys*) *aurolimbata* Förster, 1853, and *Megachile* (*Eutricharaea*) *apicalis* Spinola, 1808.

Introduction

Megachilidae (Hymenoptera) with more than 4000 described species worldwide (Michener, 2007) is a large family of specialized, morphologically rather uniform bees found in a wide diversity of habitats on all continents except Antarctica, ranging from lowland tropical rain forests to deserts to alpine environments (Litman *et al*., 2011). The front wings without exception have got two marginal cells, and the stigma is small. The pollen-collecting scopa of all nonparasitica females is located on the abdominal sterna (Stephen *et al*., 1969 and Özbek and van der Zanden, 1992). It has been reported that some species belonging to the Megachilidae are effective pollinators in some plants (Bosch and Blas, 1994 and Vicens and Bosch, 2000). These solitary bees are both ecologically and economically relevant; they include many pollinators of natural, urban and agricultural vegetation (Gonzalez *et al*., 2012). Furthermore, it has been reported that the Megachilidae species can be used as a commercial species when a decrease is observed in the primary pollinator belonging to the other family (Richards, 1997 and Güler and Çağatay, 2006).

The fauna of Iranian Megachilidae has been studied rather

well and several papers were published by Popov (1967), Esmaili and Rastegar (1974), Warncke (1981), Ebadi (1995), Talebi *et al.* (1995), Modarres Awal (1997), Izadi *et al.* (1998, 1999, 2000, 2004, and 2006), Karimpour *et al.* (2002), Engel (2006), Tavakkoli *et al.* (2010), Khaghaninia *et al.* (2010), Khodaparast *et al.* (2011), Monfared and Khodaparast (2012), Rasekh Adel *et al.* (2012 a, b and c), Salehi Sarbijan *et al.* (2012), Soraya Mohtat *et al.* (2012), Keshtkar *et al.* (2012 and 2015), Khodaparast and Monfared (2012 and 2013), Monfared *et al*. (2012) and Nadimi *et al.* (2013a, b and 2014).

The aim of this research is a partial faunistic survey on Megachilidae of Golestan and Mazandaran provinces (North of Iran).

Material and methods

The specimens of this research were collected by sweeping net and Malaise traps from some regions of northern Iran (Golestan and Mazandaran provinces). The collected specimens were placed in ordinary paper envelopes after being killed with cyanid, and then placed in a desiccator to prepare them for morphological study. The materials were pinned and labeled according to current taxonomic rules and were examined with a stereomicroscope. For the determination of the genera and species, the keys developed by Osychnyuk *et al.* (1978), Dorn and Weber (1988), Warncke (1980 and 1992), Banaszak and Romasenko (1998), Scheuchl (2006), Michener (2007) and Amiet *et al*. (2004) were used. Classification of the different taxa follows Michener (2007).

Results and discussion

In this research, 24 species of Megachilidae are recorded from Golestan and Mazandaran provinces (North of Iran). Names of the valid genera within tribes, and valid species names are listed alphabetically within genera, together with general distribution.

Family Megachilidae Latreille, 1802

Subfamily Megachilinae Latreille, 1802

Tribe Anthidiini Ashmead, 1899

Genus *Anthidium* Fabricius, 1805

1. *Anthidium* (*Anthidium*) *florentinum* (Fabricius, 1775)

Material examined: Golestan province, Minudasht, 37°10′N 55°30′E, 2♀♀, 1♂, October 2012; Mazandaran province, Sari, 36°30′N 53°30′E, 1♀, June 2013.

General distribution: Asia Minor, Caucasus, Central Asian part of the former USSR, South and Central Europe, Siberia, Syria **(Banaszak and Romasenko, 1998)**, Iran **(Warncke , 1980) and** USA **(Comba and Comba 1991).**

Plant association: Polylectic (Fabaceae and Lamiaceae) **(Banaszak and Romasenko, 1998)**, *Medicago sativa* (Fabaceae), *Euphorbia* (Euphorbiaceae) and *Epilobium hirsutum* (Onagraceae) **(Khodaparast and Monfared, 2012).**

Comments: This species was collected from alfalfa and onion fields, and is a dominant species in alfalfa fields **(Rasekh Adel *et al*., 2012 b and c).**

Tribe Lithurgini Newman, 1834

Genus *Lithurgus* Berthold, 1827

2. *Lithurgus cornutus* (Fabricius, 1787)

Material examined: Golestan province, Kalaleh, 37°43′N 55°49′E, 2♀♀, July 2012.

General distribution: Iran (Warncke 1981), Asia Minor, Caucasus, Kazakhstan, North Africa, South, East and Central Europe **(Banaszak and Romasenko, 1998)**, China, Greece, Hungary, Italy, Japan, Morocco, Romania, Taivan, Turkey, the former USSR, and former Yugoslavia **(van den Zanden, 1986)**.

Plant association: Oligolectic (Asteraceae) **(Banaszak and Romasenko, 1998 and Güler and Sorkun, 2007).**

Tribe Megachilini Latreille, 1802

Genus *Coelioxys* Latreille, 1809

3. *Coelioxys* (*Coelioxys*) *aurolimbata* Förster, 1853

Material examined: Mazandaran province, Savadkooh, 36°05′N 52°55′E, 1♂, August 2014.

General distribution: Caucasus, Central Asian part of the former USSR, Europe, North Africa and Turkey **(Banaszak and Romasenko, 1998).**

Comments: New record for Iran.

Genus *Megachile* Latreille, 1802

4. *Megachile* (*Eutricharaea*) *apicalis* Spinola, 1808

Material examined: Mazandaran province, Behshahr, 36°41′N 53°44′E, 2♀♀, 1♂, June 2013.

General distribution: Canada, Caucasus, Central Asian part of the former USSR, North Afiica, South and Central Europe **(Banaszak and Romasenko, 1998).**

Comments: New record for Iran.

5. *Megachile* (*Eutricharaea*) *leachella* Curtis, 1828

Material examined: Mazandaran province, Ramsar, 36°47′N 50°32′E, 1♂, September 2012.

General distribution: Asia, Caucasus, Europe, North Africa, North America, Russain Far East, Siberia **(Banaszak and Romasenko, 1998)** and Iran **(Khaghaninia *et al.*, 2010).**

Plant association: Polylectic (mainly Fabaceae) **(Banaszak and Romasenko, 1998).**

6. *Megachile* (*Xanthosarus*) *nigriventris* Schenck, 1870

Material examined: Golestan province, Kordkoy, 36°41′N 54°12′E, 1♀, 2♂♂, September 2009.

General distribution: North, South and Central Europe **(Banaszak and Romasenko, 1998).**

Plant association: Polylectic (Rosaceae, Fabaceae and Caprifoliaceae) **(Banaszak and Romasenko, 1998).**

7. *Megachile* (*Megachile*) *pilicrus* Morawitz, 1878

Material examined: Golestan province, Kordkoy, 36°41′N 54°12′E, 2♀♀, August 2009.

General distribution: Caucasus, Central Asian part of the former USSR **(Banaszak and Romasenko, 1998)**, South, Eastern and Central Europe **(Comba and Comba, 1991).**

8. *Megachile* (*Eutricharaea*) *rotundata* (Fabricius, 1787)

Material examined: Mazandaran province, Savadkooh, 36°05′N 52°55′E, 4♀♀, 3♂♂, August 2014; Golestan province, Minudasht, 37°10′N 55°30′E, 1♀, 1♂, October 2012.

General distribution: Caucasus, Central Asian part of the former USSR, Europe, Far East Russia, Kazakhstan, North Africa, North and South America, New Zeland **(Comba and Comba, 1991 and Banaszak and Romasenko, 1998)**, Turkey **(Özbek and van der Zanden, 1994)**.

Plant association: Polylectic (Asteraceae, Fabaceae and Lamiaceae) (**Banaszak and Romasenko, 1998).**

Tribe Osmiini Newman, 1834

Genus *Chelostoma* Latreille, 1809

9. *Chelostoma* (*Chelostoma*) *emarginatum* (Nylander, 1856)

Material examined: Mazandaran province, Behshahr, 36°41′N 53°44′E, 1♀, 1♂, June 2013.

General distribution: Austria, Azerbaijan, Bulgaria, Bosnia-Herzegovina, Croatia, Czech Republic, France, Greece, Hungary, Iran, Italy, Macedonia, Portugal, Romania, Serbia

and Montenegro, Sicily, Slovakia, Slovenia, Spain, Switzerland, Turkey **(Grace, 2010 and Müller, 2012).**

Plant association: Oligolectic on *Ranunculus* (Ranunculaceae) and possibly also on closely related genera **(Amiet *et al*., 2004; Sedivy *et al*., 2008; Grace 2010 and Müller 2012).**

10. *Chelostoma* (*Gyrodromella*) *proximum* Schletterer, 1889

Material examined: Golestan province, Kalaleh, 37°43′N 55°49′E, 2♀♀, September 2012.

General distribution: Azerbaijan, Caucasus, China, Far East, Georgia, Iran, Russia, Turkmenistan, Turkey, Ukraine **(Banaszak and Romasenko 1998 and Grace, 2010).**

Plant association: Probably Oligolectic on Campanulaceae **(Banaszak and Romasenko, 1998 and Müller, 2012).**

Genus *Haetosmia* Popov, 1952

11. *Haetosmia vechti* (Peters, 1974)

Material examined: Mazandaran province, Savadkooh, 36°05′N 52°55′E, 1♀, 2♂♂, August 2014.

General distribution: Greece, Iran, Palestine, Turkey **(Banaszak and Romasenko 1998; Grace 2010 and Müller, 2012).**

Plant association: Oligolectic on *Heliotropium* (Boraginaceae) **(Mavromoustakis, 1954).**

Genus *Hoplitis* Klug, 1807

12. *Hoplitis* (*Hoplitis*) *adunca* (Panzer, 1798)

Material examined: Golestan province, Gorgan, 36°50′N 54°30′E, 1♀, spring 2012.

General distribution: Asia Minor, Caucasus, Central Asian part of the former USSR, North Africa **(Warncke 1992; Banaszak and Romasenko, 1998 and Amiet *et al*., 2004)**, South, Eastem and Central Europe **(Comba and Comba, 1991).**

13. *Hoplitis* (*Hoplitis*) *flabellifera* (Morice, 1901)

Material examined: Mazandaran province, Amol, 36°28′N 52°21′E, 3♀♀, 1♂, April 2013.

General distribution: Armenia, Iran, Jordan, Palestine, Syria, Turkey **(Grace, 2010 and Müller, 2012).**

Plant association: Polylectic with a strong preference for *Anchusa* (Boraginaceae) **(Müller, 2012)**, *Vicia* (Fabaceae), *Borago officinalis* (Boraginaceae), *Centuria* (Asteraceae) **(Khodaparast and Monfared, 2012)**, *Vicia* (Asteraceae), *Borago officinalis* (Boraginaceae), *Centaurea* (Asteraceae) **(Khodaparast and Monfared, 2013)**.

Genus *Osmia* Panzer, 1806

14. *Osmia* (*Monosmia*) *apicata* Smith, 1853

Material examined: Mazandaran province, Behshahr, 36°41′N 53°44′E, 2♀♀, June 2013.

General distribution: Albania, Armenia, Bulgaria, Croatia, Iran, Italy, Jordan, Georgia, Greece, Macedonia, Palestine, Russia, Serbia and Montenegro, Slovenia, Syria, Turkey **(Grace, 2010 and Müller, 2012).**

Plant association: Oligolectic on *Onosma* sp. (Boraginaceae) **(Müller, 2012).**

15. *Osmia* (*Osmia*) *bicornis* (Linnaeus, 1758)

Material examined: Mazandaran province, Qaemshahr, 36°28′N 52°52′E, 2♀♀, 2♂♂, August 2014.

General distribution: Algeria, Cyprus, Europe, Far Eastern Siberia, Iran, Kazakhstan, Kyrgyzstan, Morocco, Tunisia, Turkmenistan, Palestine, Syria, Turkey **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller 2012).**

Plant association: Polylectic, prefer Rosaceae and Fabaceae **(Banaszak and Romasenko, 1998 and Müller, 2012).**

16. *Osmia* (*Metallinella*) *brevicornis* (Fabricius, 1798)

Material examined: Golestan province, Minudasht, 37°10′N 55°30′E, 2♂♂, October 2012.

General distribution: Algeria, Caucasus, Cyprus, Iran, Morocco, Northern Asia, South eastern- and Central-Europe, Tunisia, Turkey **(Banaszak and Romasenko, 1998; Grace, 2010; and Müller, 2012).**

Plant association: Oligolectic on Brassicaceae **(Banaszak and Romasenko, 1998 and Müller, 2012).**

17. *Osmia* (*Helicosmia*) *caerulescens* (Linnaeus, 1758)

Material examined: Mazandaran province, Sari, 36°30′N 53°30′E, 1♀, 2♂♂, June 2013.

General distribution: Algeria, Canada, China, Cyprus, Egypt, Europe, India, Iran, Jordan, Kazakhstan, Kyrgyzstan, Morocco, Syria, Tajikistan, Tunisia, Turkey, Turkmenistan, USA and Uzbekistan **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012).**

Plant association: Polylectic, prefers Fabaceae, Lamiaceae, Boraginaceae and Antirrhineae **(Banaszak and Romasenko 1998; Grace, 2010 and Müller, 2012)**, *Vicia* sp. (Fabaceae), *Borago officinalis* (Boraginaceae), *Medicago sativa* (Fabaceae), *Euphorbia* sp. (Euphorbiaceae), *Epilobium hirsutum* (Onagraceae) **(Khodaparast and Monfared , 2012, and 2013).**

18. *Osmia* (*Pyrosmia*) *cephalotes* Morawitz, 1870

Material examined: Mazandaran province, Amol, 36°28′N 52°21′E, 2♀♀, 1♂, April 2013; Golestan province, Gorgan, 36°50′N 54°30′E, 2♀♀, July 2009.

General distribution: Algeria, Caucasus, Cyprus, Iran, Jordon, Libya, Morocco, Palestine, South- and Eastern-Europe, Syria, Tunisia, Turkey and Turkmenistan **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012).**

Plant association: Polylectic with a preference for Fabaceae **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012)**, *Vicia* (Fabaceae), *Borago officinalis* (Boraginaceae) **(Khodaparast and Monfared, 2012 and 2013).**

19. *Osmia* (*Osmia*) *cornuta* (Latreille, 1805)

Material examined: Mazandaran province, Qaemshahr, 36°28′N 52°52′E, 1♀, August 2014.

General distribution: Algeria, Cyprus, Egypt, Europe, Iran, Tunisia, Turkmenistan, Turkey **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012).**

Plant association: Polylectic; prefers Rosaceae **(Westrich, 1989; Banaszak and Romasenko, 1998 and Amiet *et al.*, 2004).**

20. *Osmia* (*Helicosmia*) *dimidiata* Morawitz, 1870

Material examined: Golestan province, Gonbad, 37°30′N 55°00′E, 1♀, 2♂♂, September 2013.

General distribution: Asia minor, Caucasus, Cyprus, Iran, Morocco, Kyrgyzstan, Lebanon, Palestine, South Europe, Turkey and Turkmenistan **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012).**

Plant association: Probably oligolectic on Asteraceae, visiting *Cirsium syriacum*, *Calendula persica*, *Centaurea hyalolepis*, *Statice sinuata*, *Echium sericeum*, *Scolymus hispanicus* and *Marrubium vulgare apolum* **(Grace, 2010 and Müller, 2012).**

21. *Osmia* (*Helicosmia*) *melanogaster* Spinola, 1808

Material examined: Mazandaran province, Qaemshahr, 36°28′N 52°52′E, 1♂, August 2014.

General distribution: Algeria, Caucasus, Cyprus, Egypt, Iran, Jordan, Libya, South, Eastern and Central Europe, Morocco, Palestine, Syria, Tunisia and Turkey **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012).**

Plant association: Oligolectic on Carduoideae (Asteraceae) **(Müller, 2012).**

22. *Osmia* (*Helicosmia*) *niveata* (Fabricius, 1804)

Material examined: Golestan province, Gonbad, 37°30′N 55°00′E, 1♀, 1♂, September 2013.

General distribution: Cyprus, Europe, Iran, Jordan, Lebanon, Northern Africa, Palestine, Syria, Turkey, Turkmenistan **(Grace, 2010 and Müller, 2012).**

Plant association: Oligolectic on Asteraceae with a distinct preference for Carduoideae **(Westrich, 1989; Amiet *et al.*, 2004 and Müller, 2012).**

23. *Osmia* (*Allosmia*) *rufohirta* Latreille, 1811

Material examined: Mazandaran province, Sari, 36°30′N 53°30′E, 1♀, June 2013.

General distribution: Algeria, Caucasus, China, Jordan, Morocco, South, Central and Eastern Europe, Syria, Turkmenistan, Tunisia and Turkey **(Banaszak and Romasenko, 1998; Grace, 2010 and Müller, 2012).**

Plant association: Polylectic with a preference for Fabaceae **(Banaszak and Romasenko, 1998 and Müller, 2012).**

24. *Osmia* (*Helicosmia*) *signata* Erichson, 1835

Material examined: Mazandaran province, Amol, 36°28′N 52°21′E, 2♀♀, April 2013.

General distribution: Albania, Algeria, China, Cyprus, Egypt, France, Greece, Corsica, Crete, Iran, Italy, Jordan, Morocco, Palestine, Portugal, Sardinia, Sicily, Spain, Syria, Turkey, Turkmenistan and Ukraine **(Grace, 2010 and Müller, 2012).**

Plant association: Oligolectic on Asteraceae **(Müller, 2012).**

Upon the results of this research together with other works on Megachilidae of Northern Iran (e.g. Tavakkoli *et al.*, 2010 and Nadimi *et al.*, 2013 a, b and 2014) indicate that there is a diverse fauna of these beneficial insects in northern Iran. Although the fauna of Megachilidae of southern Iran was studied rather well (see references) but the fauna of northern Iran was poorly studied so far. Regarding to the diverse flora in northern Iran, we expect much more species of Megachilidae in the mentioned area. The megachilids are important pollinators of several wildflowers, vegetables and fruits, and are used as pollinators by commercial growers of blueberries, onions, carrots and alfalfa (Bohart, 1972 and Pitts-Singer and Cane, 2011). In addition to the species diversity of Iranian Megachilidae, there are many other unknown data such as the diversity of nesting biology and floral relationships. Diverse materials are used in nest building and the inclusion of these foreign materials in nest construction may have promoted a massive range expansion and diversification within the family (Cane *et al.*, 2007; Litman *et al.*, 2011 and Gonzales *et al.*, 2012). Also, many insects (e.g. Chrysididae, Mutillidae, Formicidae, Rhipiphoridae, Meloidae, Cleridae, etc.) attack the nests of leafcutting bees (Ahmed Khattaby, 1992 and Woodward, 1994), which determining of these natural enemies can

be an interesting research work in different regions of Iran.

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A faunistic study on Chrysididae, Dryinidae, Ichneumonidae and Proctutropidae (Hymenoptera) from Iran

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Abstract:

In this faunistic paper, 7 species in 4 genera of Chrysididae, 3 species in 2 genera of Dryinidae, 11 species in 9 genera of Ichneumonidae, and one species of Proctutropidae were collected and identified from different regions of Iran. *Apltelopus melaleucus* (Dalman, 1818) (Dryinidae) and *Nothoserphus mirabilis* Brues, 1940 (Proctutropidae) are new records for the fauna of Iran.

Introduction

The family Chrysididae (Cuckoo-wasps) is distributed all over the world and contains more than 3,000 species (Tyrner, 2007). They are colourful insects, which fall into categories of cleptoparasites and parasitoids. Larvae of cuckoo-wasps develop in brood cells of nesting Hymenoptera, cocoons of sawflies and Lepidoptera and eggs of Phasmatodea (O'Neill, 2001 and Orlovskyte *et al*., 2011). Chrysidids are distributed over all zoogeographical regions but mainly in subtropical and tropical zones (Tyrner, 2007).

The family Dryinidae with more than 1600 species within 12 subfamilies and 45 genera is a medium-sized cosmopolitan group of Aculeata (Olmi and Bechly, 2001 and Klejdysz *et al*., 2018). More than 230 species are represented in Palaearctic Region, mostly of them occurring also in Europe. These wasps are parasitoids of Hemiptera (including Cicadomorpha and Fulgoromorpha) (Guglielmino and Olmi, 2006 and 2007 and Turrisi and Olmi, 2009). The family Ichneumonidae is a large family of parasitic wasps comprises

more than 25,300 described species in 1,601 genera worldwide (Yu *et al.*, 2016) but the estimation is over 100,000 species (Gauld, 2000). These wasps are powerful natural enemies of agricultural and forest pests and have efficient role in biological control of insect pests (Turnock *et al.*, 1976; Gupta, 1988 and Wahl, 1993).

The family Proctotrupidae is a relatively small taxon of parasitic wasps (Proctotrupoidea) with a worldwide distribution, especially in temperate and humid climate regions. These wasps are most diverse in the Holarctic, where they occur mainly in shadowed forests. Proctotrupids are larval endoparasitoids of several Coleoptera families, as well as the dipteran families Mycetophilidae and Sciaridae, the lepidopteran family Oecophoridae, and centipedes of the family Lithobiidae. Proctotrupid fauna consists of over than 320 species in 27 genera (Johnson, 1992; Kolyada and Mostovski, 2007 and Kolyada and Perkovsky, 2011).

The aim of this paper is introducing of 22 species in 4 hymenopteran families which were collected under different faunistic investigations. The specimens of this research were collected by sweeping net and malaise traps from different regions of Iran.

Results and discussion

This paper comprises 22 species of 4 hymenopteran families, Chrysididae (7 species), Dryinidae (3 species), Ichneumonidae (11 species) and Proctutropidae (single species). The list of species is given below alphabetically with distributional data.

Family Chrysididae Latreille, 1802

Genus *Chrysis* Linnaeus, 1761

1. *Chrysis castigata* Linsenmaier, 1959

Material examined: Golestan province, Golestan National Park, 2♀, August 2010.

General distribution: Kazakhstan, Kyrgyzstan, Russia, Turkmenistan and Uzbekistan **(Rosa *et al*., 2017a).**

2.*Chrysis consanguinea* Mocsáry, 1889

Material examined: Guilan province, Talesh, Gisum Park, 2♀, September 2014.

General distribution: Southern Europe, Caucasus, North Africa and Russia **(Rosa *et al*., 2017a).**

Genus *Chrysura* Dahlbom, 1845

3. *Chrysura radians* (Harris, 1776)

Material exxamined: Qazvin province, Taleghan, 2♀, 1♂, August 2012.

General distribution: Palaearctic, Turkey **(Yildirim and Strumia, 2000).**

Genus *Cleptes* Latreille, 1802

4. *Cleptes semiauratus* (Linnaeus, 1761)

Material exxamined: Kordestan province, Kavaneh, 2♀, 2♂, September 2013.

General distribution: Palaearctic, Turkey **(Yildirim and Strumia, 2000).**

Genus *Holopyga* Dahlbom, 1845

5. *Holopyga generosa asiatica* Trautmann, 1926

Material examined: Semnan province, Shahrud (Jangal-e Abr), 1♀, June 2011.

General distribution: Russia; Trans-Palaearctic, from southern Europe and Caucasus to China **(Rosa *et al*., 2017b).**

6. *Holopyga ignicollis* Dahlbom, 1854

Material examined: Isfahan province, Chadegan, 2♀, April 2008.

General distribution: Russia; West-Palaearctic: from South Europe to Middle East, Caucasus, Kyrgyzstan and Kazakhstan **(Rosa *et al*., 2017b).**

7. *Holopyga lucida* (Lepeletier, 1806)

Material examined: Kermanshah province, Sonqor, 3♀, April 2011.

General distribution: Russia, Central and South Europe and Turkey **(Rosa *et al*., 2017b).**

Family Dryinidae Haliday, 1833

Genus *Anteon* Jurine, 1807

8. *Anteon arcuatum* Kieffer, 1905

Material examined: Golestan province, Golestan National Park, 1♀, 1♂, July 2011.

General distribution: This species is widely distributed almost throughout the Palaearctic region, from Mongolia to Spain **(Olmi and Xu, 2015).**

9. *Anteon brachycerum* (Dalrnan, 1823)

Material examined: Guilan province, Talesh, Gisum Park, 1♂, September 2014.

General distribution: This species is widely distributed almost throughout the Palaearctic region, from Japan to France, but it is rare in Western Europe **(Olmi and Xu, 2015).**

Genus *Apltelopus* Dumeril and Bibron 1841

10. *Apltelopus melaleucus* (Dalman, 1818)

Material examined: West Azarbaijan Province, Mahabad, 2♀, 1♂, 22-24 June 2012.

General distribution: This species is the most common European *Aphelopus* species and is widely distributed throughout the Palaearctic region, from Japan to Spain **(Olmi and Xu, 2015).**

Family Ichneumonidae Latreille, 1802

Genus *Absyrtus* Holmgren, 1859

11. *Absyrtus vernalis* Bauer, 1961

Material examined: Azarbaijan-e Sharghi province, Horand, 1♀, August 2013.

General distribution: Bulgaria, France, Germany, Norway, Switzerland, Turkey, Ukraine and United Kingdom **(Yu *et al.*, 2016).**

Genus *Acaenitus* Latreille, 1809

12. *Acaenitus dubitator* (Panzer, 1800)

Material examined: Semnan province, Shahrud, 4♀, 1♂, August 2015.

General distribution: Albania, Austria, Belarus, Belgium, Bulgaria, China, Czech Republic, former Czechoslovakia, France, Germany, Hungary, Italy, Latvia, Moldova, Morocco, Netherlands, Poland, Portugal, Romania, Russia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom and former Yugoslavia **(Yu *et al.*, 2016).**

Genus *Achaius* Cameron, 1903

13. *Achaius oratorius* (Fabricius, 1793)

Material examined: Kurdistan province, Bijar, 3♀, August 2015.

General distribution: Austria, Azerbaijan, Belarus, Belgium, Bulgaria, former Czechoslovakia, Denmark, Finland, France, Germany, Hungary, Ireland, Japan, Korea, Latvia, Luxembourg, Netherlands, Norway, Poland, Romania, Russia, Spain, Switzerland, Ukraine and United Kingdom **(Yu *et al.*, 2016).**

Genus *Aclastus* Förster, 1869

14. *Aclastus gracilis* (Thomson, 1884)

Material examined: Zanjan province, Abhar, 3♀, 4♂, June 2014; Chaharmahal & Bakhtiary province, Borujen, 2♀, 1♂, May 2015.

General distribution: Austria, Azerbaijan, Belgium, Bulgaria, Canary Islands, former Czechoslovakia, Denmark, Faeroe Islands, Finland, France, Germany, Greenland, Hungary, Iceland, Ireland, Italy, Madeira Islands, Netherlands, Norway, Poland, Russia, Spain, Sweden, Switzerland, Turkey and United Kingdom **(Yu *et al.*, 2016).**

Genus *Gnathochorisis* Förster, 1869

15. *Gnathochorisis crassulus* (Thomson, 1888)

Material examined: Golestan province, Kordkoy, 3♀, 28 August 2009.

General distribution: Eastern Palaearctic, Europe, Nearctic, Western Palaearctic **(Yu *et al.*, 2016).**

Genus *Medophron* Förster, 1869

16. *Medophron afflictor* (Gravenhorst, 1829)

Material examined: West Azarbaijan Province, Miandoab, 2♀, 14-16 April 2013.

General distribution: Austria, former Czechoslovakia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Norway, Poland, Romania, Russia, Sweden and United Kingdom **(Yu *et al.*, 2016).**

Genus *Oxyrrhexis* Förster, 1869

***17.Oxyrrhexis carbonator* (Gravenhorst, 1807)**

Material examined: Lorestan province, Aligoodarz, 2♀, June 2009.

General distribution: Eastern Palaearctic, Europe, Nearctic, Western Palaearctic **(Yu *et al.*, 2016).**

Genus *Phaenolobus* Förster, 1869

18. *Phaenolobus fulvicornis* (Gravenhorst, 1829)

Material examined: Lorestan province, Kamandan, 3♀, 2♂, April 2012; Kerman province, Jiroft, 2♀, October 2014.

General distribution: Albania, Algeria, Austria, Belarus, Bulgaria, former Czechoslovakia, France, Georgia, Germany, Hungary, Israel, Italy, Latvia, Lithuania, Morocco, Netherlands, Poland, Portugal, Romania, Russia, Spain, Switzerland, Turkey, United Kingdom and former Yugoslavia **(Yu *et al.*, 2016).**

19. *Phaenolobus terebrator* (Scopoli, 1763)

Material examined: West Azarbaijan province, Ourmieh, 2♀, 2♂, 3-5 August 2013.

General distribution: Albania, Austria, Belarus, Belgium, Bulgaria, former Czechoslovakia, Finland, France, Georgia, Germany, Hungary, Italy, Kazakhstan, Korea, Latvia, Moldova, Morocco, Netherlands, Norway, Poland, Romania, Russia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom, Uzbekistan, and former Yugoslavia **(Yu *et al.*, 2016).**

Genus *Rhembobius* Förster, 1869

20. *Rhembobius quadrispinus* (Gravenhorst, 1829)

Material examined: Semnan province, Damghan, 2♀, May 2011.

General distribution: Austria, Belgium, Bulgaria, former Czechoslovakia, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Norway, Poland, Portugal, Romania, Russia, Spain, Sweden, Switzerland, Turkey, Ukraine and United Kingdom **(Yu *et al.*, 2016).**

21. *Rhembobius perscrutator* (Thunberg, 1824)

Material examined: Kordestan Province: Qorveh, 1♀, September 2012.

General distribution: Belgium, Bulgaria, former Czechoslovakia, Denmark, Finland, France, Germany, Hungary, Japan, Latvia, Norway, Poland, Romania, Russia, Sweden and United Kingdom **(Yu *et al.*, 2016).**

Family Proctotrupidae Latreille, 1802

Genus *Nothoserphus* Brues, 1940

22. *Nothoserphus mirabilis* Brues, 1940

Material examined: Razavi Khorasan province, Chenaran (Nobahar), 3♀, 2♂, ex larvae of *Coccinella septempunctata* (Linnaeus, 1758), 15.vi.2010.

General distribution: China, India, Java, Nepal, Taiwan **(Ceryngier and Hodek ,1996)** and Pakistan **(Bodlah *et al.*, 2019).**

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Survey and distribution density of genus *Brachymeria* species (Hymenoptera: Chalcididae) in Egypt

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Abstract:

Surveys of *Brachymeria* (Hymenoptera: Chalcididae) parasitoids attack larvae and pupae of Lepidoptera, Diptera and Coleoptera were conducted in the Egypt between 2014 and 2018. The population density of *Brachymeria* was counted in Egypt. Data on distribution of 12 *Brachymeria* wasp species provides. In this study, field experiments were undertaken during 2014 and 2016 seasons in Monoufia, Qalubiya and Giza Governorates. The obtained results indicated that pupae of *Pieris rapae* (Linnaeus))Lepidoptera: Pieridae) and *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae), were obtained. The highest mean parasitism percentage was recorded at sowing during September 2014 and 2015 cabbage growing seasons (28.49% at 2014 and24.46 % in 2015) respectively by *Brachymeria femorata* (Panzer) (Hymenoptera: Chalcididae). The highest mean parasitism percentage was recorded in Qalubiya Governorate during 2015 in cotton growing seasons (4.76%) followed by Giza Governorate during 2016 in cotton growing seasons (4.47%) by *Brachymeria brevicornis* (Klug) (Hymenoptera: Chalcididae).

Introduction

Hymenopterous parasitoids have immense importance in natural and agricultural ecosystems, where they influence or regulate the population density of many pests (Godfray, 1994). Chalcidids comprise a very important beneficial group of parasitoids as many species of the family are important parasitoids that have been used successfully for the biological control of many insect pest species. The genus *Brachymeria* Westwood, 1829 belongs to the subfamily Chalcidinae. Apparently, there are almost 300 species of *Brachymeria* in the world (Noyes, 2011) of which many are economically

important as they are used in the biological control as entomophagous against insect pests. These chalcids parasitize the mature larvae and pupae of the wide range species of various orders. They play significant role in the ecosystem of various economically important crops.

In Egypt *Brachymeria* includes the most common and widely taxa distributed in the family Chalcididae and worldwide. Many species of this genus are primary endoparasitoids of lepidopterous families; Diptera (Fam. Sarcophagidae) and Coloeopterous families. On the other hand, sometime hyperparasitic species are found to parasitise Diptera (Tachinidae) and Ichneumonid (Hymenoptera). *Brachymeria* taxa look very much alike, but they differ widely in habits. Therefore, the precise determination of the species concerned is highly important in any host-parasite study for biological control involving this genus (Joseph *et al.*, 1973). Accurate techniques to detect and identify parasitoids are a prerequisite for understanding and managing host–parasitoid interactions: for example, they are needed to measure and monitor parasitism rates (Agusti *et al.,* 2005). Studies on the ecology of parasitoids can supply the basic information necessary for biological control and for its efficient operations as strategy point undertaking integrated control plan in Egypt, where the losses suffered due to damage to crops by insect pests are often enormous in addition to prolonged effects and hazards of chemical control on the ecosystem .

Materials and methods

1. Survey of parasitoid with host's identification:

Throughout the period from September , 2013 to May 2018, inspections of cultivated plants covered some Egyptian area that have variable climate due to different ecological zones (Beheira , Qalubiya, Monoufia, Giza, Asiut, Fayoum, Cairo , Kafr El Sheikh, Sharqia, Mersa Matruh, Arish) .The represented crops were; cotton (*Gossypium hirsutum*), cabbage (*Brassica oleracea*), vegetable marrow (*Cucurbita pepo)*, sunflower (*Helianthus annuus*), faba bean *(Viciab faba*), Egyptian mallow (*Malva parviflora*), okra (*Abelmoschus esculentus*), oboe cane (*Arundo donax*), clover (*Trifolium alexandrinum*) , wheat (*Triticum aestivum*) , rice *(Oryza sativa*), peanuts *(Arachis hypogaea*) , pampas grass (*Cortaderia selloana*) plants and maize (*Zea mays*), sesame (*Sesamum indicum*), some fruit trees , some weeds and some ornamental plants, In addition to chicken wastes , the waste of the altars and some animal jeff . The host plants were examined with the naked eye to collect Lipedopteran, Coelopteran and Dipteran last stage larvae and pupae (Figure, 1). Sampling was carried out using sweeping net in the morning (Figure, 2). The pitfall traps were used to collect occurring chalcidid parasitoids especially *Brachymeria* wasps. Light traps were also used in the following areas (Armey, Khorshed, Quture, Al Santah, El Qanater El Khayreya, Dishna, Desert Research Center Farm (El Sadat City), International Raghy Farm, Marsa Alam, Abo Simbol, Qus, Edfu, Safaga and Aswan). The traps covered all months of this year. Survey operations were usually carried out on experimental plots that are often not treated with pesticides as well as months or period in which the pesticide use is less or less effective during the period of plant life .In order to obtains the parasitoids, the trapped were insects, were gently placed

in taken by glass tubes (10cm) (containing KCN at the bottom) until mortality of insects inside. killing purpose. Specimens were preserved in 75% alcohol until they were mounted on cards. Different specimens were collected from diverse ecological zones of Egyptian Governorates. The collected specimens were labeled, indentified and recorded along with the relevant data of localities, date of collection and hosts. The identifications or compare of specimens were, mostly, carried out using Bouček (1952, 1956,1988); Habu (1960); Masi (1929a,b, 1936); Nikol'skaya (1952); Steffan (1959); Joseph *et al.* (1973) and Narendran and Achterberg (2016). Material examined in this study was deposited in the collection of Agriculture Research Center, Researches Institute of Plants Protection Insect, Taxonomy Department (Giza). All *Brachymeria* species that saved in main collection of Egyptian Agriculture Ministry collected by both Mabrouk, Alfieri, Farag, Breeding, Adier, Kasim, Rabinovitch, Breeding, Hayweerd, Priesiner, Ali and Husny during the period from 1913-1934. All species collected by the mentioned authors were identified by L. Masi. In addition to the previous field survey a literature review survey carried out including available insect reference collections in Egypt (Plant Protection Research Institute Collections, Ain Shams Univ. Collection, Cairo Univ. Collection and Al-Azhar Univ. Collection) to determine *Brachymeria* hosts, monthly occurrence and geographical zone of species in Egypt.

2. *Pieris rapae* and *Brachymeria femorata* parasitoid :

An experimental area of 40 kirats located at Toukh Tanbisha village, Berkat El-Sabaa region, Monoufia Governorate was chosen during 2014 and 2015 cabbage (*Brassica oleracea var. capitata*) growing seasons as host plant. The whole area was divided into 4 replicates (10 kirats each). All replicates were planted with cabbage seedling. In the first season, sowing dates were February 3rd, May 1st, July 2nd and September 5th. In the 2nd season, the sowing dates were February 2nd, May 3rd, and September 5th. Sowing was carried out at intra-ridge spacing 70 cm on average, with an average of 10000 seedlings per feddan. All the recommended agricultural practices were followed, except any pesticide application. After 7 weeks of sowing date, the samples were collected weekly during the 8 weeks and 250 cabbage plants were chosen randomly represented one replicate was checked weekly. Last immature larval and healthy pupal stages of cabbage worm

Figure (1): **Collected pupae belonging to different insect species:**

a. ***Musca domestica*** **and blowflies pupae**
b. ***Pieris rapae*** **pupae**
c. ***Earias insulana*** **pupae**
d. ***Vanessa cardui*** **pupae**

Pieris rapae (Linnaeus))Lepidoptera :Pieridae) was collected in cotton and kept in cloth bags which were closed with rubber bands and transferred to laboratory where the bags were put in small lb glass jars capacity covered with muslin cloth under laboratory conditions of 25 ± 2°C and 65± 5% R.H. The number of emerged parasitoids was recorded and tabled.

3.Spiny bollworm *Earias insulana* and *Brachymeria brevicornis* parasitoid*:*

The experimental area of this study was 12 kirats located at three districts; Ibnahs village, Qewaisna region, Monoufia Governorate, Qaha, Qalubiya Governorate and Saft El Laban road, Giza Governorate cultivated with cotton during 2015 and 2016 (*Gossypium hirsutum*) growing seasons of host plant. the area 12 kirats was divided into 3 replicates and planted of cotton var. Giza 86. In the first and 2nd seasons, sowing dates were March17th and 18th and the beginning of April. Cotton seeds were sown on one side of rows at 50 - 60 cm between rows and 15 cm between pits by placing 10 seeds in each pit at 4- 6 cm. depth in soil. The normal recommended agricultural practices were followed, except for the absence of any pesticide application. starting from the beginning of August and on 10 day intervals, a number of 25 fully grown bolls / karat were examined at the surface of the bottom of each boll and the healthy full-grown larvae of the spiny bollworm, *Earias insulana* (Boisduval) (Lepidoptera: Nolidae) were collected and placed in test tubes which were stopper with pieces of cotton , then transferred to laboratory. Tubes were put in small lb glass jars capacity covered with muslin cloth under laboratory conditions (25 ± 2°C and 65± 5% R.H.). The numbers of emerged parasitoids were counted, recorded and tabled at 24 hrs.

Percentages of parasitism ratios were calculated according to the following formula:

$$\% \text{ of Parasitism} = \frac{\text{No. of emerged parasitoids}}{\text{Total no. of collected host insects}} \times 100.$$

The emerged parasitoids were prepared for microscopic inspection according to the following steps:

a. Kill of parasitoid
b. Conservation in alcohol 70% until the time of mounting.
c. Boiling in NaOH 20%, using water bath for 5 to 20 minutes
d. Washing in running water to get rid of NaOH**.**
e. Dipping successively in different concentrations of alcohols from 30 %,50%, 70%, 90%, 95 and 100% for 3-5 minutes in each concentration.
f. Quick wash with xilon, then clove oil.
g. Anatomy and preparation of slides by placing Canada balsam.
h. Finally, use the oven at 40-60° C.
i. Slides photos use light microscope.

Figure (2): Collected Brachymeria species by sweeping net.

Results and discussion

1.Field survey:

The field survey of *Branchiomeric* wasps was carried out during the period from 2013-2018. The survey covered some Egyptian areas including different ecological zones. Data in Table (1) showed that the parasitoids collected by pupae and sweep net from Pomegranate, Cabbage, Olive, Cotton fields and the rabbits corpse during three successive seasons. The captured parasitoids were found belonging to 5 species which were identified as; *B. albicrus*, *B. femorata*, *B. minuta*, *B. aegyptiaca* and *B. brevicornis* . Most species were in September. *B. femorata, B. aegyptiaca, B. minuta* and *B. brevicornis*, respectively were the most widely distributed species in terms of number of localities, While *Brachymeria* was not captured the Light trap. Data in Table (2) and Figure (3) showed that 12 species were collected belonging to of *Brachymeria* distributed in Egypt. included 176 specimens was the most representative number of species collected. In similar studies, Kamal (1937) pointed out that *B. femorata* (Panzer) is widely distributed at various localities of Lower Egypt, and he was astonished about the aestivation of this insect. Gray and Treloar (1933) carried out a detailed study in order to show how many sweeps are necessary to afford a real able index of the population density. Noyes (1982) showed the ways for the ways for collecting and preserving chalcid wasps. The author discussed that the most profitable ways for collecting chalcids, those included sweeping, pitfall traps and extraction from leaf litter or grass tussocks. The techniques of sweeping, card mounting specimens and slide preparation are described in detail. Zhao *et al*. (1986) surveyed the natural enemies of the crucifer pest (*Pieris rapae* L.) in China. They found seven parasitic species (*T. evanescens, Apanteles rubecula, Apanteles glomeratus, Hyposter* sp., *Mermis* sp., *Pteromalus puparum* and *B. fermata*). Moursy *et al.* (1996) reported that most species of *Brachymeria* are parasitoids on larvae and pupae of Diptera, Coleoptera and Lepidoptera. Rasmi *et al.* (2011) surveyed the parasitoid species that were found attacking larvae and pupae of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) in the *Brassica* agro-ecosystem in the Urmia region, northwest of Iran, between 2008 and 2009. Parasitized hosts were collected from infested plants placed in *Brassica* crops during the growing season (June-October), and from the natural *P. brassicae* population on the common weeds *Capparis spinosa* L. (Capparaceae) *Crambe orientalis* L. and *Raphanus raphanistrum* L. (Brassicaceae) during the summer production break (December). Ten hymenopterous species of primary parasitoids – *Cotesia glomerata* (L.) (Braconidae), *Brachymeria femorata* Panzer (Chalcididae), *Aprostocetus taxi* Graham (Eulophidae), *Agrothereutes adustus* Grav., *Blapsidotes vicinus* Grav., *Hyposoter clauses* Brischke (Ichneumonidae), *Pteromalus puparum* (L.) (Pteromalidae) and three dipterous species *Exorista larvarum* (L.), *Exorista segregata* Rondan, *Phryxe vulgaris* Fallén (Dip.: Tachinidae) were identified. Sarcophagidae, Calliphoridae, *Tachinid puparia*, Muscidae, Trypetidae, Psychidae, Yponomeutidae. and Lymantriidae Zhao *et al.* (1986) surveyed the natural enemies of the crucifer pest (Pieris *rapae* L.) in China. They found seven natural enemies (*T. evanescens, Apanteles rubecula, Apanteles glomeratus, Hyposter* sp., *Mermis* sp., *Pteromalus puparum* and *Brachymeria*

fermata . Narendran and Rao (1987) showed that Chalcididae hosts belong to Lepidoptera, Diptera, Hymenoptera, Neuroptera, Coleoptera and Stresiptera.). Andriescu (1988) recorded that *B. femorata* was a frequent parasite of *Aporia crataegi* and mention the parasitization of pupae of Cassida by *B. inermis*, of pupae of *Mamestra suasa* and *Aphelia vibumana* by *B. intermedia*, and of puparia of Tabanidae and *Musca domestica* by *B. minuta*. Moursy *et al.* (1996) remember that the most species of *Brachymeria* were parasites on larvae and pupae of Diptera, Coleoptera and Lepidoptera. Shaw *et al.* (2009) showed a few species of *Brachymeria* were solitary primary parasitoids of Lepidoptera pupae, and attacked butterflies in grassland habitats regularly. *B. femorata* (Panzer) separated from pupae of *Pieris brassicae, Melitaea didyma, M. deione* and *Maniola jurtina*; On other hand B. *tibialis* (Walker) get from *Euphydryas aurinia* and *E. desfontainii*. Maosheng (2015) observed that *Brachymeria* sp. ovipositing on a Calliphoridae and Sacorphagidae larvae on the rotting carcass of The young brahminy kite (*Haliastur indus*) in Singapore Island. Chakraborty *et al*. (2015) Illustrate that endoparasitoids: *B. minuta* (Hymenoptera: Chalicididae) in forensic indicator *Sarcophaga* (*Parasarcophaga*) *albiceps.* Hasanshahi *et al.* (2013) recorded that *B. albicrus* (Klug) (Hymenoptera: Chalcididae) a pupal parasitoid of the cabbage white butterfly, *Pieris rapae* (Linnaeus, 1758) from Iran. Roberts (1933) decided *that B. fonscolombei* (Dufour) a hymenopterous parasite of blowfly larvae, on (*Sarcophaga plinlhopyga*) Wied, *Phormia regina* Meig, *Lucilia unicolor* Towns, *Calliphora coloradensis*, *Synlhesiomyia nudiseta*, Lucilia *sericata* Meig and *Cochliomyia macellaria*.

2.Economic importance of *Brachymeria* species in Egypt:

Data presented in Table (3) are concerned with *Brachymeria* species which attack pests belonging to various insect Orders ; Lepidoptera and Diptera .The highest number of *Brachymeria* are species recorded on order Lepidoptera, where the number reached 10 species that attacked more than one host species . Hosts from Order Lipdoptera included 7 families (Lycaenidae, Pieridae, Nymphalidae, Nolidae, Noctuidae, Pyralidae and Lymantriidae). The Order Diptera came the second as hosts of the *Brachymeria* species. Dipterous hosts included 7 species belonging to 3 families (Calliphoridae, Muscidae and Sarcophagidae). The parasitoid *B. minuta* (Linn.) was very close resemblance with *B. fonscolombei* in hosts. These results were in agree with Thompson (1954) who reported *Brachymeria* as one of hymenopterous parasitoids that attacked some insect species belong to Orders Lepidoptera, Diptera and Hymenoptera . In Egypt *B. brevicornis* Klug was attacks *Earias insulana* Boisd. (Lep. Arctiidae). Ferrierre and Kerrich (1958) indicated that *Brachymeria* species are parasitoid emerging from pupae, more often of Lepidoptera or Diptera, but sometimes from Hymenoptera or Coleoptera. Leonard (1966) mentioned that *B. intermedia* (Nees) was established in North America. *B. intermedia* was a parasitoid on Porthetria *dispar* and other lepidopteran pupae in Southern Europe and northern Africa. Joseph *et al.* (1973) recorded that *B. minuta, B. fonscolombei, B. femorata, B. excarinata* hosts belonging to the Pieridae (*P. rapae*).

3. Geographic distribution of *Branchiomeric* species in Egypt:

Data show in Tables (4 and 5) and Figure (4) that the most *Brachymeria* spp. appears to have a strictly or mainly Lower of Egypt, it represented in the following Governorates, Giza , Helwan, Sharqia, Qalyubia , Dakahlia , Kafr El Sheikh, Gharbia, Monoufia and Ismailia respectively. From the few specimens available these species appear in Upper Egypt (Qena, Bahariya Oasis, Aswan) and Costal stripes (Beheira, Mersa Matruh, Red Sea) distribution. It is probable that its distribution may extend to desert areas near the newly reclaimed agriculture in Egypt. The only species was recorded with slightly number in Sinai from near the coast is *B. aegyptiaca.* on the other hand, *B. minuta , B. brevicornis , B. fonscolombei , B. femorata , B. albicrus,* and *B.aegyptiaca* more monthly present or more adapted to the Egyptian environment .In the same context *B. minuta , ,B. fonscolombei , B. aegyptiaca ,B. femorata , B. albicrus, B. excarinata, B. kassalensis , B. vicina and* B. *somalica* were worldwide distribution other than *B. brevicornis* and *B. ancilla.* These results agreed by Masi (1929a) who studied on the genus *Brachymeria* West., from the Ethiopian. region, species *B. leighi* (Cam.), *B. feae, B. bottegi, B. cowani, B. magrettii, B. paolii, B. somalica, B. bayoni, B. afra, B. spilopus, B. dumbrodyensis* (Cam.), *B. capensis and B. beccarii*. Roberts (1933) showed that *B. fonscolombei* was generally distributed over central Europe, extending into Russia and Asia. Specimens collected from Batavia, Java, and deposited in the National Museum of United States in North America. Also, it had been found throughout the southern part (Florida to California and as far north as Illinois). The previous specimens are known in Mexico and Haiti. Kamal (1937) pointed out that *B. femorata* (Panzer) was widely distributed at various localities of Lower Egypt, and he was astonished about the aestivation of this insect. Leonard (1966) mentioned that B. *intermedia* (Nees) (Hymenoptera: Chalcididae) established in north America. Also *B. intermedia* was a parasite of Porthetria *dispar* and other lepidopteran pupae in southern Europe and northern Africa. Habu (1960) explained the distribution of *B. minuta, B. fonscolombei, B. femorata* and B. *excarinata* distribution in Japan. Joseph *et al.* (1973) explained the oriental species of *Brachymeria: B. minuta, B. fonscolombei, B. femorata, B. excarinata* distribution in India, Japan, Korea, Manchuria, North China (Mongolia, Siberia, Formosa, Botel-tobaco Is.) Centeral Asia, Asia Minor, Europe, North Africa. Vietnam, Philippines, Java, Cambodia, B.N. Borneo. North America and Thailand. Karrom (1974) mentioned *B. minuta* and *B. vicina* distribution in Syria. Klncer (1982) studied the field populations of the parasite complex associated with *Artogeia rapae* (L.) (*Pieris rapae*) on cabbage in Turkey and reared these parasites in the laboratory. *Trichogramma evanescens* west, was the only egg parasite. *Apanteles glomeratus* (L.) and Pteromalus *puparum* (L.) parasitzed the larvae. On the other hand, *Pteromalus puparum* (L.) and *B. femorata* (Panz.) were the pupae parasites. Zhao *et al*. (1986) surveyed the natural enemies of the crucifer pest (*Pieris rapae* L.) in China. They found seven natural enemies (*T. evanescens, Apanteles rubecula, Apanteles glomeratus, Hyposter* sp., *Mermis sp., Pteromalus puparum* and *B. fermata*). El-Moursy *et al.* (1996) pointed that most species of *Brachymeria* are

distribution in Lower Nile and parasites on larvae and pupae of Diptera, Coleoptera and Lepidoptera. Andriescu (1988) studied the faunistic, biogeographical and economic of family chalcididae in Rumania, the most abundant and frequent species were *Chalcis sispes*, *B. minuta*, and *B. intermedia*, however the rarest were *Neochalcis fertoni*, *Lasiochalcidia guineensis*, *Invrea subaenea* and *I. mirabilis*. Hasanshahi *et al.* (2013) studied new record of *B. albicrus* (Klug) (Hymenoptera: Chalcididae) a pupal parasitoid of the cabbage white butterfly, *P. rapae* from Iran.

4. Seasonal abundance of Brachymeria *brevicornis* parasitoids of in different crops and locations during 2015 and 2016 on cotton growing seasons:

Data present in Table (6) and Figures (5 and 7) show that the highest mean parasitism percentage was recorded at Qaha , Qalyubia Governorate, sowing during 2015 cotton growing seasons (4.76%) followed by Saft El Laban road , Giza Governorate, sowing during 2016 on cotton growing seasons (4.47%). While the ratio was almost halved at Qaha ,2016 to become (2.38%) and exceeded half at Saft El Laban road, Giza Governorate, sowing during 2015 was (2.85%); There was no recorded percentage of *B. brevicornis* Klug parasitism in Menoufia governorate. although the largest population of *B. brevicornis* insects was in Giza Governorate, sowing during 2015 and 2016 by four insects followed by Qaha, 2015 and 2016 by 3 insects. These results agreed with Thompson (1954), he mentioned that *Brachymeria* was one of hymenopterous parasitoids that attacking some insects on some families specially orders Lepidoptera. In Egypt *B. brevicornis* attaching *E. insulana*.

5.Seasonal abundance of *Brachymeria femorata* parasitoid of *Pieris rapae* in different locations during 2014 and 2015 cabbage growing seasons:

The data which presented in Table (7) and Figures (6 and 8) show *B. femorata* .The highest mean parasitism percentage was recorded at sowing during September 2014 and 2015 cabbage growing seasons (28.49% in 2014) and (24.46 % in 2015), respectively , followed by the mean percentages of parasitism sowing during July 23.95 and 17.28% respectively , followed by the mean percentages of parasitism sowing during May 10.18% and 3.23% respectively. The mean percentages of parasitism sowing during February 2014 and 2015 were 0.00. Finally, the solitary parasitoid *B. femorata* was recorded in *P. rapae* pupae at the. numbers of the mentioned parasitoid along the months of Late December to June, while it was found at the rest months (July to beginning of December). The highest parasitism percentage were recorded at September, October and November compared with other months. The highest mean parasitism percentage were recorded at 2014 specially during November compared with 2015 year. These results agree with Kamal (1937) pointed out that *B. femorata* (Panzer) allied ones are extremely important as natural agents of control on some of our most important crop pests and seem to survive very well under the mild climate of this country. Hu in China (1983) carried a survey during (1978-1972) on the pest *Artogeia rapae* (L.) and found *Apanteles glomeratus* (L.), *Ptreromalus puparum* (L.) and *B. femorata* (Panz.) species as primary parasites with (0-64.5% and 0-97%) parasitism percentage of larvae and pupae , respectively . Hussain *et al.*

(1992) indicated that *B. femorata* and *Pteromalus puparum* are founded to be effective in controlling *Pieris rapae* (L.) in Egypt. Parasitoids were found in crucifer fields parasitizing the pupal stage at 2 distinct periods: the 1st extended from October to mid – January and the 2nd from March to April. The percentage of parasitism by *B. femorata* and *P. puparum* ranged from 13.7 to 20.7%, respectively. Youssef and Moursi (1988) mentioned that, a field experiment was conducted in Alexandria, Egypt, to determine the natural mortality factors affecting *Pieris rapa*e population in cabbage fields. The results showed that parasitism by *P. puparum* and *B. femorata* was the major mortality factor, causing up to 83.7 and 10.9 % mortality of larvae and pupae, respectively. Karrom (1974) pointed out the seasonal abundance of the Chalcidids which were mainly endoparasites in other insects, depended on the both seasonal abundance of these hosts and their life cycles.

Many species of this genus spread in different places in Egypt and were mostly primary parasitoids in pupae of holometabolous insects, especially of Lepidoptera, but some species attack Diptera ,which contain many of the pests that were harmful to humans, Therefore the precise determination species concerned is highly important in any host –parasite study for biological control. Most of the species *Brachymeria* wasps of found in Egypt are concentrated in the agricultural areas, especially the Nile Delta region and around it. Most of them were recorded from the Palearctic region**.** Some wasps of the genus *Brachymeria* were primary parasitoids of butterfly from the families Pieridae and Nolidae, More than one egg may be inserted into a single maggot by the female wasp. The wasp larvae feed on the maggot from the inside, but only one adult wasp will emerge when the host dies. *B. femorata* and *B. brevicornis* species as well as its allied ones were extremely important as natural agents of control on some important vegetables and crop pest and it survived very well under the mild climate in Egypt.

Table (1): Field survey of *Brachymeria* species in Egypt (2013-2018).

Species	Date	Year	Locality	Plant	Remarks
B. aegyptiaca	September, October and November	2014 ,2015 ,2016 and 2017	Cairo - Alexandria Desert Road, Wadi al-Arish, and Kom Oshim	Olive and Pomegranate	by sweeping and from pupae
B. albicrus	September	2014	Cairo - Alexandria Desert Road	Pomegranate	from pupae
B. brevicornis	August and September	2015, 2016	Qaha and Saft El Laban Road	Cotton	from pupae
B. femorata	July, August, September, October and November	2013, 2014 ,2015 ,2016 and 2017	Kom Halin, TokhTanabsha, Kafr Alaym, Berkat as Sabee, Shibin El Kom, Quesna, Qaha, Mansoura, Habbes valley, Sidi Salem, Kotor and Damanhur.	Cabbage	by sweeping and from pupae
B. minuta	March and April	2014	Cairo and zigazig	The rabbit's corpse	from pupae

Table (2): Numbers or population number of *Brachymeria* species in Egyptian Collections.

Collections Species	Ministry of Agriculture	Ain Shams University, Department of Entomology	Alfieri	Society of Entomology in Cairo	Total	State
B. aegyptiaca	18	0	1	0	19	Moderate
B. albicrus	4	5	0	0	9	Moderate
B. ancilla	3	0	0	0	3	Rare
B. brevicornis	25	1	1	0	27	Common
B. excarinata	2	0	0	0	2	Rare
B. femorata	48	2	0	0	50	Common
B. fonscolombei	5	1	0	0	6	Moderate
B. kassalensis	2	0	0	0	2	Rare
B. libyca	1	0	0	0	1	Rare
B. minuta	71	16	11	4	102	Common
B. somalica	4	0	0	0	4	Moderate
B. vicina	2	0	0	0	2	Rare
Total	185	25	13	4	227	

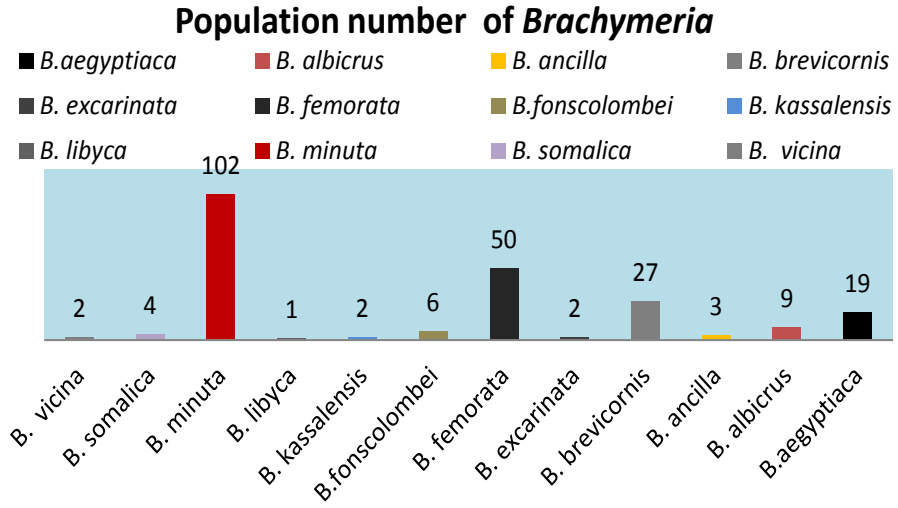

Figure (3): Population number of *Brachymeria* species in Egyptian collections.

Table (3): Economic importance of Brachymeria species in Egypt.

***Brachymeria* species**	**Host insect species record**		
	Species	**Family**	**Order**
B. aegyptiaca	*Virachola livia*	Lycaenidae	Lepidoptera
	Palpita unionalis	Pyralidae	
B. albicrus	*Danais chrysippus*	Nymphalidae	
B. brevicornis.	*Earias insulana*	Nolidae	
	Virachola livia	Lycaenidae	
B. excarinata	*Spodoptera litura*	Noctuidae	
B. femorata	*Pieris rapae*	Pieridae	
B. fonscolombei	*Lucilia sericata*, *Chrysomya albiceps* and *Synthesiomyia nudiseta*	Calliphoridae and Muscidae	Diptera
B. kassalensis	*Chaerocampa elpenor*	Sphingidae	Lepidoptera
B. libyca	*Wohlfahrtia argentifrons*	Sarcophagidae	Diptera
B. minuta	*Lucilia cuprina*	Calliphoridae	
	Sarcophaga hertipes	Sarcophagidae	
B. somalica	Snout moths	Pyralidae	Lepidoptera
B.vicina	*Lucilia sp*	Calliphoridae	Diptera
	Porthetria dispar	Lymantriidae	Lepidoptera

Table (4): Monthly occurrence and geographical zone of *Brachymeria* species in Egypt.

Species	Months												Geographical Zone
	Jan.	Feb.	Mar.	Apr.	May	June	Jul.	Aug.	Sep.	oct.	Nov.	Dec.	
B. aegyptiaca	_	_	_	_	_	_	_	_	+	+	++	+	Costal stripes, Lower Egypt and Sinai
B. albicrus	_	_	_	_	_	_	+	_	+	+	+	++	Lower Egypt and Upper Egypt
B. ancilla	_	_	_	_	_	_	_	_	_	+	_	_	Lower Egypt
B. brevicornis	_	_	+	+	+	+	+	++	+	+	–	–	Costal stripes, Lower Egypt and Upper Egypt
B. excarinata	_	_	_	_	_	_	_	_	+	_	_	_	Lower Egypt
B. femorata	_	_	+	_	_	_	++	+	++	+++	++	+	Costal stripes and Lower Egypt
B. fonscolombei	_	_	_	+	+	+	+	+	+	+	+	_	Lower Egypt
B. kassalensis		+	_	_	_	_	_	_	_	_	_	_	Upper Egypt
B. libyca	_	_	_	_	_	+	_	_	_	_	_	_	Lower Egypt
B. minuta	_	_	+	++	++	++	+	+	++	+++	+++	++	Costal stripes, Lower Egypt and Upper Egypt
B. somalica	_	_	_	_	_	_	_	_	_	+	+	+	Lower Egypt
B. vicina	_	_	_	_	_	_	_	_	_	+	_	_	Lower Egypt

***(-) indicate to specimens lack (+) indicates to the increase in specimens within the species.**

Table (5): Distribution of *Brachymeria* species in Egyptian Governorates.

Governorate	Species										
	B. albicrus	***B. excarinata***	***B. femorata***	***B. minuta***	***B. ancilla***	***B. fonscolombei***	***B. kassalensis***	***B. somalica***	***B. aegyptiaca***	***B. vicina***	***B. brevicornis***
Dakahlia			+						+		
Sharqia			+	+					+		
Qalyubia			+	+							+
Kafr El Sheikh			+	+							
Gharbia			+								
Monoufia			+								
Beheira	+		+	+					+		+
Ismailia				+							
Helwan		+		+		+		+			
Giza			+	+	+			+	+	+	+
Qena											+
Sinai									+		
Bahariya Oasis				+							
Red Sea							+				
Aswan	+										
Mersa Matruh			+	+		+					

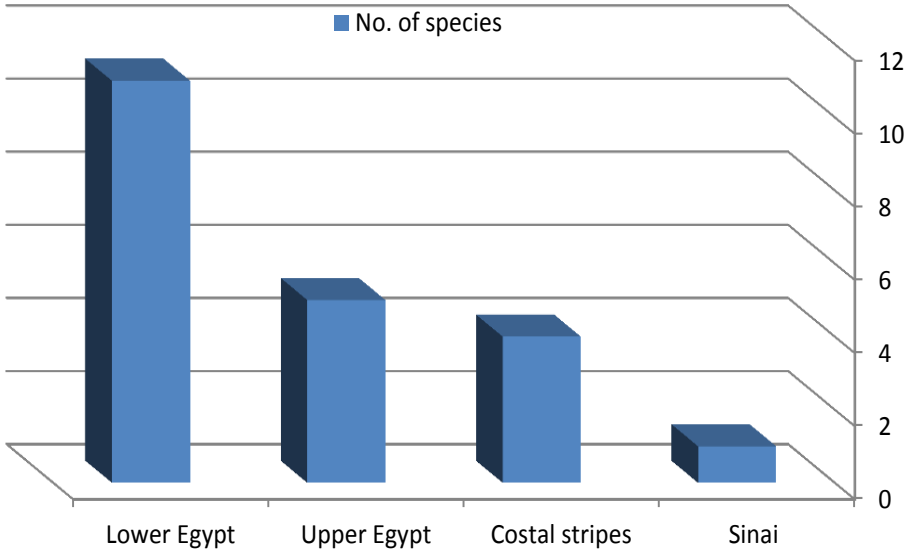

Figure (4): Number of *Brachymeria* species and geographical zone in Egypt.

Table (6): The percentage of *Brachymeria brevicornis* parasitism in spiny bollworm *Earias insulana* pupae, sowing during 2015 and 2016 cotton growing seasons.

Date and Governorates	2015			2016		
	Monoufia	Qalyubia	Giza	Monoufia	Qalyubia	Giza
1 August	0	0	0	0	0	0
11 August	0	0	0	0	0	0
21 August	0	0	0	0	0	9.09
31 August	0	0	20	0	16.66	22.22
10 September	0	33.33	0	0	0	0
20 September	0	0	0	0	0	0
30 September	0	0	0	0	0	0
Mean		4.761429	2.85714		2.38	4.47285

Table (7): The percentage of *B. femorata* (Panz.) parasitism in *Pieris rapae* pupae at the Toukh Tanbisha village, Berkat El-Sabaa region, Monoufia Governorate sowing during 2014 and 2015 cabbage growing seasons.

Sawing date / Weeks after sowing	2014			2015		
	February	May	July	February	May	July
7 Weeks	0	30	33.33	0	9.09	50.00
8 Weeks	0	0	50.00	0	0	33.33
9 Weeks	0	12.5	11.11	0	0	0
10 Weeks	0	11.11	14.28	0	0	20
11 Weeks	0	0	12.5	0	20	0
12 Weeks	0	12.5	28.57	0	0	20
13 Weeks	0	25.55	20	0	0	0
14 Weeks	0	0	33.33	0	0	22.22
15 Weeks	0	0	12.5	0	0	10
Mean		10.18444	23.95778		3.232	17.28333

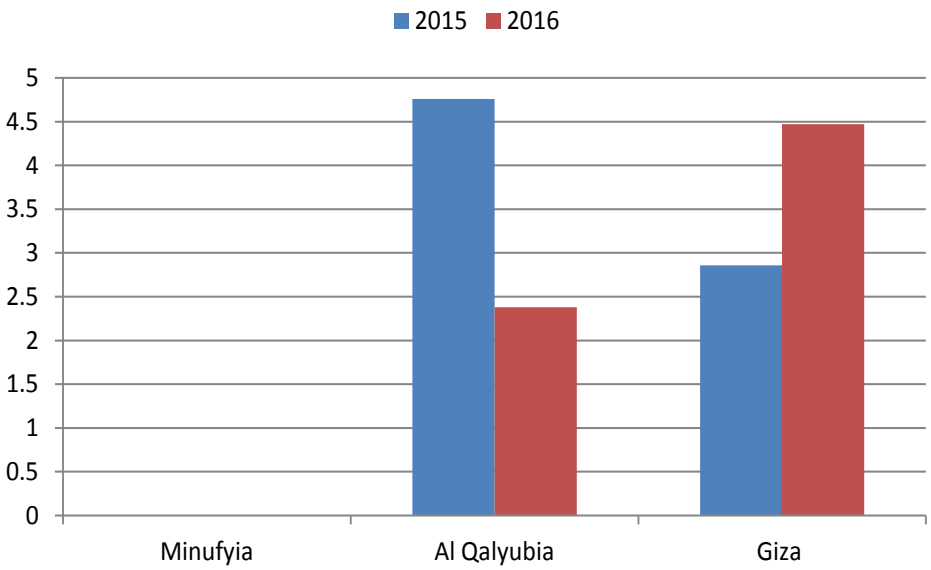

Figure (5): Percentage of *Brachymeria brevicornis* parasitism in spiny bollworm *Earias insulana* pupae, sowing during 2015 and 2016 cotton growing seasons.

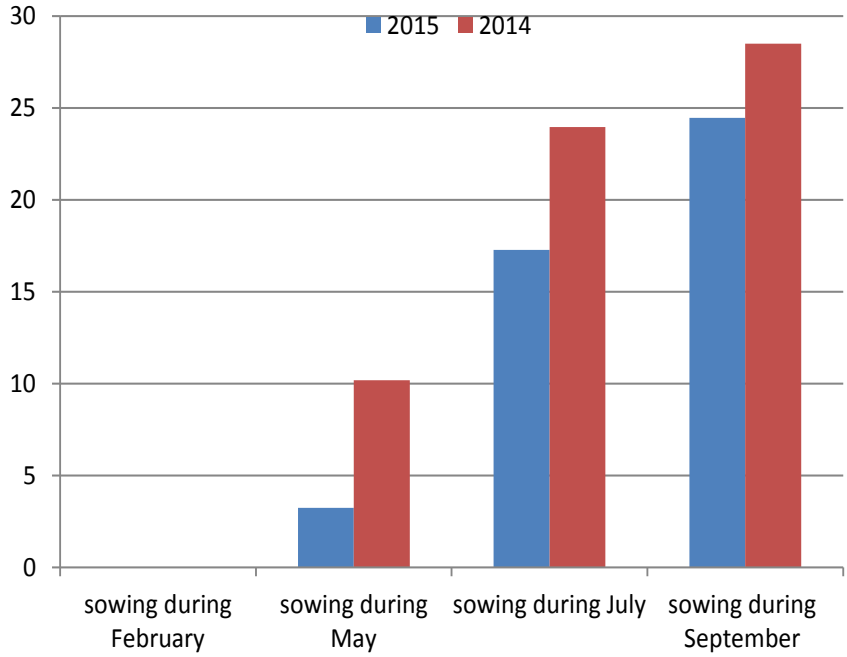

Figure (6): Percentage of *Brachymeria femorata* parasitism in *Pieris rapae* pupae at the Toukh Tanbisha village, Berkat El-Sabaa region, Monoufia Governorate.

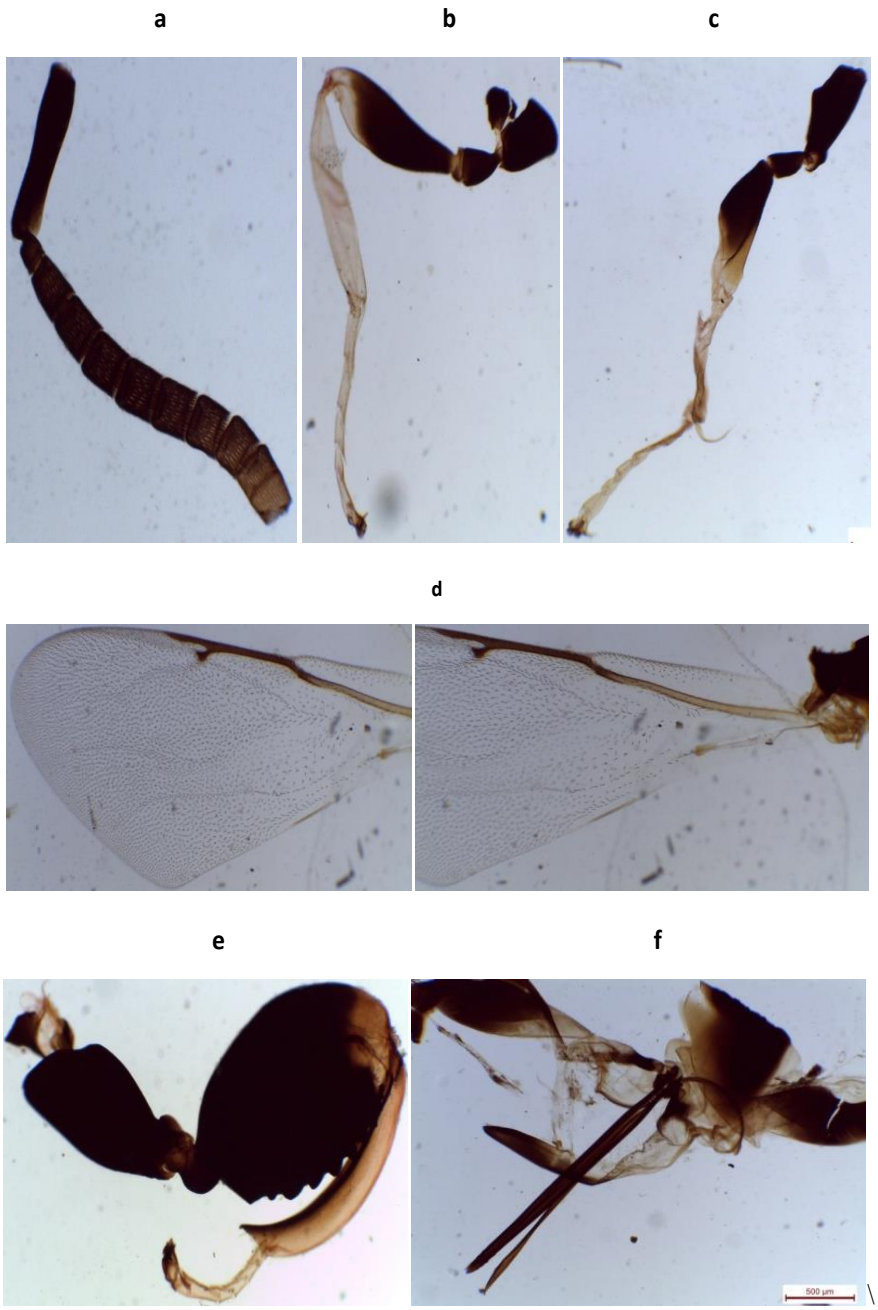

Figure (7): *Brachymeria bravicornis* .

a. Antenna b. Foreleg c. Mid leg d. Forewing e. Hindleg f. Ovipositor sheath

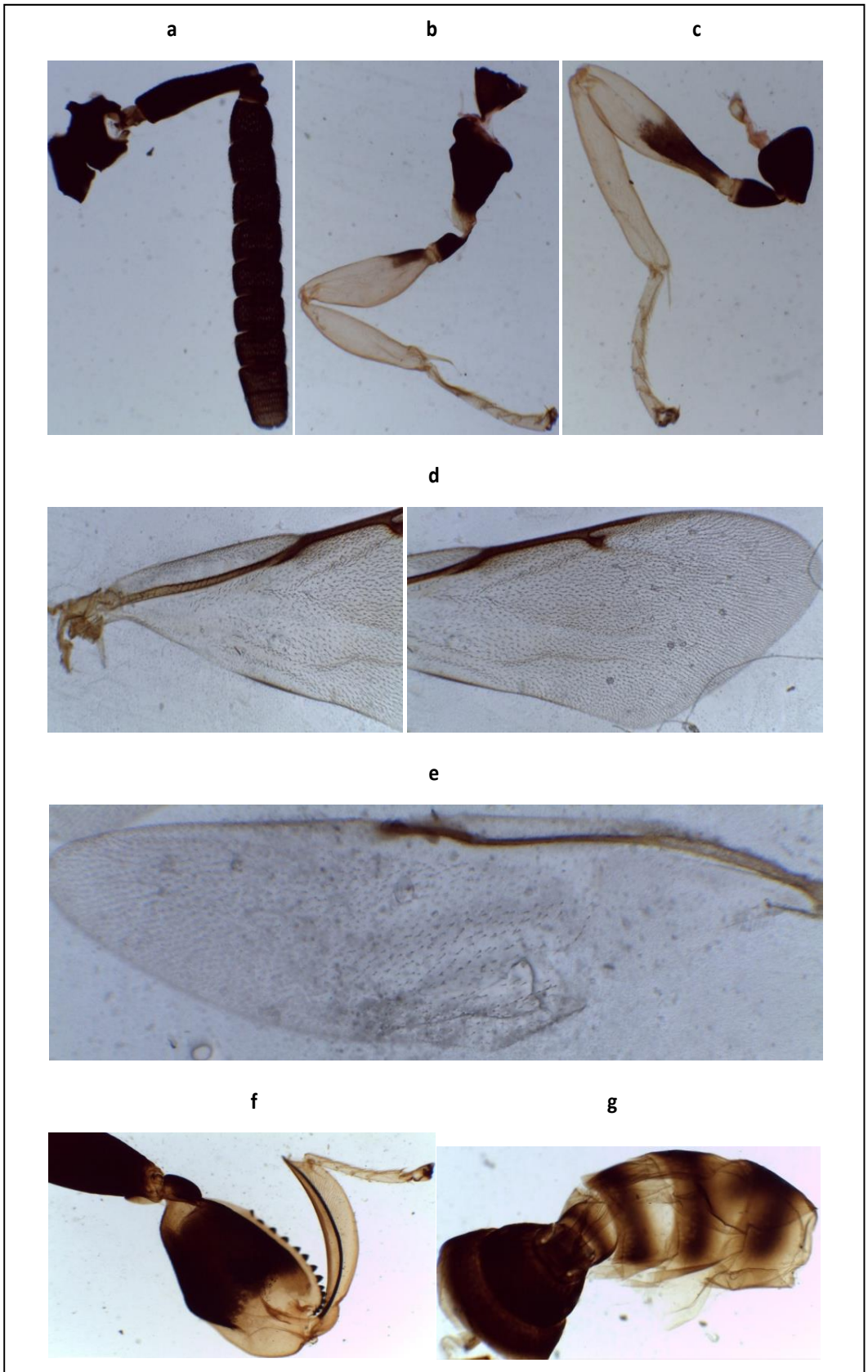

Figure (8): *Brachymeria femorata* .

a. Antenna b. Foreleg c. Mid leg d. Forewing e. Hind wing f. Hind leg g. Gaster

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Formulation of the newly synthesized arylidene derivative as 10 % flowable and evaluation of their insecticidal efficacy on cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract:

Five new acrylamide derivatives were synthesized according to standard method, their structure was elucidated using spectral techniques (IR, Mass and ^{1}H-NMR). Acrylamide derivatives were tested against the 2nd instar larvae of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) under laboratory conditions. Acrylamide (2a) showed the highest efficacy, as its LC_{50} was 0.967 mg/ml. It was then formulated as 10 % flowable (suspension concentrate). The new formula passed successfully all physical tests specified for flowables. It was then also tested against the 2nd instar larvae of the cotton leafworm *S. littoralis* under laboratory conditions; it inhibited the 2nd instar larvae of the cotton leafworm markedly, as its LC_{50} was 4.494 mg/ml.

Introduction

The cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is the most common, serious and devastative pest which attack large scale of economic crops as cotton, clover, maize and different vegetable crops (Moawad and Sadek, 2018). The noctuid moth of the cotton leafworm *S. littoralis* is found widely in Mediterranean Europe and Africa (Ahmed *et al.*, 2019). Many crops in Egyptian fields, as well as various vegetables are attacked by numerous insect pests. The lepidopterous insects in general and the cotton leafworm *S. littoralis*, are the most dangerous in this respect. On cotton, the pest may cause considerable damage by feeding on the leaves, fruiting points, flower buds and occasionally, also on bolls. Pods of cowpeas and the seeds they contain are also often badly damaged. In tomatoes, larvae bore into the fruit, which become unsuitable for consumption (Osman and Mahmoud, 2009). Generally, the larvae prefer young leaves and, while they are consuming these, they are also feeding on other parts of the plant. Infestation frequently leads to complete defoliation and devouring of the leaves. The larvae interfere with plant development by destroying growth points and flowers as well as hollowing out the

seed bolls, which often causes them to wilt and drop (Croft, 1990).

Insect resistance is a major problem generated by the frequent use of the conventional pesticides for controlling the insect pests (Nkya *et al.,* 2014). In Egypt *S. littoralis* was held in check by methyl-parathion, but then resistance to this compound developed. Since then, numerous other organophosphorus, synthetic pyrethroid and other insecticides have been used, with appearance of resistance and cross-resistance in many cases (Issa *et al.,* 1984 a and b and Abo-El-Ghar *et al.,* 1986). Agrochemicals have been critical to the production of food and fiber, as well as the control of vectors of disease. The need for the discovery and development of new agrochemicals continues unabated due to the loss of existing products through the development of resistance (Sparks and ALorsbach, 2017).

Acrylamide is an organic compound with the chemical formula C_3H_5NO. Acrylamide can be found as monomers (single units) or polymers (Kusnin *et al.,* 2015). Polyacrylamide is also used as a thickening agent in pesticides. In herbicides, polyacrylamides are used to increase its surfactants capabilities and to reduce spray drift (Smith *et al.,* 1996). In addition, it was reported by Fadda *et al.* (2017) that arylidene derivatives containing acrylamide portion has an insecticidal activity on the second instar larvae of cotton leafworm *S. littoralis.* Formulation means the combination of various ingredients designed to render the product useful and effective for the purpose claimed and for the envisaged mode of application (FAO and WHO, 2014). The basic objectives of formulation technology are to optimize the biological activity of the pesticide. In the past "old technology", most of the agrochemical formulation technologies were based on simple solutions in water miscible solvent (SL), emulsifiable concentrates in a petroleum-based solvent (EC), or dusts (DP) and wettable powders (WP). The presence of petroleum-based solvents and dusty powders in these conventional formulations generally create safety hazards in use and have a negative impact on the environment (Green and Beestman, 2007). Most Government regulatory authorities are now encouraging the pesticide industries to develop formulations, which are cleaner and safer for the user (Mulqueen, 2003). This has led to the development of water based liquid formulations such as flowable (suspension concentrates, SC), oil-in water emulsions (EW) and microcapsules (CS) etc. (Hazra, 2015).

The scope of the present study was to implement prototype for obtaining a new active ingredient containing acrylamide portion, formulating it in the form of commercial formulation for use in the control of cotton leafworm *S. littoralis* after completing the other required laboratory and field experiments.

Materials and methods

1. Tested chemicals:

1.1. Fine chemicals:

o-aminophenol (2-aminophenol, molar mass 109.13 $g.mol^{-1}$), ethanamide (acetamide, molar mass 59.068 $g.mol^{-1}$), triethylamine base (*N*, *N*-diethylethanamine, molar mass 101.193 $g.mol^{-1}$) and aromatic aldehydes were supplied by Sigma - Aldrich Co.

1.2. Solvents:

Benzene, toluene and absolute ethanol were supplied by EL-Gomhoria Co., Cairo, Egypt.

1.3. Surface active agents:

Sodium lauryl sulfate (SLS), Span 20 and Tween 20 were supplied by EL-Gomhoria Co., Cairo, Egypt.

1.4. Poly ethylene glycol 600 diolate (P.E.G 600 Do.) was supplied by the Egyptian Starch, Yeast and Detergents Co., Alexandria, Egypt.

2.The physico-chemical properties of the basic formulation components:

2.1. Active ingredient:

The physico-chemical properties of the newly synthesized (E)-2-cyano-3-(4-(dimethylamino) phenyl)-N-(2-hydroxyphenyl) acrylamide (**2a**) as an active ingredient were:

2.1.1. Solubility:

It was determined by measuring the volume of distilled water, acetone, DMF, ethanol and xylene for complete solubility or miscibility of one gram of active ingredient at 20 ˚C (Nelson and Fiero, 1954). The % solubility was calculated according to the following equation:

% solubility = W/V x 100

[Where; W = active ingredient weight, V = volume of solvent required for complete solubility].

2.1.2. Free acidity or alkalinity: It was determined according to the method described by WHO (1979).

2.1.3. Melting point:

It was determined on an electric digital melting point (Gallenkamp) 9200 A apparatus.

2.2. The physico-chemical properties of surface-active agents:

2.2.1. Free acidity or alkalinity: it was determined as described before.

2.2.2. Hydrophilic-lipophilic balance (HLB): The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance (Lynch and Griffin, 1974).

2.2.3. Critical micelle concentration (CMC): The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentration, (CMC) of the tested surfactants was determined according to the method described by (Osipow, 1964).

2.2.4. Surface tension: It was determined by using Du-Nouy tensiometer for solutions containing 0.5 % (W/V) surfactant according to ASTM (2001).

2.3. Preparation of acrylamide derivative (**2a**) as flowable (suspension concentrate, SC)**:**

Base mill was prepared by adding active ingredient, arylidene (**2a**), dispersing agent, wetter if necessary and defoamer in water. The premix was homogenized with high shear mixer or homogenizer for few minutes. The slurry was milled until desired particle size is achieved. Stabilizer was added and mixed properly with mill base. Other ingredients were added such as in-can adjuvant, anti-freeze, thickener and biocide as necessary (wet grinding processes). The obtained formula was subjected to the specified test methods for flowable formulations.

2.4. Determination of the physico-chemical properties of the local 10 % flowable (SC) formulation:

2.4.1. Suspensibility: It was determined to demonstrate that an enough the active ingredient is suspended in the spray liquid to give a satisfactory, homogeneous mixture during spraying. It was determined according to (**Dobrat and Martijn, 1995)**.

2.4.2. Free acidity or alkalinity: It was determined as mentioned before.

2.5. Determination of the physico-chemical properties of the spray solution at the field dilution rate (0.5 %):

2.5.1. Viscosity: It was determined by using Brookfield viscometer Model DVII+Pro, where centipoise is the unit of

measurement according to **ASTM** (**2005**).

2.5.2. Surface tension: It was determined as mentioned before.

2.5.3. PH: It was determined by using Cole-Parmer PH/conductivity meter 1484-44 according to **Dobrat and Martijn** (**1995**).

2.5.4. Electrical Conductivity: It was determined by using Cole-Parmer PH/Conductivity meter 1484-44, where µmhos is the unit of electrical conductivity measurements according to **Dobrat and Martijn** (**1995**).

3. Bioassay:

The present study was conducted to investigate the susceptibility of a laboratory strain of the 2nd instar larvae of the cotton leafworm *S. littoralis* to the newly prepared acrylamide derivatives (**2a-e**). It was carried out using leaf dip technique **(Sadek, 2003)**

Cotton leafworm *S. littoralis* was rearing in the Laboratory of Plant Protection Research Institute, Egypt. It was cultured under controlled conditions (30±2 ºC and 65±5 % RH.) on castor-bean leaves for several generations. A series of different concentration (10, 8, 4, 2, 1 and 0.5 mg/ml) for each compound was prepared by dissolving in DMSO then the volume was completed by water. Four castor-bean leaves dipped inside every single attentiveness for 30 seconds after which, it was left to dry. The 2nd instar larvae could feed on the treated leaves. Four replicates of 10 larvae were used for each concentration in addition to the control. Control tests were carried out using the same technique without the addition of the tested compound. Castor-bean leaves were dipped in a solution of 0.1 % Triton X-100 and solvent.

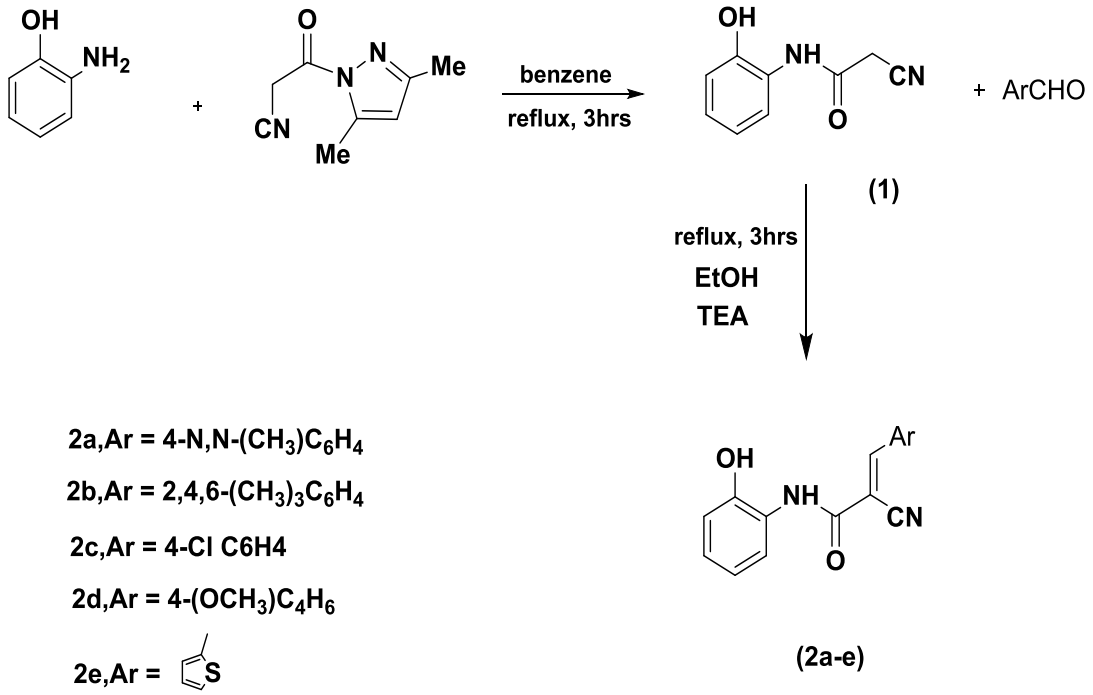
4. Statistical analysis:

The average regarding mortality portion had been determined by employing Abbott formula (1925). The actual remedied mortality portion of each compound had been statistically computed according to the method of Finney (1971), Toxicity index was calculated by the following equation; Toxicity index = LC_{50} of the most powerful compound / LC_{50} of the screened compound × 100 according to (Sun, 1950).

Results and discussion

1. Chemistry part:

2-cyano-N-(2-hydroxyphenyl) acetamide derivative (1) was prepared through the reaction of *o*-aminophenol (as a primary aromatic amine) with 3-(3,5-dimethyl-*1H*-pyrazol-1-yl)-3-oxopropanenitrile in toluene under reflux for 3 hrs. The reaction proceeded through cyanoactylation processes of *o*-aminophenol. The obtained cyanoacetamide derivative (1) on treatment with different aromatic aldehydes in refluxing absolute ethanol for 3 hrs. afforded acrylamides (arylidenes) derivative (2a-e) (Scheme, 1) (Fadda *et al.*, 2017), the reaction that takes place according to Knoevenagel condensation giving excellent yields of Knoevenagel products that were confirmed by spectral analysis, The IR spectrum showed the characteristic spike for the secondary amine (NH) at 3375 cm^{-1} and the nitrile group (CN) at 2194 cm^{-1} , the mass spectrum of the derivative (4e) showed the correct molecular ion peak at m/z (%) 270, in addition ^{1}H-NMR spectrum in general showed a singlet signal at δ_H 8.16 ppm characteristic for the vinyl proton and another singlet corresponding to amide (NH) at δ_H 10 ppm.



Scheme (1): Synthesis of the arylidene compounds (2a-e)

All melting points were uncorrected and measured on an electric melting point (Gallenkamp) 9200 A apparatus. IR spectra (KBr) were recorded with a Perkin-Elmer model 157 infrared spectrophotometer. ^{1}H-NMR spectra were obtained from Varian Gemini 200 MHz spectrometer and chemical shifts are expressed in δ (ppm) using TMS as internal reference. Mass spectra were acquired with GCMS-QP1000 EX and Jeol JMS 600 spectrometers opening at 70 eV. Microanalytical data were obtained from the microanalytical data center of the Faculty of Science, Mansoura University.

-Synthesis of 2-Cyano -N- (2-hydroxyphenyl) acetamide (1)

A mixture of *o*-aminophenol (0.01 mole, 1.09 g), and 3-(3,5-dimethyl-*1H*-pyrazol-1-yl)-3-oxopropanenitrile (0.01 mole, 0.16 g) was heated in toluene under reflux for 3 hrs. The formed solid crystalline material was filtered off washed with toluene to afford the corresponding acetamide derivative (**1**).

Silver crystals; yield 95 %; mp 280 °C; IR (KBr): *v/cm^{-1}* : 3276 (NH), 3037 (CH-arom), 2960 (CH-aliph.), 2271 (CN), 1672 (C=O) ^{1}H-NMR (200 MHz, DMSO-d6): δ/ppm 4.00 (s, 2H, CH_2), 6.77-7.84 (m, 4H, Ar-H), 9.57 (s, H, NH), 9.93 (s, H, OH), MS m/z (%): 176 (31.01), 136 (18.33), 109 (100.00), 107 (11.56), 77 (1.41).

-Synthesis of (E)-2-cyano-3-(4-(dimethylamino) phenyl)-N-(2-hydroxyphenyl) acrylamide (2a-e):

Equimolar amounts of acatamide derivative (**1**) (1 mmol) and aromatic aldehydes (1 mmol) in absolute ethanol (15 mL) containing few drops of triethyl amine (TEA) were heated under reflux for 4 hrs. The solid product that precipitated was isolated by filtration, dried, and recrystallized from 2:1 ethanol: DMF to afford compounds (**2a- e**).

- (E)-2-cyano-3-(4-(dimethylamino) phenyl)-N-(2-hydroxyphenyl) acrylamide (2a):

Orange crystals; yield 75 %; mp 280 °C; IR (KBr): *v/cm^{-1}*: 3375 (OH), 3232 (NH), 2919 (CH-aliph.), 2194 (CN), 1664 (C=O). ^{1}H-NMR (200 MHz, DMSO-d6): δ/ppm 3.08 (m, 6H, $2CH_3$), 3.39 (s, H,

CH_2), 6.82-8.16 (m, 8H, Ar-H), 8.94 (s, H, NH), 10.18 (s, H, OH).

- **(E)-2-cyano-N-(2-hydroxyphenyl)-3-mesitylacrylamide (2b):**

Brown crystals; yield 80 %; mp 175 °C; IR (KBr): *v/cm*$^{-1}$: 3393 (OH), 3367 (NH), 2227(CN), 2917 (CH-aliph.), 1679 (C=O). MS m/z (%): 306 (6.01), 198 (51.04), 108 (100.00), 107(44.51), 108 (72.41), 77 (4.23).

- **(E)-2-cyano-N-(2-hydroxyphenyl)-3-(p-tolyl) acrylamide (2c):**

Yellow crystals; yield 85 %; mp 200-205 °C; IR (KBr): *v/cm*$^{-1}$: 3365 (NH), 3040 (CH-arom.), 2217 (CN), 1683 (C=O), MS m/z (%): 298 (M^+, 16.05), 190 (100.00), 108 (66.48), 77 (2.62).

- **(E)-2-cyano-N-(2-hydroxyphenyl)-3-(4-methoxyphenyl) acrylamide (2d):**

Yellow crystals; yield 80 %; mp 205 °C; IR (KBr): *v/cm*^{1}: 3155 (NH), 2193(CN), 1675 (C=O).

- **(E)-2-cyano-N-(2-hydroxyphenyl)-3-(thiophen-2-yl) acrylamide (2e):**

Brown crystals; yield 70 %; mp 240 °C; IR (KBr): *v/cm*^{1}: 3376 (OH), 3246 (NH), 2206 (CN), 1664 (C=O). MS m/z (%): 270 (M^+, 1.84), 162(100.00), 108(11.78), 76 (5.53).

2. Biological activity:

Data in Table (1) showed the toxicological assay of acrylamide derivatives (**2a-e**) against 2nd instar larvae under laboratory conditions, compound (**2a**) showed the most toxic effect with LC_{50} value of 0.967 mg/ml , the effect that may be attributed to the presence of N,N dimethyl amine group (N $(CH_3)_2$) followed by (**2c**) (3.31mg/ml) that comprise halogen group, followed by (**2b**), (**2d**) and (**2e**) that showed LC_{50} values 8.10, 9.30 and 25.52 mg/ml respectively.

Table (1): Effect of the newly synthesized acrylamide derivatives (2a-e) against the 2nd instar larvae of cotton leafworm *Spodoptera littoralis* under laboratory conditions.

Tested compounds	LC_{50} (mg/ml) Its limits at 95 %	LC_{90} (mg/ml) Its limits at 95 %	Slope	Toxicity index %
2a	0.967 0.303 1.498	8.72 5.05 791.2	1.3417 ±0.3666	100.00
2b	8.10 6.30 18.64	17.89 10.68 126.27	3.7272 ±1.1281	11.93
2c	3.31 2.13 7.11	74.93 21.43 2764.19	0.9464 ±0.2393	29.16
2d	9.301 8.33 11.08	16.62 13.19 27.03	5.0801 ±0.9670	10.39
2e	25.52 10.22 791.27	319.13 51.65 4.31	1.1683 ±0.3588	3.78

Compound (**2a**) showed the lowest $LC_{50,}$ it was considered as a promising compound and it was formulated as 10 % flowable. Data presented in Table (2) showed comparison between the toxicity of compound (**2a**) as an active ingredient (a.i) and its 10 % (SC) formulation against the 2nd instar larvae of the cotton leafworm, (*S. littoralis*) under laboratory condition. The active ingredient revealed LC_{50} and LC_{90} values 0.967 and 8.72 mg/ml respectively while its 10 % flowable formulation showed 4.49 and 24.79 mg/ml respectively. These results showed that, active ingredient was more efficient on the 2nd instar larvae of cotton leafworm compared to its formulation which appeared clear from its corresponding toxicity index 100 and 21.51 % respectively. The results that could be explained on the bases of how the active ingredient (a.i) reaches its target site in both cases, in case of active ingredient, it was dissolved during the

bioassay experiments in dimethyl sulfoxide (DMSO) which is classified as an organic solvent that facilitates the entering of active ingredient (solubility rule), taking the same factor into consideration in case of the new (SC) formulation, flowables are water based formulations, which means, in contrast to active ingredient, the ability of active ingredient to reach its target site in case of aqueous layer formulation containing active ingredient is difficult with a consequence difficulty to penetrate the external fatty layer of the insect under study, these results were the same as reported by (Hamouda, 2016). Although the efficacy of the new formula was decreased, it is safe and eco-friendly, as it is a water based formula, in addition, it could be possible on testing the active ingredient biologically to evaporate the dissolving solvent after treatment and the insect will uptake the pesticide from the residue already present on the treating surface.

Table (2): Comparison between the efficacy of the newly synthesized arylidene derivative (2a) as an active ingredient and its 10 % SC formulation against cotton leafworm *Spodoptera littoralis* under laboratory conditions.

Parameter / Tested compound	LC_{50} (mg/ml) Its limits at 95%	LC_{90} (mg/ml) Its limits at 95%	Slope	Toxicity index (%)
Active ingredient	0.967 0.303 1.498	8.72 5.05 791.2	1.3417 ±0.3666	100.00
10 % SC Formulation	4.49 3.42 5.97	24.79 14.94 66.91	1.7281± 0.3078	21.51

3. Formulation part:

A flowable or liquid formulation combines many of the characteristics of emulsifiable concentrates (EC) and wettable powders (WP). Manufacturers use these formulations when the active ingredient is a solid that does not dissolve in either water or oil. The active ingredient impregnated on a substance such as clay and ground to a very fine powder. The powder is then suspended in a small amount of liquid. The resulting liquid product is quite thick. Flowables / liquids are easy to handle and apply (Fishel, 2010). The most effective derivative (**2a**) was formulated as 10 % flowable (suspension concentrate, SC) after determining the necessary physico-chemical properties of both active ingredient and surfactant.

3.1. Physico-chemical properties of arylidiene derivative (2a) as an active ingredient:

The newly synthesized arylidiene derivative (2a) showed no solubility in all solvents (aqueous and organic); in addition, it showed an alkaline property appeared from the value of free alkalinity calculated as sodium hydroxide percentage Table (3). These results showed that, it could be formulated as flowable.

Table (3): Physico-chemical properties of arylidene derivative (2a) as an active ingredient.

Solubility % (W/V)					Free alkalinity as % NaOH	Melting point ˚C
Water	Acetone	DMF	Ethanol	Xylene		
N.S*	N.S*	N.S*	N.S*	N.S*	0.005	280

N.S*: means insoluble.

3.2. Physico-chemical properties of surface-active agents:

The physico-chemical properties of the surface-active agents were studied to choose the most compatible surfactant with the properties of the active ingredient to be used in the processes of formulation Table (4). Four surface active agents were tested; Tween 20, span 20, sodium lauryl sulfate (SLS) and

polyethylene glycol 600 dioleate (P.E.G 600 Do.). Sodium lauryl sulfate showed the lowest surface tension (27.8 dyne/cm) followed by P.E.G 600 Do. (35.8 dyne/cm), followed by Tween 20 (50 dyne/cm) and span 20 (58 dyne/cm). Tween 20 and sodium lauryl sulfate showed HLB values greater than 13 while span 20 and P.E.G 600 Do. showed values lower than 13. The tested surfactants showed different CMC values ranging from 8 - 0.01 %. For free acidity or alkalinity; span 20 and sodium lauryl sulfate showed alkaline property while the other two tested surfactants showed acidic property. More than one surfactant could be used for the formulation of this active ingredient as flowable. Experimentation will determine the most appropriate one.

Table (4): Physico-chemical properties of the tested surface-active agents.

Surface active agent	Surface tension dyne/cm	HLB	CMC %	Free acidity as % H_2SO_4	Free alkalinity as % NaOH
Tween 20	50	>13	0.50	0.19	-
Span 20	58	6-8	0.01	-	0.224
Sodium lauryl sulfate	27.8	>13	8	-	0.48
P.E.G 600 Do. *	35.8	8-10	0.9	0.196	-

P.E.G 600 Do. *: poly ethylene glycol 600 dioleate.

3.3. Physico-chemical properties of the local 10 % flowable formulation before and after accelerated storage:

Table (5) showed the physico-chemical properties of the 10 % local prepared flowable formulation under normal and accelerated storage conditions. Under normal conditions, it showed 100 % suspensibility, no foam was formed, free alkalinity as sodium hydroxide (0.04) for all types of water used. Relatively the same results were obtained after accelerated storage as it showed more than 95 % suspensibility in different types of water with no foam formed. Although it showed an alkaline property as before storage, but the value of free alkalinity was increased after accelerated storage. These results showed that the new formula can retain its properties before and after accelerated storage.

Table (5): Physico-chemical properties of the 10 % local prepared flowable formulation before and after accelerated storage conditions.

Type of water	Before storage			After storage		
	Foam	Suspensibility %	Free alkalinity as NaOH	Foam	Suspensibility %	Free alkalinity as NaOH
Hard water	0.00	100.00	0.04	0.00	95.00	0.32
Soft water	0.00	100.00		0.00	99.00	
Tap water	0.00	100.00		0.00	96.80	

3.4. Physico-chemical properties of spray solution at field dilution rate (0.5 %):

Spray solution plays an important role in the determination of the biological efficacy of the newly prepared formula, as their physico-chemical properties are closely related to the expected biological efficiency. The spray solution at the field dilution rate (0.5 %) showed high viscosity (10.24) centipoise, the increase in viscosity causes reduction drift, retention sticking and increased insecticidal efficacy (Spanoghe *et al*., 2007). Also it showed high electrical conductivity (351 µ mhos), (Twifik and

El-Sisi, 1987) reported that increasing electrical conductivity would lead to deionization of insecticide, increase its deposits and penetration in the tested surface with a consequence increase in its insecticidal efficacy. It showed an alkaline PH value, and low surface tension (49 dyne/cm) compared to that of water (72 dyne/cm) The decrease in surface tension can improve wettability and spreading on the treated surface then increase deposit and activity of pesticide (Osipow, 1964) (Table,6).

Table (6): Physico-chemical properties of the spray solution at field dilution rate.

Viscosity centipoise	Electrical conductivity µ mhos	PH	Surface tension dyne/cm
10.24	351	8.79	49

New arylidene derivatives were prepared, their structures were elucidated and it were tested against the 2nd instar larvae of the cotton leafworm *S. littoralis* under laboratory conditions. Compound (**2a**) was the most effective compared to the other prepared compounds. It was then considered as candidate compound and formulated as 10 % flowable. The new formulation passed all reported tests for flowables; it showed good inhibition on using against the 2nd instar larvae of the cotton leafworm, *S. littoralis* under laboratory conditions. It could be used in the control of cotton leafworm *S. littoralis* after completion of the other required studies in the future.

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Physicochemical characteristics of some Egyptian honey from different botanical origins

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Bee honey, physicochemical, botanical origins and Egypt.

Abstract:

Eight of honey types were collected from different apiaries located in Egypt country during seasons of year 2018, depending on floral sources, banana (*Musa* sp.), bardkoush (*Origanum majorana*), camphor (*Cinnamomum camphora*), mesteka (*Pistacia lentiscus*), sidr (*Ziziphus spina-christi*), black seed (*Nigella sativa*), north Egyptian cotton Giza 94, 86 and upper Egyptian cotton Giza 90,95 (*Gossypium barbadense*). Pollen investigate of honey samples showed a wide variability, with samples from different honey sources being collected from geographical origins. The tested parameters viscosity, specific gravity, moisture content, electrical conductivity, total soluble solids, pH, lactone, free acidity, Total acidity, proline content, HMF and sugar (Fructose, glucose, sucrose, and maltose) are useful to determine the botanical origin of Egyptian honeys and their quality. The present study concluded that, the quality and physicochemical properties of honey were varied based on the geographical and botanical origins

Introduction

Determination of the standard criteria of food products is the most important process, since consumption, quality and validity of these products depend on it. Honey is one of the most important global natural products. Honey comes in the first order of these products, since it has many benefits in foods, and medicine (Hassan, 1985). Honey is defined as the natural sweet substance produced by honey bees from the nectar of plants, or from secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and matured (Rodriguez *et al*., 2004). Honey is always a mixture of different sources and no honey is completely the same as another (Oddo and Bogdanov, 2004). Honey contains approximately carbohydrates 80% (glucose 35 %, fructose 40 %, and sucrose 5 %) and water 20 %, serving as an excellent source of energy. In addition, it constitutes more than 200 components, including amino acids, vitamins, minerals, enzymes, organic acids, and phenolic compounds (Rodriguez *et al*., 2004 and Kahraman *et al*., 2010). Pollen investigation is the official method for the botanical origin determination of honey (Noaman *et al*.,

2004). Properties and compositions of bee honey depend on its geographical floral origin, season, environmental factors and treatment of beekeepers (Kasˇkonien *et al*., 2010 and El-Metwally, 2015). The identification of honey plant sources is a subject of a great deal of interest since many years. There are various reasons why the floral origin of honey may be wanted to be known, such as, for quality control in marketing and where there is regulatory concern about the country of origin of honey (Molan, 1998).

This study aims to identify the authenticity and investigating the safety of representing various types of honey products sold in Egypt (24 samples). For this purpose, physicochemical properties, pH, HMF, and pollen test were performed. Sugar composition was also evaluated by means of high-performance liquid chromatography (HPLC) technique. All results were assessed based on Egypt standards, Codex Alimentarius Commission (CAC) (2001).

Materials and methods

1. Honey samples:

Twenty four samples of honey were harvested from apiaries located in different regions of Egypt during seasons of the year depending on floral sources , banana (*Musa* sp.), bardkoush (*Origanum majorana*) , camphor (*Cinnamomum camphora*), mesteka (*Pistacia lentiscus*), sidr (*Zizyphus vulgaris*), black seed (*Nigella sativa*) , North Egyptian cotton Giza 94,86 and upper Egyptian cotton Giza 90,95 (*Gossypium barbadense*) (Table,1). Honeys were collected from different location in Egypt regions were Ismailia, Kafr El-Sheikh, Beni Sweif, El-Minia and Assuit. Honey samples were collected in dark jars kept in freezing conditions until analyses.

Table (1): Types and floral sources of Egyptian honeys.

No. of samples	Local or English name of honey	Floral sources
Sample 1	Banana	*Musa* sp.
Sample 2	Bardkoush	*Origanum majorana*
Sample 3	Camphor	*Cinnamomum camphora*
Sample 4	mesteka,	*Pistacia lentiscus*
Sample 5	Sidr	*Ziziphus spina-christ*
Sample 6	black seed	*Nigella sativa*
Sample 7	north cotton	*Gossypium barbadense* (Giza94,86)
Sample 8	upper cotton	*Gossypium barbadense* (Giza90,95)

2. Physical properties:

2.1. Viscosity of honey was measured according to (Munro, 1943), the **specific gravity** was measured according to Wedmore (1955).

2.2. Determination of Color: The optical density of all the samples was determined and the color was measured by using the relation between optical density and USDA standards, as indicated by White (1978).

2.3. Determination of electrical conductivity (EC): According to the method of Vorwhol (1964).

3.Chemical properties:

3.1. Determination of moisture content: Determination of moisture content of honey was carried out by measurement its refractive index value (Abbe refractometer at 20ºC) **(A.O.A.C, 1995).**

3.2. Determination of total soluble solids (TSS) of honey by (**Association of Official Analytical Chemists** (**A. O. A. C.)**, **1980**). Equipment: Abbe refractometer was used and expressing the T.S.S. in honey in percentage.

3.3. Determination of pH, free acids, lactone content and total acidity

according the method of **White *et al.* (1962)**.

3.4. Determination of Proline content in honey samples. The proline content was measured according to (**Association of Official Analytical Chemists** (**A. O. A. C.)**, 1990).

3.5. Determination the quantity of sugars by High Performance Liquid Chromatography (HPLC). The concentration of fructose, glucose, sucrose and maltose in honey samples were determined by HPLC according to the method of Bogdanov and Baumann (1988).

3.6. Determination of Hydroxymethyl furfural (HMF). Hydroxymethyl furfural (HMF) was determined by using the standard method **Association of Official Analytical Chemists** (**A. O. A. C.) (1990)** Official Method 980.23.

3.7. Determination of pollen sediment content, according to the method of **Louveaux *et al*. (1978).**

Results and Discussion

1. Physical properties of honey:

Data in (Table, 2) showed some physical properties of honeys under investigation. The viscosity of honey types were ranged between 13.60±05 to 69.00±11 poise, there were significant differences among honey types, while no difference was recorded between the honeys of black seed and camphor also between banana and mesteka ,sidr and upper cotton .From the previous results it could be observed that the viscosity value of caplets was near to the maximum range in comparison with the normal values , while the other kinds recorded high values more than normal one , this may be due to the dried and hot atmosphere at the site where the caplets was planted that the high temperature degrees increase the values of this property .As pointed out by (White, 1975) the variations in viscosity of honey types are due primarily to temperature and water content where the values were highly different recording: 2.6, 10.7, 21.4 63.4 189.6 and 600 poise. Thawley (1969) and Crane (1990) related high viscosity of honeybee content of water, and (Pierro, 1994) reported that the viscosity is reduced when the temperature raises to 30°C. Moreover, Abd-EI-Bary and Meshrif (1993) found that the viscosity in clover and cotton honeys were 24.34 and 31.52 poise , respectively , where Meshrif *et al*. (1997) found that the viscosity of clover and cotton and sunflower honeys were 55.56, 63.48 and 116 poise .respectively . Al-Arify (1998) found that viscosity of some Saudi Arabian honeys ranged between 103.86 - 367.71 CP with mean value of 229.88 CP at 40°C.

1.1. Specific gravity in all honey types were nearly equal 1.40 it was ranged between 1.390±05 to1.42±0.36 with no significant differences. Regarding to specific gravity values at all tested honeys Table (2), it was noticed that, these values agreed with the normal degrees and fall within those found by White (1975); ranging between 1.421 to 1.423. Al-Arify (1998) found that specific gravity of Saudi honeys ranged from 1.42 to 1.44 with mean value of 1.432. Also, this result agrees with (El-Sharawi *et al*., 2009) that the specific gravity ranged between 1.39 to 1.42.

1.2. Electrical conductivity (EC):

As show in table 2, EC ranged between 110.0 ±10 to 520.0 ±10 ppm with significant differences among honey samples, while no difference was recorded between the honeys of black seed and bardakosh also between mesteka and sidr honeys ($P < 0.05$). EC is a good criterion of the botanical origin of honey and it is determined in routine honey control instead of the ash content (Adenekan *et al*., 2010). This measurement depends on the ash and acid content of honey, the higher ash and acid content, the higher the resulting conductivity. There is a linear relationship between the ash content

and the EC. As for EC% it could be concluded that all tested honeys agreed with the ideal one. These results were less than Meshrif *et al.* (1997) who found that the electrical conductivity of Egyptian honeys was (0.45, 0.72, 0.87%) for clover, cotton and sunflower, respectively. The high EC values are attributed to high minerals content (Nour, 1988). Laurino and Gelli (2002) found that electrical conductivity of citrus honey was 0.185%. Tharwat and Nafea (2006) recorded that the EC ranged between 0.01 to 0.09 in some Saudi Arabia Honeys.

1.3. Color [Optical density (OD)]:

The color of honey usually ranges from light yellow to amber, dark amber and black in extreme cases and sometimes even green or red hues Bogdanov *et al*. (2008). Data presented in Ttable 2, showed that the color range of the eight Egyptian honeys was from 0.02 ±0.01 to 0.38±0.01 OD, the minimum value was detected in black seed honeys, while the maximum was detected in the banana honeys. There were significant differences among honey types, while no difference was recorded between the honeys of north cotton and upper cotton also between bardakosh and sidr honey.

Changes in color might be attributed to beekeeper's interventions and different ways of handling the combs such as the use of old wax combs for producing honey, minerals content contamination of heavy metals and exposure to either high temperature or light (El-Banby *et al*., 1989; Moniruzzaman *et al.,* 2013 and El-Metwally, 2015). Color classification of monofloral honeys is very important for commercial activities. The pruned value of Saudi and Kashmiri honey is like Gelam and Manuka honeys, which were amber, with pruned values of 122 and 110 respectively Moniruzzaman *et al.* (2013). According to the mentioned measures, it could be concluded that banana honey contains high ash than other honeys.

2.Chemical properties of honey:

2.1. Moisture content:

Data in Table (3), revealed that the moisture percentages of honey samples ranged between 17.25±0.66 to 21.0±1.11%, the lowest percentage was found in camphor honey and black seed honey, while the highest percentage was found in mesteka honey. There were significant differences among honey types, while no difference was recorded between the honeys of sidr, north cotton and upper cotton ($P < 0.05$). The higher the moisture content is the higher probability of honey fermentation during storage (Singh and Bath, 1997). Lower moisture limits (<20%), elongates honey shelf life which would be met by a large majority of the commercial honeys (Terrab *et al*., 2003). These results were accepted by the international regulations for honey quality (Codex Alimentarius Commission (CAC) , 2001) and Council Directive of the European Union, 2001). However, moisture content depends on the temperature and relative humidity in the geographical origin during honey producing in honey colonies (Crane, 1979). Moisture content is an important quality parameter, important above all for honey shelf-life (Bogdanov *et al*., 2008).

These results are in symmetry with the values obtained by Sancho *et al*. (1991) mentioned that the moisture content ranges from 12.4 to 20.3 %, Foldhazi (1994) reported a range of 16.46 to 17.70 %, while Ihtishamulhaq *et al.* (1998) reported higher ranges of 17.6 to 21.83 %. Finally, Al-Arify (1998) found that moisture of Saudi honey ranged from 14 to 16.9 % with mean value 15.26%. El-Sharawi *et al.* (2009) found that the moisture content ranged from 17.5 to 23.0% in honeys

collected from different location in Aswan.

2.2. Total soluble solids (TSS):

Percentage of honey samples ranged between 79.0±0.7 to 87.75±0.92%. It could be noticed that all honey content of TSS located at the normal rate of honeys. In table 2, showed that the lowest percentage of honeys (79.0 %) was found in mesteka honey, while the highest percentage (87.75 %) was found in black seed honey. There were significant differences among honey types. While no difference was recorded between the honeys of sidr, banana and upper cotton (P<0.05) (Table, 3). The TSS which should be 77% or more, is responsible for protecting honey from fermentation. In this respect, these results are in harmony with those obtained by Minh *et al*. (1971) who reported that 79.34 % TSS was recorded in honeys from Philippines. Hussein (1989) mentioned 76.83 % TSS in honey from Oman, and finally, Al-Arify (1998) found that the TSS of Saudi honey ranged from 81.73 to 84.33 % with mean value 83.26%.

As for the values of pH, it could be concluded that all collected honeys recorded pH values ranged between 3.7±0.17 to 4.7±0.26 found within the normal values of honeys (3.42 to 6.1). All tested samples were acidic table 3, and within the standard limit (pH 3.40 to 6.10) (Codex Alimentarius Commission (CAC), 2001) that insures honey samples' freshness. There were no significant differences among all honey types, except for sidr honey recorded highly significant value 4.7 (P <0.05).The pH values of four tested types of honey samples were close to those previously reported in Indian, Algerian, Brazilian, Spanish and Turkish honeys (between pH 3.49 and 4.70) (Azeredo *et al*., 2003; Ouchemoukh *et al*., 2007; Kayacier and Karaman, 2008 and Saxena *et al*., 2010). The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: flavor and stability against microbial spoilage (Bogdanov *et al*., 2008). Furthermore, it might also indicate that the honey samples have high content of minerals (Mohammed and Babiker, 2009 and El-Metwally, 2015).

Acidity in honey is calculated as free acidity, lactonic and total acidity. Specifics a free acidity of not more than 50 meq/1000 g (meq/kg) (European Commission, 2002). Some factors affecting bee honey acidity e.g. harvest seasons and floral types (El-Sherbiny and Rizk, 1979 and Pe´rez-Arqullue *et al*., 1994). The ranged values for free acidity in honey samples between 11.0±1.32 to 68.3±0.85 (meq/kg). There were significant differences among all honey types, except for banana honey recorded highly acidity significant value 68.3 (P =0.000) (Table,3). Lactonic acid ranged from 7.5±0.7 to 17.5±0.70 meq/kg and found highly significant between all samples (P=0.000) (Table, 3). Total acidity detected highly significant between all samples (P=0.000 (Table, 3), it's ranged from 18.51. ±1.05 to 86.0±0.7 meq/kg; The present investigations are quite in agreement with Ouchemoukh *et al*. (2007).

2.3. Sugar (Fructose, glucose, sucrose, maltose) content of collected honey samples indicated that most of tested samples contain ideal values representing normal values of honeys. In addition, it could be observed that all tested samples of fructose sugar were ranged between (38.2±0.66 to 41.2±0.30%) while the normal content is (42.5 to 50.8%). There were significant differences among honey types. And no difference was recorded between the honeys of camphor, banana and upper cotton (P <0.05) (Table,3). Glucose values of all tested honeys

were ranged between (28.0 ±1.23 to 32.0±1.61%), it means that the honey content of glucose is partially like normal ones. There were significant differences among honey types, while no difference was recorded between the honeys of black seed, mesteka and north cotton (P <0.05) (Table, 3).

Regarding to sucrose values of all tested honeys, it was ranged between (1.1±0.09 to 5.1±0.30%), it means that the honey content of sucrose is partially like normal ones. There were significant differences among honey types, while no difference was recorded between the honeys of black seed and north cotton and between camphor, bardakosh and sidr honey (P <0.05) (table3). The international normal established by Codex Alimentarius Commission (CAC) (2001) that a good quality honey should not contain more than 5 % sucrose. The values obtained for sucrose contents of the honey samples were all within the limits of international standards. According to White and Doner (1980), the sucrose level in honey never arrives at zero. The sucrose contents obtained in this realization are within the range of values stated for Argentine and Turkish (Cantarelli *et al*., 2008), Venezuelan (Vit *et al*., 2009), American (White and Doner, 1980), Algerian (Makhloufi *et al*., 2007), Pakistani (Zafar *et al*., 2008) and Spanish (Cavia *et al*., 2006) honeys.

As for, maltose values of all tested honeys, it was ranged between (4.5±0.20 to 10.0±0.62%), it means that the honey contents of maltose sugar are within the normal values. The statistical analyses show significant differences among honey types, while no difference was recorded between the honeys of black seed, mesteka, north cotton and upper cotton and between camphor, bardakosh honey (P <0.05) (Table,3). Comparable results are reported by the previous several studies on different honey types (Buba *et al*., 2013 and El-Metwally, 2015). Fructose/ glucose ratio (F/G) indicates the ability of honey to crystallize. F/G ratio of honey samples were ranged from 1.45 to 1.9 and the glucose/water (G/W) ratio of honey samples were ranged from 1.25 to 1.4(Table, 3). White and Doner (1980) noticed that even though honey has less glucose than fructose, the honey was granulated because glucose less soluble in water than fructose. When the F/G ratio is high, honey remains liquid. Honey crystallization is slower when the F/G ratio is more than 1.3 and it is rapid when the ratio is below 1.0. However, the G/W ratio is considered more suitable than the F/G ratio for the forecast of honey crystallization. It has been stated that when the G/W ratio is <1.3 honey crystallization is very slow or even zero and it is complete and rapid when the ratio is >2.0 (Amir *et al*., 2010). Glucose, which is a major sugar in honey, can spontaneously crystallize from honey solutions in the form of its monohydrate (White and Doner, 1980). This sometimes occurs when the moisture level in honey can drop below a certain level; i.e., when the moisture content is very low.

2.4. Amino acid proline content of honey samples were ranged from 316.67 ±8.01 to 566.7±2.05ppm, the statistical analyses shows significant differences among honey types, while no difference was recorded between the honeys of bardakosh and sidr (P<0.05) (Table,3). From the foregoing findings it could be concluded that proline is the predominant essential amino acid in floral and non-floral honeys, the literature contains variable results regarding the amino acids distribution in multifloral honeys from different geographical areas (White, 1975).

Data in Table (3), indicated to HMF concentrations of the honey samples ranging from 2.0±0.17 to

23.04±0.30 mg/kg Notably all HMF concentrations were within the recommended range set by the Codex Alimentarius Commission (CAC) (2000) at 80 mg/kg. The values are also within the allowed maximum limit of 40 mg/kg, as recommended by the Turkish Alimentarus Codex Commission (2003) for honey samples from tropical countries. The HMF content, which is used as an index of heat treatment of honey, indicated that this honey with highest HMF. The accumulation of HMF was due to processing of honey at high temperature above 75°C or storage above 27°C for months Turkish Alimentarus Codex Commission (2003). Analysis of variance of HMF reveals that there is a significant difference among HMF of different honey (Table,3), except for black see , camphor and bardakosh honey shows no significant differences between them (P <0.05).Overall, the low HMF concentrations of the tested Egyptian honey confirm that these samples are of good quality.

Abselami *et al*. (2018) found that except for lavender honey that contained 56.14 mg/kg of HMF, the HMF concentrations of the remaining honey samples ranging from 3.98 to 38.55 mg/kg. Laurino and Gelli (2000) reported that the values of HMF ranged between 2.0 to 26.0mg/kg. Nour *et al.* (1991) found that HMFvalues ranged between 2.0 to 19.13mg/kg. In freshness honeys. Tharwat and Nafea (2006) found that HMF in Saudi honeys ranged between 0.48 to 21.12 mg/kg.

It is concluded that, the quality and physicochemical properties of honey were varied based on the geographical and botanical origins.

Table (2): Physical properties of different of some Egyptian honey types.

	Honey types							
Properties	**Black seed**	**Camphor**	**Banana**	**Bardkoush**	**Mesteka**	**Sidr**	**Cotton Nourth**	**Cotton Upper**
Viscosity (Poise)	69.0 ±0.11 a	69.0 ±0.05 a	20.0 ±0.10 e	48.1 ±0.05b	13.6 ±0.05 e	34.9 ±0.10 d	36.9 ±0.07 c	34.9 ±0.9 d
Specific gravity	1.42 ±0.36 a	1.42 ±0.53 a	1.4 ±0.53 a	1.4 ±0.30 a	1.39 ±0.50 a	1.41 ±0.56 a	1.41 ±0.50 a	1.41 ±0.89 a
Color	0.02 ±0.01 f	0.19 ±0.01 c	0.38 ±0.01 a	0.16 ±0.03 d	0.23 ±0.01 b	0.16 ±0.017 d	0.12 ±0.01 e	0.13 ±0.03 e
EC %	170.0 ± 5.00 e	200.0 ±21.7 d	520.0 ±10.0 a	170.0 ±10.0 e	380.0 ±10.0 b	470.0 ±5.00 b	110.0 ±10.00 f	260.0 ±0.50 c

Different letters indicate in the row significant difference (P<0.05).

Table (3): Chemical composition of some Egyptian honey types.

Parameters	Black seed (multifloura)	Camphor	Banana	Bardkoush	Mesteka (multifloura)	Sidr	Cotton North	Cotton Upper
Moisture (%)	17.25 ±0.66 cd	17.0 ±0.26 d	20.0 ±1.11 ab	18.5 ±0.70 bc	21.0 ±1.11 a	19.5 ±0.70 b	19.0 ±0.70 b	19.50 ±0.75 b
Tss (%)	87.75 ± 0.92a	83.0 ±0.50 b	80.0 ±1.00c d	81.5 ±0.50 c	79.0 ±0.7 d	80.5 ±1.00 cd	81.0 ±0.89 c	80.5 ± 0.04 cd
pH	4.1 ±0.20 b	4.1 ±0.30 b	3.7 ±0.17 b	3.8 ±0.20 b	4.1 ±0.36 b	4.7 ±0.26 a	3.9 ±0.30 b	4.0 ± 0.3 b
Free acidity	19.0 ±2.00d	11.0 ±1.32 f	68.3 ±0.85 a	21.0 ±2.65 d	33.5 ±101 b	16.0 ±0.70 e	13.5 ±1.32 ef	25.0 ±0.01 c
Lacton (meq/kg)	12.5 ±0.70 c	10.0 ±0.70 d	17.5 ±0.70 a	12.5 ±0.62 c	15.0±0.70 b	7.5 ±0.70 e	10.0 ±0.36 d	12.5 ±8.66 c
Total acidity	31.5 ±0.92 d	21.5 ±1. 05 f	86.0 ±0.70 a	33.5 ±0.92 c	18.5 ±1.05 g	23.5 ±1.05 e	23.5 ±0.53 e	37.5 ±1.0 b
Fractuse	41.2 ±0.30 a	38.5 ±0.40 e	38.2 ±0.66 e	39.9 ±0.44b c	40.5 ±1.05 ab	39.2 ±0.30de	39.5 ±0.40 cd	38.2 ±1.0 e
Glucose	31.00 ±1.67 ab	32.00 ±1.61 a	29.4 ±1.15 bc	28.00 ±1.23 c	31.1 ±0.87 ab	28.4 ±1.08 c	31.5 ±1.15 ab	30.1 ±0.2 abc
Sucrose	4.00 ±0.50 b	5.00 ±0.50 a	1.1 ±0.09 d	5.1 ±0.30 a	1.3 ±0.17 d	5.00 ±0.46 a	3.5 ±0.40 b	2.8± 2.65 c
Maltose	5.00 ±0.62 d	7.4 ±0.46 b	10.00 ±0.62 a	7.2 ±0.36b	4.5 ±0.20 d	6.1 ±0.36 c	4.5 ±0.5 d	0.46 ±0.3 d
Glucose/Water	1.8 ±0.12 ab	1.9 ±0.07 a	1.5 ±0.12 cd	1.5 ±0.07 cd	1.5 ±0.12 cd	1.45 ±0.07 a	1.7 ±0.01 bc	1.5 ±0.62 cd
Fructose/glucose	1.3 ±0.08 abc	1.2 ±0.05 d	1.3 ±0.03 bc	1.4 ±0.05 a	1.3 ±0.07 bc	1.4 ±0.01 ab	1.25 ±0.03 cd	1.3 ±4.41 cd
Proline (ppm)	366.67 +8.01 e	316.67 +8.01 g	450.0 +4.36 c	566.67 +8.01 a	550.0 +4.86 b	566.7 +2.05 a	350.0 +4.36 f	383.33 +0.46 d
HMF (ppm)	7.68 ±0.40 d	7.6 ±0.46 d	13.4 ±0.46c	7.7 ±0.46 d	17.3 ±0.40 a	2.0 ±0.17 f	23.04 ±0.30 a	5.7 ± 0.36 e

Different letters indicate in the row significant difference (P<0.05).

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Occurrence of major mite species and their biocontrol agents on soybean *Glycine max* crop

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Abstract:

This study conducted to incidence and determined the levels of infestation of phytophagous mites and its predators associated with soybean. There were five species belonging to three families were determined in total collected samples can be classified according to their feeding habitats to two major groups *i.e.* phytophagous mites represented by two species, *Tetranychus urticae* Koch and *Oligonychus pratensis* (Banks) (Acari: Tetranychidae) and predaceous mites represented by three species, *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae), *Agistemus exsertus* Gonzalez (Acari: Stigmaeidae) and *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) . Frequency of occurrence for mite species associated with soybean were determined. The most distributed species was *T. urticae* followed by *E. scutalis, A. exsertus, T. pyri* while, *O. pratensis* was the lowest species in occurrence. Their percentages of relative occurrence were 34.92, 25.40, 20.63, 11.91 and 7.14%, respectively. *T. urticae* showed two peaks of abundance. The highest peak was 29.25 individual/leaf appeared in the beginning of August month. There were significant differences in population between the infestation % and damage of *T. urticae* during the growth stages of soybean. The percent of protein contents was high through flowering and reproductive stage compared with vegetation stage. Multiplication of *T. urticae* number on soybean, 10, 20 and 30 mites/leaf in greenhouse during growing season, 35 days old soybean plants infested with spider mites at the rate of 30 mites/leaf produced significantly a greater number of spider mites. the age of the soybean plant had no effect on the predator population.

Introduction

Soybean *Glycine max* L. is considered the most important cash crops in many countries and one of the summer legumes crops, with great nutritive value, containing relatively high percentage of oil and proteins many essential amino acids (Badenhop and Hacker, 1971). It is used for feeding human and animals. It can substitute for meat production pross and for some extent for milk and its remains are used as fertilizer to enrich the soil with the steady of increase in world population, the demand for both Soybean oil and protein is still increasing. Many pests infesting soybean crop specially, piercing sucking insects are of the major

insect pests which attack this crop in the fields causing severe damage (El-Kifil *et al*., 1974; Hamed, 1977; Metwally, 1989; Awadallah *et al*., 1991) and El-Khouly *et al*., 1998). *G. max* is subjected to attack by many pests among of these pests, mites throughout the growing seasons. Other mite species may be predators which play an important role in biological control of certain agricultural pests. For example, species belonging to family phytoseiidae, that release of these mites have been shown experimentally to reduce spider mite densities in many crops (Nyrop *et al.,*1998). There is a relationship between both morphology and occurrence of phytoseiid mites and leaf surface texture in addition to weather factors. *Tetranychus urticae* Koch (Acari: Tetranychidae) is the most injurious phytophagous mite on leguminous plants. It feeds on the plant sap causing serious damage according to the rate of infestation. It is also the most abundant species on leaves of vegetable plants in both open field and plastic houses. The population growth of *T. urticae* densities on bean plant at different ages, was studied by Bustos *et al*. (2009) where, bean plants of four weeks of age were an excellent substrate for the development of *T. urticae* population. The present study was carried out to shed light on the mites associated with *G. max*, the study of population dynamics of these phytophagous and predaceous mites during the growing season of *G. max* in addition to the effect of plant age on population and multiplication of *T. urticae* and its predator, *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae).

Materials and methods

1.Occurrence of mite species associated with soybean crop:

Samples of plant leaves were randomly collected every week from soybean *G. max* during growing season 2018. Samples of crop was obtained from district i.e. Zagazig. The total number of collected samples from crop was 123 samples.

1.1.Samples procedures:

Samples were collected in early morning. Each sample consisted of 25 leaves, collected randomly. All collected samples were kept in paper bags. The necessary information including crops, locality and date of collection were recorded. Samples were directly transferred to the laboratory and examined using stereomicroscope. To compare occurrence of the identified species on each crop, percentage of absolute and relative frequency occurrence were calculated according to **Norton (1978)** as follows:

$$\% \text{ Absolute frequency occurrence} = \frac{\text{Number of samples containing species}}{\text{Number of samples collected}} \times 100$$

$$\% \text{ Relative frequency occurrence} = \frac{\text{Frequency of species}}{\text{Sum of frequency of all species}} \times 100$$

1.2.Mounting and identification of surveyed mites:

Collected mite individuals were cleared in Nesbit's solution and mounted in Hoyer's medium on glass slides for identification with the aid of research microscope. The identification according to Krantz (1970).

2.Population dynamics of mites on soybean crop:

Samples were collected in early morning, each one consisted of 25 leaves collected randomly from infested field; all the collected samples were kept in paper bags. Necessary information including crop locality, date of collection and sowing date were directly transferred to the laboratory and examined using stereo microscope to compare occurrence of the identified mite species on the crop,

this process was repeated every week, the recorded temperature and relative humidity associated with the plant in this day was listed. Sample correlation was calculated according to Little and hills (1978). Growing stages of the crop were divided into two stages, vegetation and flowering and reproductive stages to study the effect of plant age on the population of the two spotted spider mite, *T. urticae* to calculate the damage (number of mite individuals/leaf) and the percent of infestation (number of infested leaves/number of total leaves×100).

3. Multiplication of *Tetranychus urticae* on soybean crop:

Soybeans were planted in greenhouses during season 2018 and were divided into 4 treatments according to their age to 25, 35, 45 and 55 days after planting and each treatment included 60 plants were infected with three levels of infection 10, 20 and 30 individuals by 20 plants / level and was taken 5 leaves weekly randomization of each level within each treatment and then counting the mobile individuals.

4. Chemical analysis of soybean leaves:

Soybean leaves were analyzed and estimated, total carbohydrates %, total k (ppm), total p%, total nitrogen %, and total protein %. The leaves were analyzed at agriculture faculty, Benha university, Egypt.

5. Statistical analysis:

Data were subjected to statistical analysis, Duncan (1955), multiple range test was used to determine the significant of the difference between mean values of the treatments.

Results and discussion

Data in Table (1) and Figure (1) showed that five species belonging to three families were determined in total collected samples can be classified according to their feeding habitats to two major groups i.e. phytophagous mites represented by two species, *T. urticae* and *Oligonychus pratensis* (Banks) (Acari: Tetranychidae), and predaceous mites represented by three species, *E. scutalis*, *Agistemus exsertus* Gonzalez (Acari: Stigmaeidae) and *Typhlodromus pyri* Scheuten (Acari:Phytoseiidae).Frequency of occurrence for mite species associated with soybean were determined. The most distributed species was *T. urticae* followed by *E. scutalis, A. exsertus, T. pyri* while, *O. pratensis* was the lowest species in occurrence. Their percentages of relative occurrence were 34.92, 25.40, 20.63, 11.91 and 7.14%, respectively. Species of mite were present with high, moderate and rare abundance. *T. urticae* and *E. scutalis* were most dominant species. These results are similar with Eleawa (2007) reported that the most frequently species was *T. urticae* followed by the predatory mite *E. scutalis* and *Typhlodromis* sp. on soybean plants.

Table (1): Incidence of frequency occurrence (F.O) for mite associated with soybean crop.

Mites	Absolute F. O.%	Relative F. O.%	Frequency
Phytophagous	-	-	-
Family: Tetranychidae	-	-	-
Tetranychus urticae	71.54	34.92	+++
Oligonychus pratensis	14.63	7.14	+
Predaceous	-	-	-
Family: Phytoseiidae	-	-	-
Euseius scutalis	52.03	25.40	+++
Typhlodromus pyri	24.39	11.91	++
Family:Stigmaeidae	-	-	-
Agistemus exsertus	42.27	20.63	++

+++ Highly (10-15) individuals/leaf ++ Moderate (5-10) individuals/leaf + Rare (>2) individuals/leaf

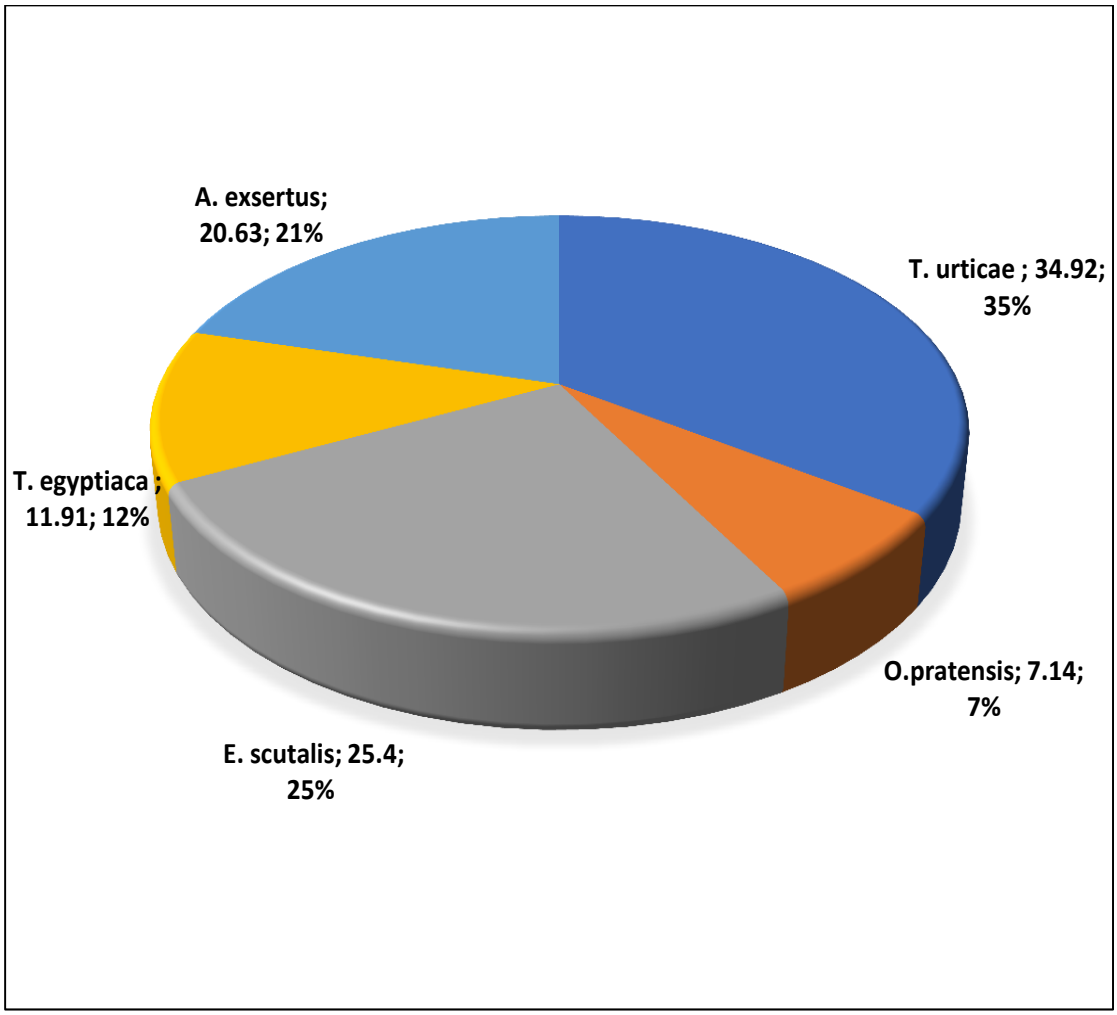

Figure (1): Relative frequency occurrence of mite species associated with soybean crop.

Data in Table (2) illustrated the population dynamics of *T. urticae* on soybean crop during season 2018 weekly the examination was conducted at Zagazig area during fourteen consecutive weeks. Results indicated that *T. urticae* showed two peaks of abundance. The highest peak was 29.25 individual/leaf appeared in the beginning of August month. *O. pratensis* is represented by six peaks the highest peak was 2.53 individual/leaf appeared in 26 May on the other hand in predaceous mite's population fluctuated during the growing season. *E. scutalis* was the most dominant species that appeared with highly population 8 individual/leaf at 11 August while, the population of other predaceous mites appeared with approximate number. The relation between two weather factors temperature (°C) and relative humidity (R53H.) and mite population in Soybean plant indicated significant positive correlation between the population of *T. urticae* and the two weather factors, r=0.72 and 0.39 for temperature and R.H., respectively. while, the correlation was negative between the population of all predaceous mite and R.H. These results are similar with Romeih *et al*. (2013) recorded that the highest peak for adult (26.4, 49.14 and 37.14 individual/leaf for immature and egg, respectively, infestation by *T. urticae* on Faba bean occurred at March.

Table (2): Population dynamics of mites on soybean crop during season 2018.

Date of inspection	Phytophagous mites/leaf		Predaceous mites/leaf		
	Tetranychus urticae	*Oligonychus pratensis*	*Typhlodromus pyri*	*Euseius scutalis*	*Agistemus exsertus*
12/5	0.00	0.23	0.00	0.00	0.00
19/5	2.53	1.50	1.30	0.00	1.50
26/5	7.19	2.53	2.00	0.00	1.00
2/6	10.03	1.37	1.31	1.96	2.30
9/6	15.11	2.04	1.60	2.48	3.20
16/6	12.63	1.56	2.12	3.36	2.24
23/6	5.23	1.80	2.64	4.64	1.60
30/6	5.13	1.32	3.12	5.40	1.28
7/7	9.57	2.24	4.40	6.80	2.80
14/7	14.75	1.16	3.84	4.92	1.96
21/7	18.39	2.16	5.40	4.20	1.80
28/7	21.15	1.68	4.00	5.56	2.80
4/8	29.25	1.20	2.88	7.16	3.32
11/8	21.10	1.96	3.44	8.00	3.96
18/8	13.73	2.44	4.08	7.28	4.20
25/8	5.00	1.52	2.40	3.48	3.20
1/9	2.89	0.88	1.76	2.40	2.88
8/9	2.04	0.76	0.96	1.28	3.20
Mean	10.87	1.57	2.62	3.83	2.40
Correlation coefficient values(r)	**°C**	0.72	0.31	0.66	0.41	0.22
	RH.	0.39	0.45	-0.11	-0.65	-0.28

Data in Table (3) showed that there were significant differences in population between the infestation % and damage of *T. urticae* during the growth stages of Soybean. The infestation% was high 87.27% in flowering and reproductive stage. On the other hand, damage (number of mite/leaf) was high 13.00 individual/leaf in flowering and reproductive stage. These results similar with Anuradha *et al*. (2014) they reported that number of *T. urticae* increased gradually after sowing reached maximum in early growing stage and decreased in delayed growing stage on *Phaseolus vulgaris* L.

Table (3): Damage and infestation % of *Tetranychus urticae* through growing stages of soybean crop during season 2018.

Date of inspection	Growing stage	Infected leaves number/25 leaves	Infection %	Individuals number/leaf (Damage)	Infection %	Damage
12/5	**Vegetative**	00	00	0.00	70.85[b]	7.53[b]
19/5		23	92	2.53		
26/5		20	80	7.19		
2/6		20	80	10.03		
9/6		19	76	15.11		
16/6		18	72	12.63		
23/6		24	96	5.23		
30/6	**Floweringand reproductive**	22	88	5.13	87.27[a]	13.00[a]
7/7		23	92	9.57		
14/7		25	100	14.75		
21/7		21	84	18.39		
28/7		19	76	21.15		
4/8		17	68	29.25		
11/8		23	92	21.10		
18/8		20	80	13.73		
25/8		24	96	5.00		
1/9		23	92	2.89		
8/9		21	84	2.04		

Means in columns followed by the same letter are not significantly different at p≤5% (Duncan's multiple range test, 1955)

Data in Table (4) showed the high content of carbohydrates in flowering and reproductive stage, while it was poor in the vegetation stage. The percent of protein contents was high through flowering and reproductive stage compared with vegetation stage. These results are in harmony with those obtained by Najafabadi *et al*. (2011), they reported that numbers of adult or immature stages of *T. urticae* showed significant variations among nitrogen treatments, where these stages of the mite increased with high nitrogen level and enhanced the mite population on bean leaves in field. These results near similar with Saleh (2017) cleared that the population of *T. urticae* was increased in the vegetation stage of soybean compared with growing stage he reported that protein content was highly in vegetation stage.

Table (4): Chemical composition of soybean crop leaves during plant growing stages.

Growing stage	Total carbohydrates%	Total potassium (K ppm)	Total phosphor P%	Total nitrogen N%	Total protein%
Vegetation	12.02[a]	267.41[b]	0.79[a]	3.59[a]	14.18[b]
Flowering and reproductive	14.17[a]	379.50[a]	0.40[b]	2.09[b]	23.20[a]

Means in columns followed by the same letter are not significantly different at p≤5% (Duncan's multiple range test, 1955).

Results in Table (5) conducted to determine the appropriate crop stage and the optimal number of spider mite to be initially released on soybean crop, at the age of 35 days, the bean infestation reached a maximum 102.2 individuals/leaf after 53 days followed by 92 individuals/leaf at the age of 60 days,

at the level of infection of 30 individuals/leaf. We find that at the age of 25 days for the plant we have another peak as much as 84.6 individuals/leaf after 60 days of planting, while the rest of the treatments appeared in fluctuation numbers during the growing season, which lasted up to 88 days, which started decreasing in individuals in all treatments. The treatment, 55 days was the lowest number of treatments, where, appeared 3.8 individuals/leaf at the level 10 individuals/leaf. These results near similar with Bustos *et al*. (2009) where, bean plants of four weeks of age were an excellent substrate for the development of *T. urticae* population. Generally, 35 days old soybean plants infested with spider mites at the rate of 30 mites/leaf produced significantly a greater number of spider mites. Therefore, aiming to produce a greater number of predator mites.

Table (5): Number of *Tetranychus urticae* on soybean at different crop growth stages and mite infestation densities during 2018.

Treatments		Mean number of spider mites / leaf								
Days after sowing	MD	32 Days	39 Days	46 days	53 days	60 days	67 days	74 days	81 days	88 days
25 days	10	18.40^{d}	22.60^{d}	34.20^{d}	44.20^{e}	30.60^{d}	25.40^{d}	26.80^{c}	20.60^{b}	10.20^{d}
	20	38.00^{bc}	43.40^{bc}	51.20^{c}	63.20^{c}	48.60^{c}	40.40^{c}	32.00^{b}	20.80^{b}	18.60^{c}
	30	42.60^{b}	51.40^{b}	75.00^{a}	78.20^{b}	84.60^{a}	70.00^{a}	31.20^{b}	29.60^{a}	26.20^{b}
35 days	10	26.20^{c}	29.60^{c}	40.20^{d}	52.00^{d}	36.20^{d}	28.80^{d}	30.20^{b}	24.40^{b}	19.60^{c}
	20	44.80^{b}	54.00^{b}	60.60^{b}	69.00^{b}	64.00^{b}	57.40^{b}	41.40^{a}	30.00^{a}	18.00^{c}
	30	60.00^{a}	76.20^{a}	80.80^{a}	102.20^{a}	92.00^{a}	53.40^{b}	40.00^{a}	34.80^{a}	30.60^{a}
45 days	10	14.00^{d}	20.20^{d}	26.00^{e}	34.80^{e}	20.00^{e}	18.40^{e}	14.80^{d}	16.00^{c}	09.20^{d}
	20	25.80^{c}	34.00^{c}	40.20^{d}	50.60^{d}	42.20^{c}	34.00^{c}	32.20^{b}	24.00^{b}	14.40^{c}
	30	38.00^{bc}	42.40^{bc}	52.00^{c}	56.20^{d}	62.20^{b}	63.80^{a}	24.80^{c}	34.00^{a}	36.80^{a}
55 days	10	00.00^{e}	00.00^{f}	08.20^{f}	12.80^{f}	13.40^{f}	10.00^{e}	10.60^{d}	05.20^{d}	03.80^{e}
	20	00.00^{e}	02.60^{e}	03.80^{f}	04.80^{f}	06.20^{f}	12.20^{e}	08.60^{d}	05.40^{d}	04.40^{e}
	30	00.00^{e}	00.00^{f}	00.00^{g}	07.20^{f}	06.60^{f}	11.60^{e}	10.20^{d}	11.20^{c}	09.20^{d}

Means in columns followed by the same letter are not significantly different at p≤5% (Duncan's multiple range test, 1955) MD: Soybean infested with number of spider mites / leaf.

From the predator's results in Table (6), the age of the soybean plant had no effect on the predator population. We noticed fluctuation in predator population during the growing season. However, the predator population was affected by its release rate. Three levels 2, 4 and 6 individuals were released at a fixed prey level of 30 individuals/leaf. The release rate 4 and 6 predators resulted in several individuals similar to both of levels during the growing season that extended to 88 days. The highest number of predators was 14.8 individuals at 60 days at the level of 6 predators followed by 14.4 individuals at 60 days at the level of 4 predators. The population reached 14 individuals appeared at levels 4 and 6 predators, but during different days after planting began 53 days from planting to 74 days after planting. The results obtained shows that the level of 4 and 6 predators gave the highest population during the growing season this is preferable when used and released in mite control at these levels.

Table (6): Number of *Euseius scutalis* on soybean infested with *Tetranychus urticae* at different crop growth stages during 2018.

Treatments		Mean number of spider mites / leaf								
Days after sowing	MD	32 days	39 days	46 days	53 days	60 days	67 days	74 days	81 days	88 days
25 days	2	3.40^{b}	4.60^{b}	08.00^{b}	07.00^{c}	10.00^{b}	06.60^{c}	04.40^{d}	03.20^{c}	1.20^{c}
	4	4.20^{b}	6.00^{a}	09.20^{a}	10.00^{b}	12.40^{a}	08.00^{b}	06.00^{c}	06.20^{b}	3.00^{b}
	6	5.80^{a}	6.80^{a}	10.00^{a}	11.20^{b}	14.80^{a}	10.40^{ab}	07.80^{b}	08.80^{b}	4.00^{a}
35 days	2	3.80^{b}	4.80^{b}	07.00^{b}	08.00^{c}	09.40^{b}	10.60^{ab}	06.40^{c}	03.40^{c}	2.00^{b}
	4	6.80^{a}	8.40^{a}	11.00^{a}	14.00^{a}	14.40^{a}	08.80^{b}	07.00^{b}	05.40^{b}	4.40^{a}
	6	5.40^{a}	6.80^{a}	10.00^{a}	07.60^{c}	09.00^{b}	05.80^{c}	07.40^{b}	03.80^{c}	4.60^{a}
45 days	2	2.80^{c}	5.80^{b}	11.20^{a}	12.00^{ab}	08.60^{c}	08.80^{b}	05.60^{c}	03.80^{c}	4.60^{a}
	4	4.00^{b}	5.20^{b}	09.40^{a}	12.40^{ab}	13.60^{a}	14.00^{a}	08.80^{b}	10.60^{a}	5.20^{a}
	6	5.60^{a}	8.00^{a}	09.20^{a}	09.20^{b}	11.00^{b}	12.60^{a}	14.00^{a}	07.80^{b}	4.80^{a}
55 days	2	1.40^{c}	0.00^{c}	02.60^{c}	02.80^{d}	02.20^{d}	00.00e	00.00^{f}	00.00^{d}	0.00^{d}
	4	0.00^{e}	0.00^{c}	00.00^{d}	03.00^{d}	05.00^{d}	01.60^{d}	01.80^{e}	00.00^{d}	1.00^{c}
	6	0.60^{d}	0.00^{c}	00.00^{d}	00.80^{e}	01.80^{d}	01.00^{d}	00.00^{f}	00.00^{d}	0.00^{d}

Means in columns followed by the same letter are not significantly different at p≤5% (Duncan's multiple range test, 1955) MD: Soybean infested with number of spider mites / leaf

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Evaluation the efficiency of five inorganic salts on the cowpea beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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Abstract:

The experiment was conducted to determine the efficacy of five inorganic salts viz., sodium chloride (NaCl), sodium fluoride (NaF), sodium phosphate (NaH_2PO_4), potassium chloride (KCl) and potassium phosphate (KH_2PO_4) against cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) . The effect of the salts was evaluated based on toxicity, oviposition, adult emergence and percentages of weight loss. After period 96 hrs, sodium fluoride showed the highest efficiency salt (LC_{50} 0.39 and LC_{90} 1.23%), followed by sodium chloride (0.42 and 2.33%) and potassium phosphate (0.43 and 1.39%). Sodium phosphate was the least toxic salt against the adult beetle (0.79 and 4.84%), whereas potassium chloride showed moderate toxicity (0.50 and 2.23%). The mean number of cowpea beetle eggs laid on cowpea seed treated with four concentrations of five inorganic salts, was significantly low as compared with control. Potassium phosphate and sodium fluoride affected oviposition of *C. maculatus* significantly. Results of adult emergence showed the same trend of oviposition, potassium phosphate and sodium fluoride showed high efficiency on adult emergence, sodium phosphate exhibited the lowest effective salt, whereas potassium chloride and sodium chloride were inbetween. Generally, based on direct toxicity, effect on oviposition, adult emergence and loss in seed weight, potassium phosphate and sodium fluoride showed great efficiency against the cowpea beetle, *C. maculatus*. So, it may be recommended to use these salts in control programme of this pest.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is a member of the family Fabaceae. It is a food and animal feed crop grown in the semi-arid tropics covering Africa, Asia, Europe, United States and Central and South America (Asante *et al.*, 2001), and also , a good source of energy (337.57-360.67 kcal / 100g), crude protein (25.79-29.25%), carbohydrate (53.56-57.36%), fat (0.79-3.18%), ash (2.72-3.73%) and crude fiber (1.92-3.37%), as well as small amounts

of essential micronutrients including calcium, iron, magnesium and copper (Lambot, 2002 and Chinma *et al*., 2008). The cowpea beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) has been recognized for years as the major insect pest of cowpea seeds (Ofuya, 2001; Ileke and Bulus, 2012 and Ileke *et al.*, 2013a). Huge losses of between 20 and 50% have been reported on stored cowpea due to the attack by cowpea beetle, *C. maculatus* and sometimes the loss could be complete accounting for 100% loss (Udo and Harry, 2013). Efficient control of stored products insect pests has long been the aim of entomologists throughout the world (Ileke *et al.*, 2013b). The control of stored products insects like *C. maculatus* has centered mainly on the use of synthetic insecticides (Asawalam *et al.*, 2007). However, the use of these chemicals is hampered by many attendant problems such as development of insect resistant strains, their toxic residues getting into food of animals and man, workers safety and high cost of procurement (Sighamony *et al.*, 1990 and Ileke and Oni, 2011). These problems have necessitated researcher to use an alternative eco-friendly cheaper means to control insect pests. It has been known for several years that some insects can be controlled by application of finely powdered substances, which are not chemically active. Desiccant dusts have been used traditionally as stored grain protectants. These dusts primarily exert their effects on insects through physical means. There are several group of desiccant dusts which can differentiated by their chemical composition or by their particle size (Golob, 1997 and Korunic, 1997 and 1998). The inert dusts have been used as a traditional method of insect control for thousands of years. The farmers in the developing countries had been used to mix the sand, wood ash, paddy husks etc in grains as grain protectants against stored grain insect pests. Therefore, based on degree of effectiveness against stored grain insect pests and chemical composition; these compounds can be categorized in to four groups. The first group mainly consists of the minerals such as lime (CaOH), lime stone ($CaCO_3$), salt (NaCl), dolomite, magnesite, copper, and Ketelsous (ground sulpher and rock phosphate). The sand, clay, kaolin (kaolinite, aluminum silicate hydroxide), paddy husks, wood ash and volcanic ash constitute the other group. The third group contains synthetic silica and the fourth one is of diatomaceous earth (Golob, 1997). Therefore, the objective of this study is to evaluate the efficiency of some inorganic salts on cowpea beetle.

Materials and methods

1. Rearing technique:

The beetles used in the present study were obtained from naturally infested cowpea seeds. Adult's *C. maculatus* were cultured in incubator at constant temperature of 27 ± 2°C and 70 ± 5% RH. in the Laboratory of the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Assiut Governorate. Uninfested cowpea seeds (*V. unguiculata*) were used for the experiment, purchased from a local market in Assuit city and disinfested in an oven at 60°C for 1 hour before using them as a substrate for insect rearing. Stock culture was set up by 50 pairs of *C. maculatus* introduced into the rearing bottles containing 250g seeds. The bottles were covered with muslin cloth and secured with rubber bands. The parent beetles were sieved out after 7 days of oviposition period. Later the seeds were kept in the incubator for adult emergence,

which used for the experiment (Suleiman *et al.*, 2014).

2. Inorganic salts:

Inorganic salts used in the experiments were, sodium chloride (NaCl), sodium fluoride (NaF), sodium phosphate (NaH_2PO_4), potassium chloride (KCl) and potassium phosphate (KH_2PO_4). Each material was finely grounded in a porcelain mortar and passed through 0.1mm sieve. Four concentrations of each material (0.5, 1.0, 1.5 and 2.0%), were used.

3. Experimental set up:

To determine the effect of the experimental materials against *C. maculatus*. Quantities of 100g cowpea seeds were placed in glass jars 250 ml capacity and treated with an appropriate concentration of the experimental materials. The jars were shacked manually for a suitable time to ensure even coating of seeds, and then infested with 10 pairs of 1-2 day-old adult cowpea beetles *C. maculatus* for each jar. Each jar was covered with muslin cloth. Untreated cowpea seeds (control) were used as previously described. Three replicates were made for each treatment. Experiments were carried out under laboratory conditions.

3.1. For toxicity test:

In each treatment, observations were made and recorded for toxicity effect on mortality rates in all the jars after 24, 48, 72 and 96 hrs. The content of each jar was spread out in a try and the dead insects were removed and counted. After each count, the seeds and the alive insects were returned into the glass jars. The mortality in the control was also calculated. Percentage of mortality was corrected according to Abbott's formula (1925).

$$\%\ \text{Corrected mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortaliy}}{100 - \%\ \text{control mortality}} \times 100$$

3.2. Effect on the oviposition and progeny emergence:

After 14 days of infestation with the cowpea beetles, three replicates of random samples each containing 20 seeds of cowpea were removed from each jar and the number of eggs oviposited on them were counted and returned into the glass jars until progeny emergence. Progeny emergence in each replicate was taken for the first generation. At each time of observation, the newly emerged progenies were sieved out, counted and recorded.

3.3. Weight loss:

After 4 weeks of treatment, the weight loss of the seeds was evaluated by weighing the entire cowpea seeds in each jar and the difference from the initial weight of 100g was transformed into weight loss. According to the method of Mebarkia *et al.* (2010) used to calculate the loss in seeds:

$$WL = Wh - Wd$$

Where, WL = Weight loss.

Wh = Weight healthy seeds before infestation.

Wd = Weight damaged seeds after infestation.

4. Statistical analysis:

Data were analyzed using a one-way analysis of variance by MSTAT-C (1988) software package and means were separated using the least significant differences method only when a significant "F" test was obtained. Probit analysis was done to reckon either LC_{50} and LC_{90}, and confidence limits using SPSS V. 10 system software (SPSS Inc., 1999) and probit-line graphs were illustrated using Sigmaplot V. 8.02 Demo system software (SPSS Inc., 2002).

Results and discussion

1. Direct toxicity:

The LC_{50}, LC_{90} and their confidence limits, and slope value of LCP

lines of five inorganic salts tested against *C. maculatus* for 96 hrs are shown in Figures (1-4).

After 24 hrs exposure (Figure,1) the least LC_{50} value was recorded for potassium phosphate (1.69%), whereas the values for the rest of inorganic salts were comparable. For LC_{90}, the sodium fluoride and chloride showed the least values (4.27 and 5.60%) but for the rest of inorganic salts, the values were comparable. According to the LC_{50} and LC_{90} values, sodium fluoride and chloride, and potassium phosphate showed relatively high efficiency against *C. maculatus* adults. Comparing the slope values, adult cowpea beetle showed relative high homogeneity response to sodium phosphate (3.89), sodium chloride (3.55) and potassium chloride (3.26). Data of 48 hrs exposure showed the same trend of 24 hrs results, but all values of LC_{50} and LC_{90} were less. Sodium fluoride and potassium phosphate showed the highest effective inorganic salts (LC_{50} 0.68 and 0.79%, and LC_{90} 3.14 and 3.41%, respectively). Sodium phosphate was the least effective one, LC_{50} and LC_{90} values were 1.78 and 5.78%. However, sodium chloride and potassium chloride showed moderate efficiency, the LC_{50} value was 1.45 and 1.47% and the LC_{90} value was 5.06 and 4.37%, respectively. Comparing slope values of LCP lines, cowpea adult showed relative homogeneity response to sodium phosphate (2.51) and sodium chloride (2.18) (Figure, 2).

The values of LC_{50} and LC_{90} of the organic salts after 72 hrs exposure, were less than that at 24 and 48 hrs exposure. Sodium fluoride exhibited the highest effective salt against cowpea beetle adult (LC_{50} 0.44 and LC_{90} 1.42%), followed by potassium phosphate (LC_{50} 0.53 and LC_{90} 1.97%), then sodium chloride (LC_{50} 0.59 and LC_{90} 3.87%), whereas sodium phosphate was the least effective salt (LC_{50} 1.32 and LC_{90} 5.35%). According to the slope values of LCP lines, adult beetle showed relative high homogeneity response to sodium fluoride (2.57) and potassium phosphate (2.25), the least homogeneity response was met with sodium chloride (1.58). However, potassium chloride and sodium phosphate were of moderate responses (2.09 and 2.11) (Figure, 3).

Data (Figure, 4) show the LC_{50} and LC_{90} values, and their confidence limits, slope value of LCP lines of five organic salts applied on cowpea beetle adult after 96 hrs. As in the previous exposure period, sodium fluoride showed the highest efficiency salt (LC_{50} 0.39 and LC_{90} 1.23%), followed by sodium chloride (0.42 and 2.33%) and potassium phosphate (0.43 and 1.39%). Sodium phosphate was the least active salt against the adult beetle (0.79 and 4.84%), whereas potassium chloride showed moderate toxicity against the adult beetle (0.50 and 2.23%). The cowpea adult beetle showed relatively high homogeneity response to sodium fluoride and potassium phosphate (slope value 2.57 and 2.52), whereas, the least homogeneity response was met with sodium phosphate (1.63) and sodium chloride (1.72).

2. Oviposition:

Data Table (1) represent the mean number of cowpea beetle eggs laid on cowpea seed treated with four concentrations of five inorganic salts, compared with control. Statistical analysis showed significant variation between treatments at all concentrations tested. Potassium phosphate showed the highest effective salt in reducing eggs laid, the mean number of eggs deposited on cowpea seeds at concentration 0.5%

was 5.66 eggs/20 seeds, decreased gradually as the concentration increased to attain 1.33 eggs at 2.0%. Sodium fluoride ranked second after potassium phosphate, the mean number of eggs laid cowpea seeds at 0.5% was 12.33 eggs decreased to attain 5.00 eggs at 2.0%. The lowest effective salt was sodium phosphate, at concentration of 0.5% the mean number of eggs laid on cowpea seeds was 27.67 eggs, decreased gradually by increasing concentration to attain 10.33 eggs at concentration 2.0%. Sodium chloride and potassium chloride showed moderate effect on ovipositon of *C. maculatus*. At 0.5% concentration, the mean number of eggs laid on cowpea seeds was 26.33 and 21.00 eggs, declined by increasing concentration to attain 2.66 and 9.33 eggs at 2.0%. The mean number of eggs laid on untreated cowpea seeds was 37.67 eggs.

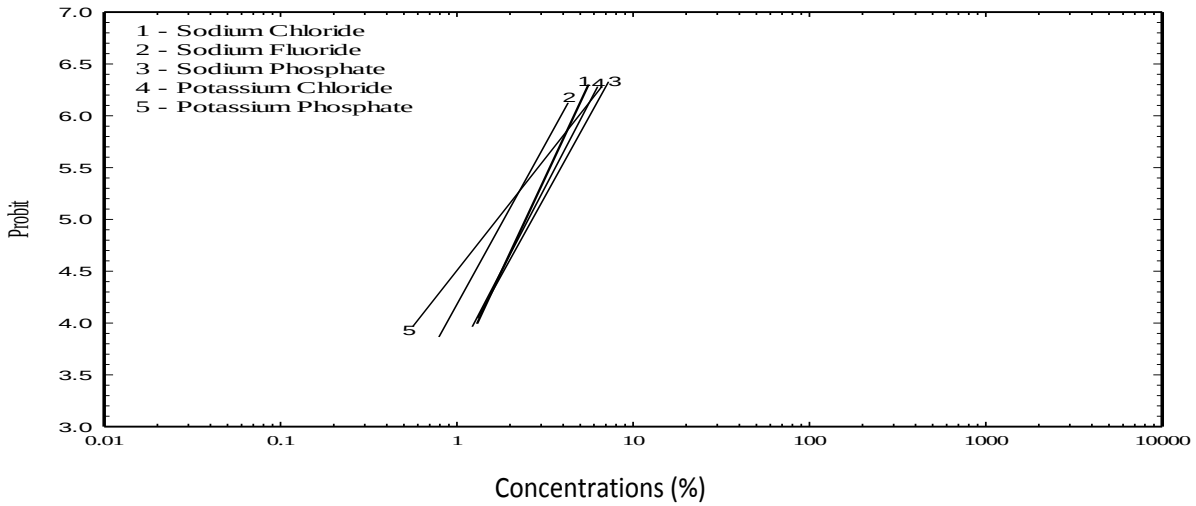

Figure (1): LCP lines of five-inorganic salts tested against C. maculatus at 24 hours

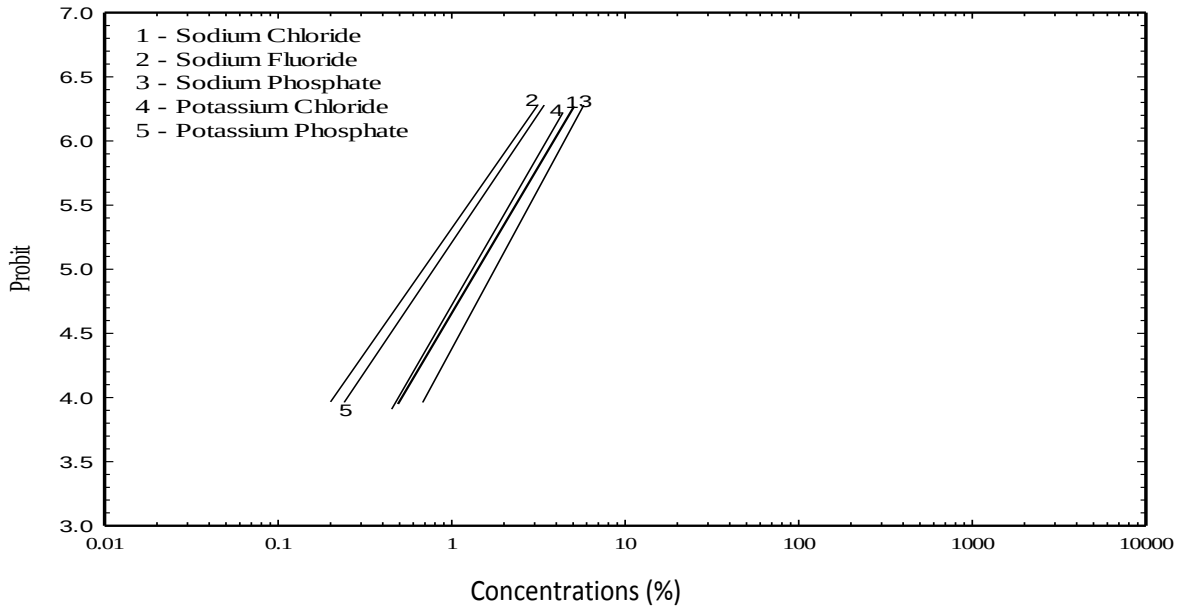

Figure (2): LCP lines of five-inorganic salts tested against *C. maculatus* at 48 hours.

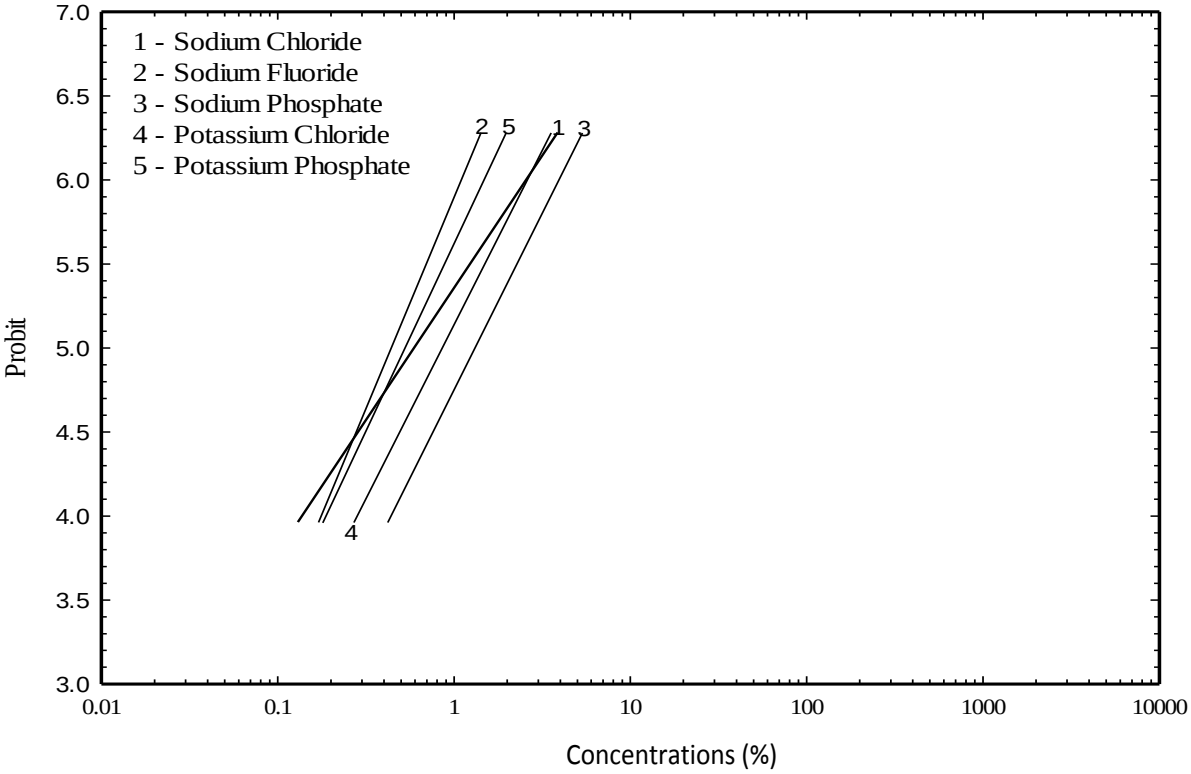

Figure (3): LCP lines of five-inorganic salts tested against *C. maculatus* at 72 hours.

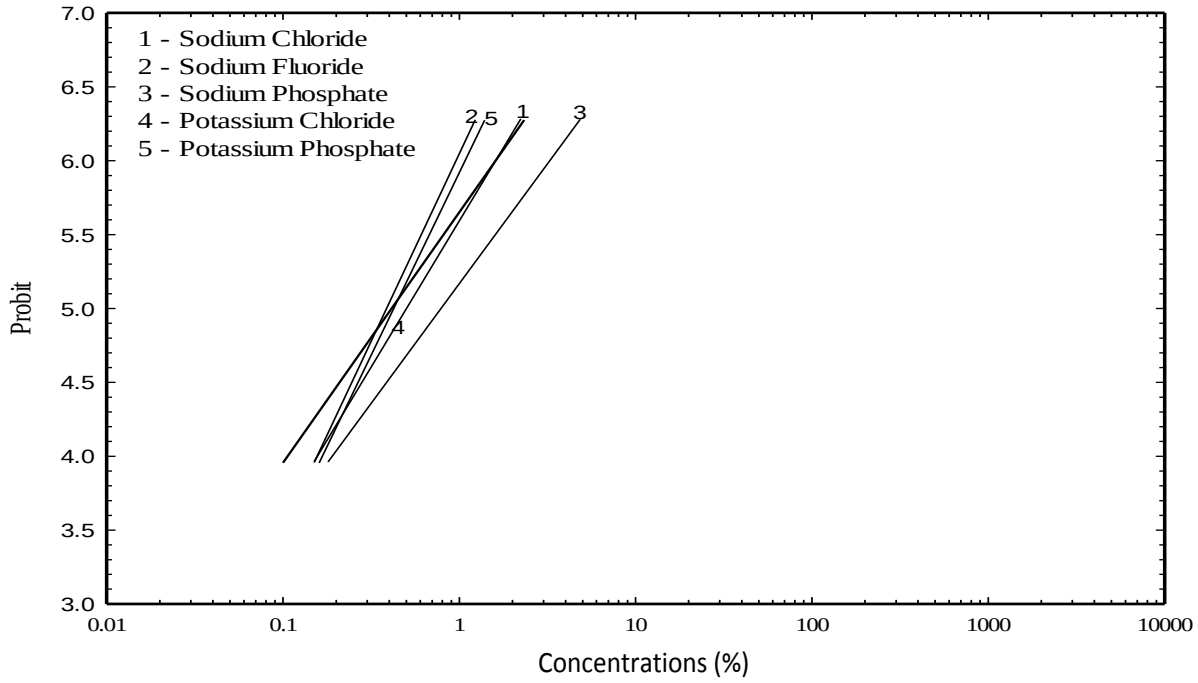

Figure (4): LCP lines of five-inorganic salts tested against *C. maculatus* at 96 hours.

3. Adult emergence:

Data Table (2) show the mean number of cowpea adults emerged from cowpea seeds treated with five inorganic salts compared with untreated seeds. In control, the mean number of cowpea adult emerged was 103.00 adult. Statistical analysis showed significant variation in adult emergence between treatments, and between concentrations. Regardless of concentrations applied, potassium phosphate and sodium fluoride exhibited the highest effective salts on adult emergence. At 0.5%, the mean number of adult emerged from cowpea seeds was 6.00 and 6.33 for the two salts respectively. The adult emergence decreased by increasing concentrations, no adult emerged at 1.5% of potassium phosphate, and at 2.0% sodium fluoride. The least efficient salt on adult emergence was sodium phosphate, at 0.5%, the mean number of cowpea adult emerged was 18.66, decreased gradually as concentration increased to attain 3.33 at 2.0%. However, potassium chloride and sodium chloride showed moderate efficiency against adult emergence, at 0.5% the mean number of adult emerged was 10.00 and 12.33, respectively, decreased gradually by increasing concentration to attain 1.66 and 0.00 at 2.0%.

4. Loss in seed weight:

Data Table (3) show the mean of loss in cowpea seed weight when treated with five inorganic salts at four concentrations compared with untreated seed. Results showed that, the mean of loss in seed weight in untreated seed was 4.84 g/100g seeds. Statistical analysis showed that treatment with inorganic salts significantly affect the seed weight loss. Potassium phosphate significantly reduced the seed weight loss, at 0.5% the mean of loss was 0.21g, declined to 0.10g at 1.0%, and no loss in seed weight was recorded at 1.5%. Sodium fluoride ranked second in reducing the mean of loss in seed weight at 0.5%, the average loss in seed weight was 0.39g decreased gradually by increasing concentration to attain 0.00g loss at 2.0%. Sodium phosphate showed the least salt in reducing seed weight loss, at 0.5% the loss was 1.15g to decrease gradually as concentrations increased to attain 0.09g at 2.0% concentration. However, potassium chloride and sodium chloride exhibited moderate effect on loss of cowpea seed weight. At concentration of 0.5% the two salts reduced the loss in seed weight by 0.62 and 0.91g. Whereas, at the highest concentration tested 2.0%, no loss in seed weight was detected for the two salts.

Table (1): Oviposition of *C. maculatus* on cowpea seeds treated with different concentrations of inorganic salts.

Concentration (%)	Mean no. of eggs /20 seeds ± SE				
	Inorganic salts				
	Sodium			Potassium	
	Chloride	Fluoride	Phosphate	Chloride	Phosphate
0.5	26.33 ± 0.88 A b	12.33 ± 0.66 C b	27.67 ± 1.20 A b	21.00 ± 1.15 B b	5.66 ± 1.20 D b
1.0	19.33 ± 1.45 B c	9.33 ± 0.66 C bc	24.33 ± 0.88 A bc	19.67 ± 0.88 B b	3.00 ± 0.57 D bc
1.5	10.33 ± 2.03 C d	6.66 ± 0.33 D cd	20.67 ± 0.33 A c	14.00 ± 0.57 B c	2.66 ± 0.66 E bc
2.0	2.66 ± 1.20 BC e	5.00 ± 0.57 B d	10.33 ± 1.45 A d	9.33 ± 1.20 A d	1.33 ± 0.88 C c
Control	37.67 ± 2.18 a	37.67 ± 2.18 a	37.67 ± 2.18 a	37.67 ± 2.18 a	37.67 ± 2.18 a

Means, in the same column / row, followed by the same letter are not significantly different at 0.05 level of probability.

- Small letter for concentrations (columns)
- Caps letter for treatments (rows)

Table (2):Adult emergence of *C. maculatus* from cowpea seeds treated with different concentrations of inorganic salts.

Concentration (%)	Mean no. of adult emerged in F_1 ± SE				
	Inorganic salts				
	Sodium			Potassium	
	Chloride	Fluoride	Phosphate	Chloride	Phosphate
0.5	12.33 ± 1.20 B b	6.33 ± 0.88 C b	18.66 ± 1.45 A b	10.00 ± 0.57 B b	6.00 ± 0.57 C b
1.0	7.00 ± 0.57 B bc	4.00 ± 0.57 CD bc	11.33 ± 0.88 A c	6.33 ± 1.45 BC bc	2.66 ± 0.33 D c
1.5	4.66 ± 0.88 AB cd	0.33 ± 0.33 C c	6.66 ± 1.20 A cd	3.00 ± 1.00 B c	0.00 ± 0.00 C c
2.0	0.00 ± 0.00 C d	0.00 ± 0.00 C c	3.33 ± 0.33 A d	1.66 ± 0.33 B c	0.00 ± 0.00 C c
Control	103.00 ± 4.04 a	103.00 ± 4.04 a	103.00 ± 4.04 a	103.00 ± 4.04 a	103.00 ± 4.04 a

Means, in the same column / row, followed by the same letter are not significantly different at 0.05 level of probability.

- Small letter for concentrations (columns)
- Caps letter for treatments (rows)

Table (3): Loss of seed weight cowpea treated with different concentrations of inorganic salts due to *C. maculatus*.

Concentration (%)	Mean (g/100g seeds) ± SE				
	Inorganic salts				
	Sodium			Potassium	
	Chloride	Fluoride	Phosphate	Chloride	Phosphate
0.5	0.91 ± 0.06 B b	0.39 ± 0.06 CD b	1.15 ± 0.07 A b	0.62 ± 0.07 C b	0.21 ± 0.03 D b
1.0	0.36 ± 0.03 B c	0.20 ± 0.02 C c	0.80 ± 0.03 A c	0.44 ± 0.05 B c	0.10 ± 0.02 C c
1.5	0.17 ± 0.05 B d	0.07 ± 0.01 BC d	0.51 ± 0.05 A d	0.11 ± 0.02 BC d	0.00 ± 0.00 C d
2.0	0.00 ± 0.00 A e	0.00 ± 0.00 A d	0.09 ± 0.06 A e	0.00 ± 0.00 A e	0.00 ± 0.00 A d
Control	4.84 ± 0.10 a	4.84 ± 0.10 a	4.84 ± 0.10 a	4.84 ± 0.10 a	4.84 ± 0.10 a

Means, in the same column / row, followed by the same letter are not significantly different at 0.05 level of probability.

- Small letter for concentrations (columns)
- Caps letter for treatments (rows)

The toxic effects of calcium phosphate have been reported by Majumder and Bano (1964). The use of mineral salts offered a new promising method of insect control (Pratt *et al*., 1972). The salt tri-calcium phosphate ($Ca_3 PO_4)_2$ or TCP) has shown promise as insect population suppressant when added to blended cereal foods. In Egypt, El-Halfawy (1977) showed that hydrated lime had the greatest inhibitory effect on the bruchid. He tested the mixing dusts of twelve inert materials with cowpea (*V. unguiculata*) at a concentration of 1.0% on the adult life-span and productivity of *Callosobruchus chinensis* L. Ignatowicz and Boczek (1978) found that iodine salts induced sterility in *Tyrophagus putrescentiae* (Schra.) by reduction of egg production rather than by reduction of their hatchability. They added that females were more susceptible than males because iodine salts exerted detrimental effect upon formation of eggs. Hassan (1981) found that NH_4NO_4 and $Ca_3 (PO_4)^2$ caused the strongest inhibition of *Batrachoides surinamensis* (Bloh and Schneider) development while KH_2PO_4 and $(NH_4)3PO_4.3H_2O$ proved to be the most stimulatory salts for this species. Davis *et al*. (1984) reported on tri-calcium phosphate (TCP) as a legume grain protectant against three bean weevils where mortality was recorded as occurring within 8 hrs. TCP at 0.1 and 0.25% by weight dusted on navy bans or cowpeas as a protectant prevented the occurrence of a F_1 generation. Research on inorganic salts as grain protectants has been conducted at the USDA-ARS (Highland *et al*., 1984 and Bookwalter *et al*., 1985). Le-Patourel (1986) assessed the toxicity of a sorptive silica dust in samples of wheat to adult populations of *S. granarius*, *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.) at grain moisture contents between 9.4 and 18.7%. The tolerance of these species to the dust treatments was found to increase with increasing moisture content and to be unrelated to their relative abilities to survive short periods at low relative humidites when provided with food. The amorphous silica dust

(Dryacide) was used by Aldryhim (1990) to treat wheat grains at concentrations of 0.0, 250, 500, 750, and 1000 ug silica dust /kg wheat. Adults of *S. granarius* and *Tribolium confusum* Duv. were placed in the grains which were then incubated at 20 or 30°C and 40 or 60% R.H. Mortality counts were taken after 48 and 168 hrs. *S. granarius* was more susceptible to silica dust than *T. confusum* under the same conditions. Silica dust reduced progeny by 100% at 40% R.H. at all used concentrations. Progeny were produced by *S. granarius* at 30°C and 60% R.H. but with significantly reduced numbers by increasing dosage. In recent study Mahmoud (2012) found that silica dust and tri-calcium phosphate had high repellent effect on the granary weevil, *Sitophilus granarius* L., at concentration of 5.0 g/kg grains.

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Thermal storage effect on some of non fumigant nematicides

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Abstract:

The effect of thermal storage on the degradation of fenamiphos ,oxamyl and ethoprophos under trade names nematop 40% EC, hidet star 24% SL and nemavet 20% EC, respectively, were studied .The results showed that the difference of the degradation rate for fenamiphos ,oxamyl and ethoprophos active ingredients in its formulation during storage stability test ,where the calculated half-life T 0.5 were 615.6, 744.6 and 350.5 dayes, respectively. It meanes that velocity decomposition can be arranged as ethoprophos > fenamiphos> oxamyl . Also, N. nitroseamine (impurity) in oxamyl was not detected during 30 days at 54±2°C. On the other, gas chromatography/mass spectrometry (GC-MS) was used to compare the fragmentation of three nematicides formulation (fenamiphos ,oxamyl and ethoprophos) and resultes showed that breakdown of phenol-3-methyl-4-methylthio (fenamiphos phenol) and methyl (2-dimethyl amino)-N-hydroxy-2-oxoethanimidothioate (oximino oxamyl)as main equivalent product for fenamiphos and oxamyl, respectively. While ethoprophos less stability than fenamiphos and oxamyl.

Introduction

Nematicides is the type of chemical pesticides used to kill nematodes,which are parasitic worms that feed on living material.They can often be harmful to plant growth and health as attack and feed on plant roots Deborah (2001), Lambert and bekal (2002) ,where numbers of eel worm get too high in the soil ,farmers sometimes apply chemicals called fumigant or non fumigant (organophosphate and carbamate) nematicides to the soil control them. However these chemicals are generally very toxic and hazardous to the health of human and environment. Some nematicides were found to easily leach through the soil and contaminate drinking water in aquifers Barbercheck (2011). Nematicides like aldicarb, caudusfos, fenamiphos and oxamyl one of the most synthetic non fumigant nematicides in the global market .Oxamyl is lipohobic are less adsorbed than more lipophilic compound such as fenamiphos or ethoprophos ,and the former are more effective in awide rang of soil types,

organic contents moisture, soil PH and some environmental parameters such humidity,temperature,sunlight Hugo et al.(2014) ,Jones and Norris (1998). The aim of this investigation is to study the effect of storage stability tests on the degradation of fenamiphos ,ethoprophos and oxamyl. Also, identification of test nematicides by gas chromatography/mass spectrometry (GC/MS).

Materaials and methodes

1.Nematicides used:

Table (1): Name of nematicide, trade name, IUPAC name and molecular formula and mass.

Name	Trade name	IUPAC name	Molecular formula and mass
Oxamyl 24% SL	Hidet star (Carb)	Methyl-2-(dimethylamino)N-(methyl carbamyl) oxy). 2-Oxo ethanimidothioate.	$C_7H_{13}N_3O_3S$ 219.26 g/mol
Fenamiphos 40%Ec	Nematop (OP)	Ethyl 3-Methyl-4(methyl sulfenyl)phenyl isopropyl phosphoramidate	$C_{13}H_{22}NO_3PS$ 303.16g/mol
Ethoprophos 20% Ec	Nemavet (op)	O-ethyl S,S-dipropyl phosphorodithioate	$C8H_{19}O_2PS_2$ 242.3g/mol

SL. Solution stability EC: emulsion concontrate Carb: carbamate Op: organophosphate

2. Storage stability test:

The tested samples were stored in oven at 54±2°C for 30 days .During storage period samples were taken at 0,3,7,14,21 and 30 days to determine the active ingredients for the above nematicides .Also ,determine impurity (N-nitrosamine) in oxamyl during storage according to **FAO (2008).**

3. Preparation of sample:

3.1. Standard preparation:

Ten mg of analytical standard from tested nematicides were weighted inside a25 ml volumetric flask then dissolved and completed to the final volume with methanol .

3.2. Sample preparation for tested nematicides :

Accurately weighed sufficient samples formulation to aquivalent 10 mg of standard in different 25 ml volumetric flask for each samples , and weight 1 gm of oxamyl (impurity) was mixed in 25 ml of methanol :

4. Determination of oxamyl , fenamiphos and ethoprophos by GLC instrument :

Hewlett- Packard GC (Modal 6890 instrument, equipped with Flame ionization detector (FID) ,capillary column 15m ,0.55mm.Nitrogen was used as carrier gas at flow rate 40 ml/min .Injector temperature 250°C,Detector temperature 300°C and Oven temperature 200°C, 200°C and 100°C ,respectively Mann (1981). At this conditions the retention times (Rt) of fenamiphos ,ethoprophos and oxamyl were 3.07 ,2.455 and 3.13 minutes ,respectively .The results of the above samples were quantitatively determined by compression with the standards of known purity under the identical GLC conditions .

5.Determination of N. nitrosamines (impurity) in oxamyl by HPLC:

An equipment HPLC (Agilent 1200 series) was used DAD detector .The wave length detector at 197 nm A C18 column was used and the flows rate 1.3 ml/ min. The mobil phase were acetonitrile : methanol (90:10w/v) .At these conditions the retention time (Rt) of N.nitroseamines was illustrated by Gamon *et al.* (1998).

6. GC-Chromtography-Mass spectrometry analysis of the some nematicides before and after storage :

Apparatures Agilent 7980 B, 5977 A MSD gas chromatography equipped with an agilent mass spectrometric detector ,with adirect capillary interface and fused silica capillary column (30mX 0.025 mm) .HP-5-0.25 microm -60 to 325°C was used .Samples were injected under the following conditions .Helium was used as carrier gas approximately 1ml/ min ,pulsed split mode ,splitle ratio (10:1) , split flow 10 ml/ min. The solvent delay was 4 min and the injection size was 1µL,Oven temperature program .50°C for 0.5 min, then 10°C / min ramp to 190°C followed by a 10°C min ramp to 210°C for 1 min followed by a 10°C / min ramp to 300°C and held for 2 min (total run time :29.5 min), the injector temperature was set at 280°C. Wily spectral data base was used in the identification of separated peaks.

7. Kinetic study :

The rate of degradtion of the tested active ingredients and half –Life T0.5 for the nematicides were calculation according to equation Moye *et al.* (1987).

T0.5 =Ln2/K

K=1/Tx LnA/ Bx

k. rate of decomposition

A: initial residue

Tx: time in day

Bx :residue at time

Resultes and discussion

1. Influnce of storage stability tests on nematicides:

The storage stability test in one of the most important tests which gave attenation on the importance of good storage conditions for pesticides, where temperature is known to be one of the most important factors influencing the stability ,persistence and degradation pesticides (Susana and Pieter , 2016).

Data present in Table (2) showed that active ingredient in tested nematicides were affected by storage conditions and periods exposure .The loss percentage of fenamiphos , Ethoprophos and Oxamyl were 2.15,12.2 and 1.87% ,respectively after 30 days of storage at 54±2°C. Decomposition of these materials can be calculating follows first order reaction. However, the half. Lives $T_{0.5}$ of these materials of Fenamiphos .Ethoprophos and Oxamyl were 615.6 ,350.5 and 744.6 days , respectively , it means that velocity decomposition can be arranged as Ethoprophos more Fenamiphos more oxamyl .The results in line with Dijksterhuis (1996) and Singh *et al.* (2005) , the degradation of oxamyl and aldicarb were still accelerated 5 years after last application ,while Ethoprophos have low environmental persistence but high toxicity. Genarally ,duration times for carbamate insecticides are usually longer than for organophosphorus insicides according to Smelt *et al.* (1996) and Hay dock *et al.* (2012).

2. Influnce of storage at 54 C on N.nitrosamines (impurity) of oxamyl :

N.nitrosamines are chemical compound of the chemical structure R_1N-(R_2)-N=O ,where R alkyl or aryl group , it is chemical are used in manufacture of pesticides like oxamyl. Most N.nitrosamines are carcinogenic is especially toxic to human either ingested ,inhalet or contact with skain , it over use can also lead to residue accumulation in food (Rostkowska *et al.* ,1998 and and Park *et al.* , 2015) .It is found that N.nitrosanines was not detected during storage for 30 days at 54±2°C.According to FAO specification (2008) which reported that maximum content of N. nitrosamines was 0.1 mg/kg of oxamyl .

Table(2) :Effect of storage at 54±2°C on the stability of some nematicides.

Storage periods (days)	Fenamiphos 40%EC	Loss%	Ethoprophos 20%Ec	Loss%	Oxamyl 24%Sl	Loss%
0	39.92	0.00	19.87	0.00	23.98	0.00
3	39.77	0.38	19.81	0.301	23.81	0.71
7	39.39	1.33	19.71	0.81	23.69	1.21
14	39.19	1.83	18.66	6.09	23.52	1.91
21	39.12	2.004	18.04	9.21	23.56	1.75
30	39.06	2.15	17.44	12.2	23.54	1.87
T 0.5	615.6		350.5		744.6	

0. Initial time one houre befor storage $T_{0.5}$ half. life

3.Identification of fenamiphos by chemical ionization GC/MS spectroscopy:

Fragmentation hypothesized to rationalize through :

Figure (1) described two possible reaction pathways leading to the degradation of fenamiphos M.F C_{13} H_{22} O_3 NPS m/z 303.4.

3.1. First cleavage phosphate ester bond and dealkylation by hydrolysis.

Results in the formation of M.F C_{12} $H_{19}O_3NPS$ m/z 288 by loss CH_3, M.F $C_{10}H_{13}O_3NPS$ m/z 260 by loss C_3H_7 and ,then loss of $C_3H_{10}N$ to $C_{10}H_{12}O_3PS$ m/z 243.03 , loss of $C_5H_{12}N$ to $C_8H_{10}O_3PS$ m/z 217.08 , loss of $C_3H_5O_3P$ to $C_{10}H_{17}NS$ m/z 195, and $C_9H_{14}NS$ m/z 180 by loss CH_3 .

Second ,oxidation of fenamiphos to fenamiphos sulfoxide phenol $C_{13}H_{22}O_4NPS$ m/z 319.3 (ethyl-3-methyl-4(methyl sulfenyl) phenyl isopropyl(amidophosphate) .

Fenamiphos phenol (phenol-3-methyl-4 methylthio) M.F $C_8H_{10}OS$ which is formed by hydrolysis of fenamiphos sulfoxide has also low detectable .Fenamiphos phenol m/z 154.04 as major degradation and loss CH_3 to phenol -4-methylthio M.F C_7H_7OS m/z 139.01 .Fragmentation 2-aminopropan-2-yl (hydroxy) phosphinite M.F $C_3H_9NO_2P$ m/z 122.03 ,phenylthio radical M.F C_6H_5S m/z 109.01 , methyl phenyl C_7H_7 m/z 91 and phosphoro-oxyazanium H_3NO_2P m/z 79.99 gm/mol is described as final product of fenamiphos as shown in Figure (1) .

Data in Table (3) referred that retention time Rt of fenamiphos was 15.507 minutes before storage and Rt of breakdown product of fenamiphos , fenamiphos sulfoxide phenol and phenol -3-methyl-4 methylthio were 20.68 ,28.936 and 12.182 minutes ,respectively after storage . The finding is similar by Singh and Walker (2006), Caceres *et al.* (2010) and Kookana *et al.* (1997). Fenamiphos will be oxidation to fenamiphos sulfone (FSO_2) The oxidation products are then converted into corresponding phenol by hydrolysis which considered to the most important step in the decontamination process in the environmental conditions.

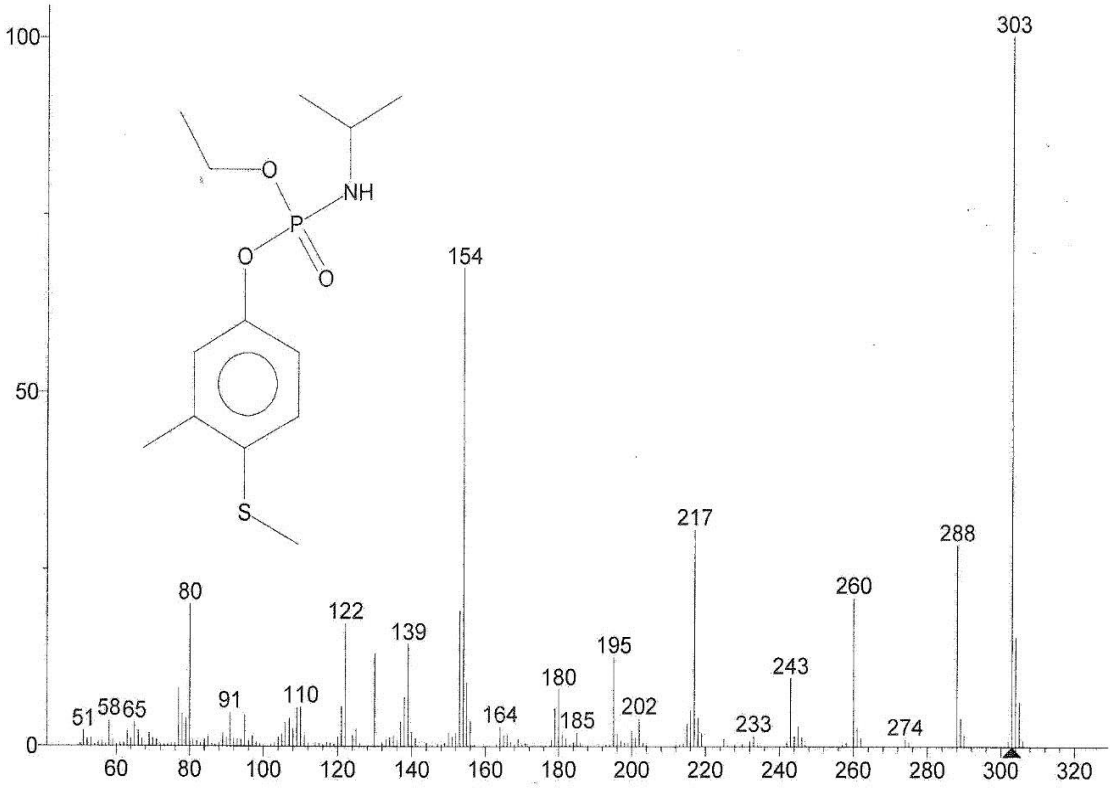

Figure (1): Mass Spectrometry (MS) of fenamiphos.

4. Identification of oxamyl by GC-Mass spectroscopy:

Degradation of oxamyl M.F $C_7H_{13}N_3O_3S$ m/z 219.26 gm/mol proceeded through hydrolysis and cleavage of the methyl carbamoyl bond CH_3NH-C=O yielded methyl (2-dimethyl amino) –N-hydroxy-2-oxoethanimidothiate known as (oximino oxamyl) $C_5H_{10}N_2O_2S$ m/z 162.2 as major product. Further hydrolysis yielded by loss of CH_3NCO and CH_3 to $C_4H_7N_2O_2S$ m/z 145 ,loss of CH_3NCO and CH_3S to $C_4H_7N_2O_2$ m/z 115 ,loss CH_3NCO ,CH_3S and HO to $C_4H_6N_2O$ m/z 98.04 as minor product . While N,N-dimethyl (oxo) methaniminum C_3H_6NO m/z 72.1 as major product by GC-Mass as shown in Figure (2) .

It is similar that MC Nalley and Wheeler (1988) cleavage of methyl carbamoyl bond to form oxamyl oxime as the primary hydrolysis product and ils geometrical isomer were observed in natural and distilled water exposed to artificial and sunlight . Further hydrolysis yielded (dimethyl amino oxacetic acid) as minor photodegradation product in natural water. Finally ,the unstable carbamic acid is rapidly decayed to formaldehyde and CO_2 (Osborn *et al.* , 2010) . Table(3) showed that retention time of oxamyl and oxamyl oxime were 12.98 and 18.26 minutes befor storage and shift to 12.97 and 18.25 min. ,respectively after 30 days of storage at 54±2°C.

Oxamyl oxime was identified as major breakdown product of oxamyl and increasing temperature after last application ,total residue for oxamyl and oxamyl oxime reported as oxamyl equivalent (Holt and Pease,1976) .

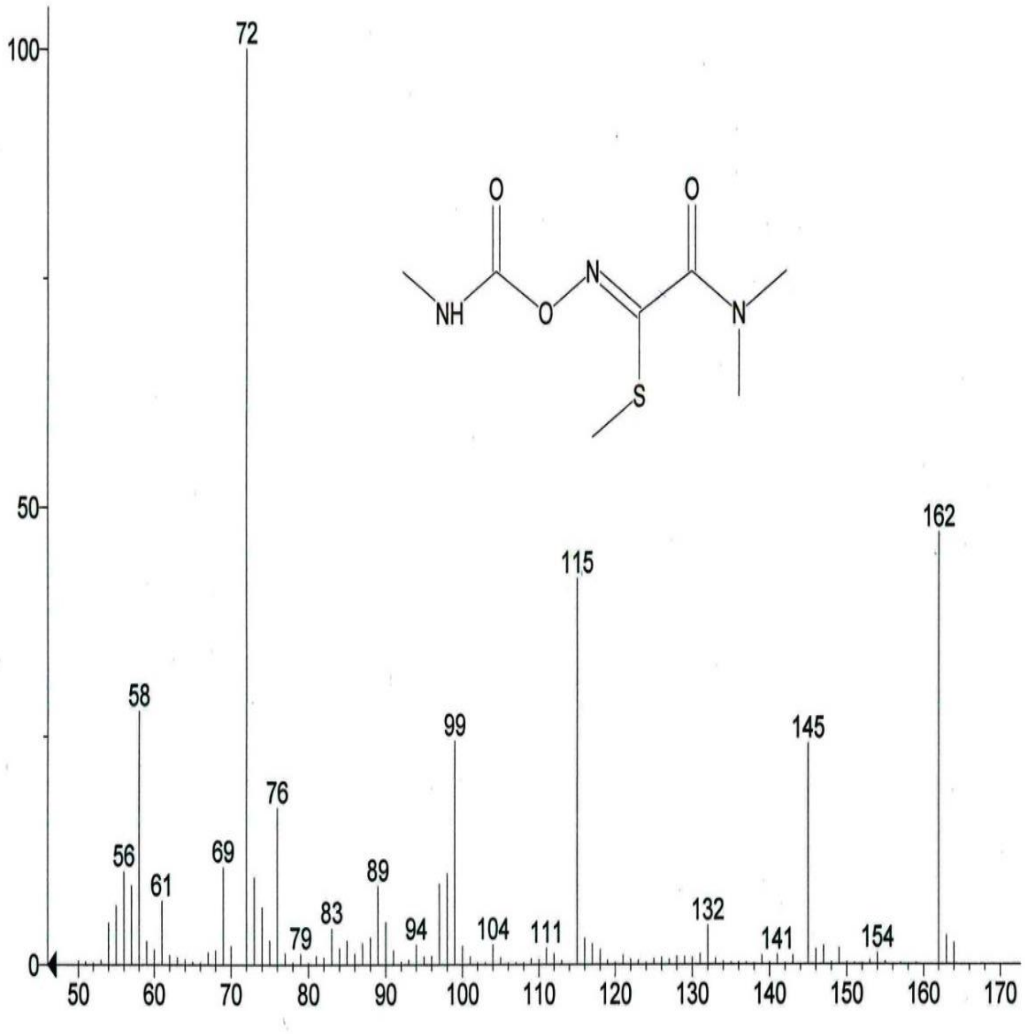

Figure (2): Mass Spectrometry (MS) of oxamyl.

5.Identification of ethoprophos by GC-Mass spectroscopy :

Mass Chromatogram is described in Figure (3) two possible reaction pathways leading to the fission of C-bond in ethoprophos m/z 242.5 ,M.F $C_8H_{19}O_2PS_2$ by hydrolysis ,results in the formation m/z 200.2 ,M.F $C_5H_{13}O_2PS_2$ by loss C_3H_6 and $C_5H_{12}O_2PS$ m/z 168 by loss C_3H_7S .Dimethylphosphorodithioate M.F $C_2H_7O_2PS_2$ m/z 158 as the major intensity by loss $2C_3H_6$ groupes in this compound as shown in Figure (3) .Another way by oxidation S is considered to be turned into m/z 139 ,M.F C_3H_7OPS and phosphenothioic acid M.F H_2O_2PS m/z 97 as the major intensity in chromatogram .Propanethial C_3H_6S ,m/z 74.1 and Propanol C_3H_6OH m/z 59.1 is described as final product of ethoprophos as shown in Figure (3) . According to Karpouzas and Walker (2000), it is found that the alkyl group attached to heteroatom S removed and oxidized to an alchol . Data in Table (3) referred that retention time (Rt) of ethoprophos was 14.022 min before storage and (Rt) of ethoprophos was 14.52 min.after 30 days at 54±2°C.

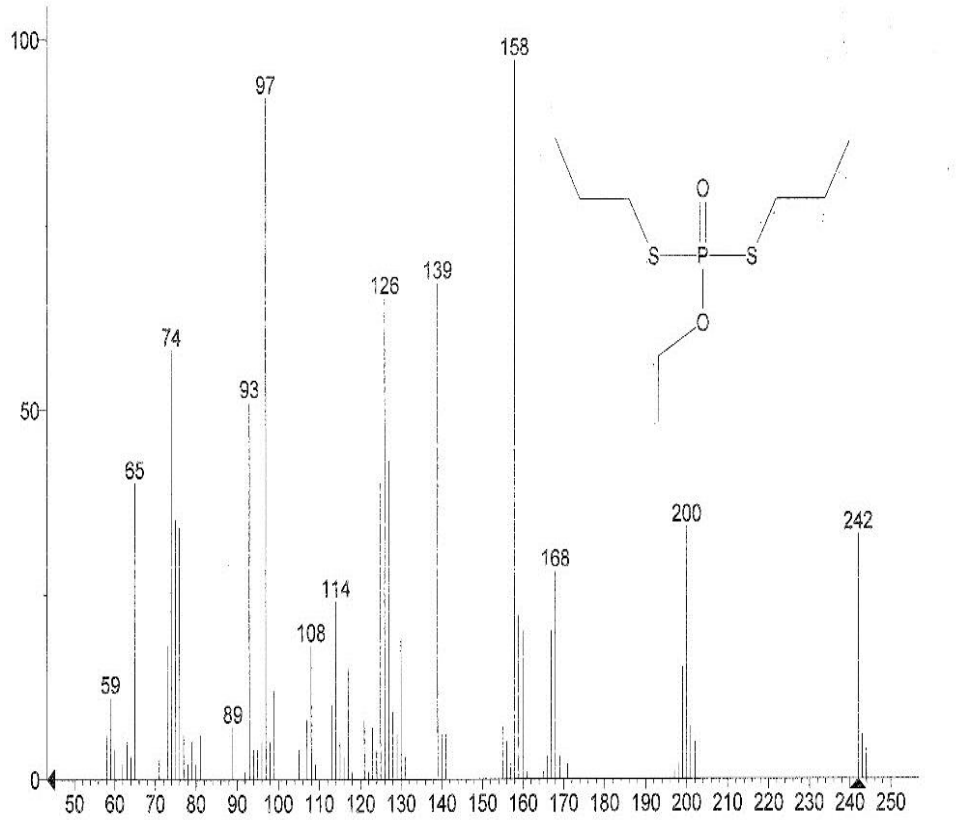

Figure (3): Mass Spectrometry (MS) of ethoprophos

Table (3): identification of fenamipho, oxamyl and Ethoprophos by chemical ionization GC/MS.

Storage period	Nematicides degradation and m/z g/mol	Retention time (min)	Molecular formula their relatively intensity
Initial	Fenamiphos m/z303.4	15.507	$C_{13}H_{22}O_3NPS$ 99%
After 30days of storage	Fenamiphos m/z 303.4	16.27	$C_{13}H_{22}O_3NPS$ 99%
	Fenamiphos sulfoxide m/z 319.3	28.936	$C_{13}H_{22}O_3NO_4PS$99% low intensity
	Fenamiphos phenol m/z 154	12.182	$C8H_{10}OS$ 80%
Initial	Oxamyl m/z 219.26	12.98	C_7H_{13} N_3O_3S 64%
	Oximino oxamyl	12.262	C_5H_{10} N_2O_2S 50%
After Storage 30days	Oxamyl m/z 219.26 Oximino oxamyl m/z 162.2	12.97 18.255	C_7H_{13} N_3O_3S 64% C_5H_{10} N_2O_2S 50%
Initial	Ethoprophos m/z 242.5	14.022	C_8H_{19} O_2S_2 98%
After Storage 30days	Ethoprophos m/z 242.5	14.52	C_8H_{19} O_2PS_2 98%

Initial . One houre before storage.

It is concluded that half- life of ethoprophos is less than fenamiphos and oxamyl because breakdown of fenamiphos phenol and oxamyl oxime are equivalent active ingredients for fenamiphos and oxamyl, respectively . While breakdown of ethoprophos was not equivalent active ingredient for ethoprophos in environment.

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Effect of jojoba and moringa essential oils and cascade on grasshoppers in the field

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Grasshoppers, Acrididae, essential oils , moringa , jojopa, cascade (IGR), mortality, trehalase, chitinase and protease.

Abstract:

Efficacy of moringa and jojopa essential oils and cascade was tested against 3rd, 4th and 5th nymphal instars of different species of grasshoppers at El-Baharia Oasis (western desert of Egypt) by using Ulva sprayer (ULVA+). Mortality percentages were calculated after 2, 4, 6, 8, 10 and 12 days post treatment. The results indicated that the mortality percentages of grasshoppers were 96, 65 and 87% by moringa, jojopa and cascade, respectively after 12 days post treatment. The effects of moringa, jojopa and cascade on trehalase, chitinase and protease activities were tested in haemolymph of some nymphal instars of grasshoppers. There was insignificant difference activity between moringa and control after 2 days. Also the difference in chitinase activity was insignificant between moringa and control after 2 and 4 days, while decreased significantly after 6 days from treatment. In cascade and jojopa increased also after all periods after treatment. The difference in protease activity was insignificant between moringa and control after 2 days while caused increase significant between jojopa and control after 4 and 6 days after treatment. There was no significant difference after 2 days but increased inactive significantly after 4 and 6 days compared with control. In cascade increased significantly after 2, 4 and 6 days after treatment. The efficacy of moringa and jojopa essential oils and cascade in all treatments can be useful for development safe elements for an IPM strategy to grasshoppers.

Introduction

Several species of grasshoppers such as; *Euprepocnemis plorans plorans*, *Heteracris annulosa*, *Acrotylus insubricus*, *Chrotogonus homalodemis*, *Acrididella nasuta*, *Catantops axillaris* and *Aiolopus strepens* are considered among the most dangerous pests that attack the agricultural crops in Egypt and many parts of the world. Also, locust and grasshopper generally have very high reproductive rates and are able to respond to unfavourable climatic conditions with rapid population increase. The most economic species that caused a serious damage is the berseem grasshopper, *E. plorans plorans* and *H. annulosa.*These species cause 95% damage to crops of the El-Farafra Oasis at the new valley, El-Baharia Oasis and Nile Delta (Abdel-Fattah, 2002). Field trials showed the

efficacy of some chemical insecticide formulations, the bioinsecticide *Metarhizium anisopliae* var. *acridum* and anti-moulting agent atabrone against different species of grasshoppers at El-Baharia Oasis western desert of Egypt by micron Ulva sprayer (Ulva+) (Abdel-Fattah and Abdel-Lattef, 2013). The biochemical effects of neem and cascade on the mortality percentages, malformations and some biochemical changes were studied by (Soltan, 2014). In Egypt, toxicity of two chemical insecticides (chlorpyrifos, es-fenvalerate); a bioinsecticide (protecto); an IGR (lufenuron) and jojoba oil against 2nd and 4th instar of larvae of *Spodoptera littoralis* and their effect on some biological characters and fecundity were studied on 4th instar larvae (Gaaboub *et al*., 2012). Jojoba oil is suggested as a safe product with a potential for use as a bioinsecticide in integrated pest management especially in urban localities where use of chemical insecticides are discouraged (Abdel-Razik and Mahmoud, 2017). Jojoba, *Simmondsia chinensis* L. is native to south western the desert, United States and northern Mexico. It is also grown in Australia, Brazil, Argentina and some Middle East countries. Jojoba has become an attractive alternative crop because of the promising commercial applications for its seed oil in cosmetics. Many countries are looking toward developing jojoba culture to solve overproduction and low price for their food and other traditional crops (Ayerza, 1996). Antifeedant and protection activity percentage were increased by increasing the concentration. The highest mortality percentage (100%) of *Schistocerca gregaria* (Forsskål) (Orthoptera: Acrididae) nymphs was recorded at 10% jojoba oil (Halawa *et al*., 2007).

The Drumstick tree *Moringa oleifera* (Lamk.) belongs to Moringaceae family commonly called miracle tree. It is an important vegetable crop and is a fast growing, drought resistant tree, native to the southern foothills of the Himalyas in North western India. It is the most widely distributed species (Sontag, 1982). Dimetry *et al*. (2017) studied *M. oleifera* leaves that decreased the weight gain significantly in the treated individuals of *S. littoralis* in comparison with the control. The relative consumption index (CI) increased in case of treated leaves in comparison with the control ones. Flufenoxuron (Cascade) is classified in the chitin syntheses inhibitors (CSIs), it caused some toxic effects on larvae of insect species (Bakr *et al*., 2010).

Therefore, the present work aims at throwing some light on the toxicity, and enzyme activities of grasshoppers due to using two botanical oils, I G R (Cascade) in the field at El-Baharia Oasis western desert of Egypt.

Materials and methods

During the seasons 2017 and 2018 many ecological surveys were carried out to evaluate the major insect pests of family Acrididae prevailing at El-Baharia Oasis western desert of Egypt. It was found that the grasshoppers, *E. plorans plorans, H. annulosa, A. insubricus, Ch. Homalodemis, A. nasuta, C.* axillaris and *A. strepens* and the local locust *Anacredium aegyptium* were existed in this area. Among these pests, the berseem grasshopper, *H. annulosa* was the most dominat. A suitable infested area characterized by high population tested nymphs were 3rd, 4th and 5th instars only.

1. Essential oils:

1.1. Jojoba oil and *Moringa oleifera*: (plant oil is formulated as EC) produced by Egyptian Natural oil Co. used at the rate of 1.2 liter/ ha.

1.2. Cascade 10% EC (Flufenoxuron) 1.5 liter/ ha. Its chemical name is: 1-{4-(2-chloro-α, α, α,-trifluoro-p-tolyloxy)-2-fluorophenyl}-3-fluorophenyl}-3-(2,6-difluorobenzoyl) urea.

2. Experimental design:

A field cultivated by alfalfa (*Medicago sativa*) in sandy loam soil, highly infested with different grasshoppers , mixed with few local locusts at the region of western desert El-Baharia Oasis was chosen in August 2019. The field was divided to plots of (35x20) = 700 m^2 each the plots were isolated by a wide belt of 10x25m= 250 m^2. Five plots were allocated randomly for each treatment. Plots laying up wind of treatment were used as a control. The untreated cheek plot was sprayed with water only. Each treatment as well as the control was represented by five replicates (cages) 0.5m x 0.5m. The cages were put in the treated plots. The insects were collected randomly from the same treatment of the pesticides after application directly by using sweep-net and introduced to the cages. The insects were kept in cages and fed with treated plants (alfalfa) from the same plot. Unfortunately, the sweeping net didn't catch any individual of locust after treatment, so, locust results were not mentioned in the tables, however, by observation after treatments, and there was no alive individual. Mortality counts were calculated after 2, 4, 6, 8, 10 and 12 days post treatment but collected haemolymph after 2, 4 and 6 days post treatment to biochemical analysis. A suitable infested area characterized by high population density of grasshoppers (more than 30 insects/m^2) was selected. The tested nymphs were 3^{rd}, 4^{th} and 5^{th} instars only (Abdel-Fattah *et al*., 2012).

Sprayer used: The micron Ulva (ULVA+),Nozzle: Red nozzle to treatments EC. Red nozzle calibrated 90 ml water/min., Spraying height: 0.5 m above the plants.,Walking speed: 40m/min = 2.4 km/hr., Swath width:3m according to wind velocity.,Weather conditions at applications: Wind: 4–6 m/sec, measured by anemometer and Temperature: 33ºC ± 2 °C, the sun rose clearly.

The spraying was done between 07 and 10 am in morning. Daily routine works includes removing the previous uneaten food, faeces and dead nymphs and counting the living insects before introducing the fresh food were conducted.

2.1.Collection of haemlymph: according to the technique was followed as described **by** (Amin, 1998).

2.2. Determination of trehalase activity: Trehalase was determined according to the method described by Ishaaya and Swiriski (1976).

2.3. Determination of chitinase activity: Chitinase was determined according to the method described by **Bade and Stinson (1981).**

2.4. Determination of Protease activity: Protease was determined according to the method described by Gatehouse *et al*. (1999).

3. Statistical analysis: Data were subjected to analysis of variance (ANOVA), and Duncan's multiple range test to differentiate between the means at $P<0.05$, using SAS program **(**SAS, 1995).

Results and discussion

The effect of jojoba, moringa and essential oils and cascade were tested under field conditions against 3^{rd}, 4^{th} and 5^{th} different nymphal instars of the grasshoppers by using ULVA+ spraying equipment after 2, 4, 6, 8, 10 and 12 days post treatment. The efficacy of tested essential oils and cascade was calculated by using equation as follow:

Efficacy% = dead treatment% - dead check% / 100- dead check%

Data in Table (1) show the efficacy of jojoba and moringa, essential oils and cascade against nymphal instars of grasshoppers after 2, 4, 6, 8, 10 and 12 days post treatment. Results showed that there is no mortality in the check (untreated after 2, 4, 6, 8, 10 and 12 days). Data cleared that the mortality percentages of nymphal instars of grasshoppers were (Jojoba 96%, moringa 65% and cascade 87%) after 12 days post treatment. The present results in this concern agreed with Aaboub *et al.* (2012) studied the toxicity of two chemical insecticides (chlorpyrifos, es-fenvalerate); a bioinsecticide (protecto); an IGR (lufenuron) and jojoba oil against 2nd and 4th of instar larvae S. littoralis and their effect on some biological characters and fecundity and found that Es-fenvalerate proved to be the most effective insecticide against 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisd.) (Lepidoptera:Noctuidae) after 24 hrs, followed by chlorpyrifos, lufenuron, jojoba oil and protecto. The highest mortality percentage (100%) of S. gregaria nymphs was recorded at 10% jojoba oil. Abd El-Rahman (2003) mentioned that jojoba oil caused 83.8 and 90.8% mortality against *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). larvae at 0.5 and 1% respectively. In the same subject, Salem *et al.* (2003) revealed that jojoba oil formulation was the potent agent against both white fly and leafhopper species where the LC50 was 5.4% for *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and 6.4% for *Empoasca decipiens* Paoli (Hemiptera: Cicadellidae), respectively. Abdel-Razik and Mahmoud (2017) showed that 2nd or 4th instar larvae of cotton leafworm, *S. littoralis* exposed to jojoba extract for 24 hrs were greatly suffered from toxic effects which give good evidence for using jojoba as an element for the integrated management of insects. The variable toxicity may be due to the constituents of each oil and disturbance or the hormonal regulations (Al-Sharook *et al*., 1991), 200 species of plants, which produce chemicals substances able to act against insects, are known. The substances can have poisonous and repellent effects and can work as phagorestrainer ovicide and can affect the insect's hormonal system. Moreover, a great number of essential oils can reduce the reproduction system of several insects and they can also hinder the growth, the development and the reproduction of some herbivore insects (Partes *et al*., 2000). Dimetry *et al.* (2017) studied that the acceptability and anti-feedant effect of *M. oleifera* leaves as host plant towards the cotton leaf worm *S. littoralis*. The obtained results showed highly significant anti-feedant effects of *M. oleifera* leaves towards studied instars in comparison with castor oil leaves as a control. Also, the percentage mortality of the larvae was very high and those of the 1st instar larvae failed to complete one generation and all of them died during 2nd and 3rd instars. The present results agree with those findings by several CSIs against the same Acrididae species, *S. gregaria*, such as Diflubenzuron which interfered with the chitin synthesis during the nymphal ecdysis to the last instar causing some mortalities (Taha and El-Gammal, 1985) and the mortal power of cascade Flufenoxuron depending on the developmental nymphal instar under treatment or its physiological age (Soltan, 2014).

Table (1): Mortality percentage of jojoba, moringa and cascade against nymphal instars of the grasshoppers, after 2, 4, 6, 8, 10 and 12 days post treatment in the field.

Days after treatment	Jojoba	Moringa	Cascade mortality %	Control mortality %
2nd	10	5	0	0
4th	30	19	22	0
6th	49	38	39	0
8th	68	46	51	0
10th	83	59	72	0
12th	96	65	87	0

Some biochemical effects (Trehalase, Chitinase and Protease) activities after treatment by jojoba, moringa and cascade on nymphal some instars of grasshoppers in the field:

Data in Table (2) showed that, The effect of jojoba, moringa and cascade on trehalase, chitinase and protease activities of haemolymph to nymphal instars of grasshoppers. Data in Table (2) showed that, jojoba highly significant increased trehalase activity after 2, 4 and 6 days compared to control. The trehalse activity difference was insignificant between moringa and control after 2 days while caused significant increase after 4 and 6 days from treatment. In cascade significant increased after 2, 4 and 6 days compared to control was observed. On the other hand Jojoba increased the chitinase activity significantly and cascade showed highly increase on chitinase activity after 2, 4 and 6 days after treatment compared with control but moringa caused significant increase in chitinase activity after 2 and 4 days but decreased after 6 days compared with control. While jojopa induced insignificant difference in activity in protease after 2 days but highly increased after 4 and 6 days compared with control. Also moringa caused insignificant difference after 2 days from treatment but induced highly significant increase compared with control after 4 and 6 days. While cascade increased protease activity significantly after 2, 4 and 6 days from treatment compared with control. These results agree with Tanani *et al.*, (2012) showed that the treatment of newly molted 5th instar of the *S. gregaria* by through fresh plant IGR tebufenozide caused statistically significant increase in trehalase activity after 4 days. Soltan (2014) observed that the difference trehalase activity of desert locust was insignificant between neem and control after 2 days while increased significantly after 4 and 6 days from treatment, but cascade and mixuture (Neem and cascade) increased after 2, 4 and 6 days. Trehalase is activated for the production of glucose needed for chitin build-up in the newly synthesized cuticle; it is generally present in large amounts in the haemolymph of most insects and its activity might be an indicator of energy reserves resulting from availability of carbohydrate nutrient (Wyatt, 1967). Ecdysis is initiated by apolysis the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysal membrane formation. The molting fluid contains protease and chitinase, enzymes that digest the main constitution of old endocuticle (Reynolds and Samuels, 1996). Accordingly mortality percentage and changes in enzymes activities of the insects was greatly affected. Thus, it could be concluded that essential oils of jojoba and moringa and cascade could be use as an effective natural products to be included in the integrated pest management program of grasshoppers in the field.

Table (2): The effect of jojoba, moringa and cascade on trehalase , chitinase and Protease activity of nymphal instars grasshoppers.

Enzymes	Trehalase (µg glucose released/min./gm fresh weight)			Chitinase (µg NAGAx10^3/min./gm fresh weight)			Protease (µmol/min./mg protein)		
Days after treat.	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**
Jojoba	600^a	445^a	417^a	509^b	849^b	979^b	5.2^b	14.1^a	17.51^b
Moringa	421^c	425^c	362^c	440^c	346^c	261^d	5.81^b	16.1^a	18.65^a
Cascade	527^b	399^b	321^c	577^a	1284^a	1843^a	8.22^a	12.42^b	14.75^c
control	410^c	330^d	273^d	451^c	335^c	318^c	5.11^b	7.5^c	9.28^d
LSD	68.89	72.33	79.55	588.4	615.2	679.1	4.05	4.12	4.68

Measurement of distance between individual distributions (P<0.05).
Means with the same letter are not significantly different.

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Evaluating the insecticidal efficiency of some legumes extracts against *Bactrocera zonata* (Diptera: Tephritidae)

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Abstract:

Insecticidal and biological activity of three legumes; *Lupinus luteus* L., *Glycine max* L. and *Cicer arietinum*; seeds ethanolic extracts against adult and egg stages of peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) was investigated under laboratory conditions. The phytochemical screening of ethanolic extract of the tested three legumes showed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins and saponins in *G. max* extract and absence of saponins in *C. arietinum* . Also, terpenoids, flavonoids and tannins are absent in *L. luteus* ethanolic extract. Ethanolic extracts of the tested legume seeds achieved variable toxicity against adult and egg stages of *B. zonata*. Ethanolic extracts of *G. max* and *C. arietinum* seeds (LC_{50} = 366.2 and 437.3 mg L^{-1}, respectively) were more toxic against insect adults than *L. luteus* seeds ethanolic extract (LC_{50} = 627.5 mg L^{-1}) after 8 days of exposure. Ethanolic extract of *C. arietinum* achieved the higher ovicidal activity (Egg hatchability reached to 0% at 1000 mg / L) compared to the other extracts. *G. max* and *C. arietinum* seeds ethanolic extracts were the most potent on the adult fecundity with 2.0 and 0.0 egg laid / female at 1000 mg / L, respectively compared to 49.0 egg / female in control. The tested ethanolic extracts of the three tested plant seeds reduced the adult longevity of *B. zonata* and the effect was concentration dependent. Results of the present study suggest that, plant extracts can be an effective tool in integrated pest management programs for the control of fruit flies.

Introduction

The peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a serious pest of fruits in Egypt and many world regions. It attacks a wide range of fruit and vegetable hosts (White and Elson-Harris, 1994). Saafan *et al.* (1993) recorded *B. zonata* in many orchards in several Egyptian Governorates. Peach fruit fly originates in South and South- East Asia (Kapoor, 1993 and Duyck *et al.*, 2004). The presence of this insect reduces the quality of fruits and subsequently negatively affects their exportation (Shehata *et al.*, 2008). Damage is caused mainly by the larvae, which feed during the growth and development of the fruit (Stonehouse *et al.*, 2005). Therefore, the control of

larvae is difficult using a single control measure (Dhillon *et al*., 2005).

Control methods of this insect are ranged from foliage and soil spraying by the specific insecticide, bait-application, male annihilation techniques, releases of sterilized flies and parasitoids, and cultural controls (Khan *et al.*, 2017). The unwise use of synthetic insecticides resulted in many environmental problems, health problems, development of insecticide resistance and natural enemies toxicity (Victor, 2009). Therefore, there is a need to search about more effective and safe alternative methods to these synthetic insecticides. Between these alternatives are the plant natural products.

Plants synthesize many aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins which can negatively affect insects (Cowan, 1999). These compounds can be used as repellents, feeding deterrents, toxins, defense chemicals and growth regulators. Many are also effective against biting Diptera, especially those volatile components (Gurjar *et al*., 2012). It is documented that, the leaf extracts of eucalyptus *(Eucalyptus globulus)* shows efficacy against *Bactrocera cucurbitae* (Ali *et al*., 2011). In addition, Mahmoud and Shoeib (2008) showed that low concentrations of neem can be applied effectively as sterilant and oviposition deterrent for the peach fruit fly populations. Neem blocks the ovarian development and can be used as safe alternative of insecticides for the control of *a* species (Mahfuza *et al.*, 2007). In the present study, the toxic effects of ethanolic extract of three legume plant seeds; *Lupinus luteus L*. (*L. luteus*), *Glycine max L* (*G. max*) and *Cicer arietinum L* (*C. arietinum*), were tested against *Bactrocera zonata* adult. Effects on the fecundity and fertility of treated adults were also evaluated.

Materials and methods

1.Tested insect: *B. zonata* pupae were obtained from the Department of Pests Horticultural Crops, Plant Protection Institute Research, Agricultural Research Center. Emerged adults were introduced to cages of 30 cm- 30 cm - 30 cm size. Cages were enclosed with mesh screens and have cloth sleeves at front and back sides for the introduction of the food (hydrolyzed yeast and sugar of ratio 1:3) (Khan *et al*., 2016) and oviposition trays (El-Minshawy *et al.*, 1999). The insect larvae were reared under the laboratory conditions (25 °C ± 2; 65% ± 5 RH.) on a semi-artificial diet (Wheat bran 500 gm, molasses 250 gm, dried yeast 150 gm, citric acid 4 gm and sodium benzoates 6 gm in 1liter water) according to Awad (1993).

2.Tested plants:

Seeds of three legumes; *L. luteus*, *G. max* and *C. arietinum*, ethanolic extracts were used to study its potential biocidal activity against the tested insect *B.zonata*. Dried legume seeds were purchased from local markets, Alexandria Governorate. The plants were identified by Department of Horticultural Crops,Agricultural Research Center, Alexandria.

3.Plant extraction:

The legumes seeds were pulverized into fine powder using a grinding mill. The extraction of the investigated plants was carried out according to the method of (Mbatchou *et al.*, 2011). Powder of each of *L. luteus* , *G.. max* and *C . arietinum* seeds (200g) were soaked in (700 ml) of ethanol 98% for two weeks with intermittent shaking. The extracts were separately evaporated to dryness at room temperature to obtain

the crude extracts (ethanol extracts). This procedure was repeated 10 times. The resulting crude extracts were stored in glass vials pack closed at (2-4°C) until used for phytochemical and bioassay assessment experiments.

4. Qualitative phytochemical screenings:

The extracts obtained from ethanol extract, were subjected to preliminary phytochemical analysis tests to identify the main chemical groups such as alkaloids, steroids, flavonoids, saponins and tannins according to **Mbatchou *et al*. (2011).**

4.1.Alkaloids test (Wagner's **test):**

One ml of each extract was treated with drops of Wagner's reagent (Dissolve 2 g of iodine and 6 g of potassium iodide in 100 ml distilled water) and observed for the formation of reddish brown precipitate.

4.2. Flavonoids test (Willistatter test):

To an ethanol solution of each extract, a piece of magnesium ribbon was added, followed by dropwise addition of concentrated HCl. Colors ranging from orange to red indicated flavonoids.

4.3. Terpenoids and steroids test (Liebermann Buchart test):

A small quantity of each extract was dissolved in trichloromethane, and a minimum volume of concentrated sulphuric acid added to its content. A blue or green color or a mixture of these two shades was taken as positive test for terpenoids compounds and the formation of dark pink or red color taken as positive test for Steroids compounds.

4.4. Tannins test:

Each plant extract (0.2 ml) was re-extracted with ethanol. The solution obtained was later treated with 5% ferric chloride. A blue-black or blue-green appearance was taken as positive test for tannins.

4.5. Saponins test:

A small portion of each extract was added to 2 ml of distilled water and boiled for 3:5 minutes. The resultant mixture was filtered, allowed to cool with the filtrate shaken vigorously. Honey comb froth higher than the aqueous layer was taken as strongly positive for saponins.

5.Insecticidal activity of legumes extract:

The bioassay experiments were carried out using the extract of the investigated plants to test its potential biocidal activity against *B. zonata*. Series of concentrations of each botanical extract from the three legumes were prepared in ethanol and tested against *B. zonata* adults. Tween-twenty was used as an emulsifier. Twenty adults of *B. zonata* (Males and Females) 10 days old gravid flies (Rehman *et al*., 2009a) put in plastic container containing food (hydrolyzed yeast and sugar of ratio 1:3) and water. (Sultana *et al*., 2013). The tested extract was put as a layer on the banana peel in the plastic container. Emulsifier and ethanol solution without extract was added to a piece of banana peel and kept as untreated control. Each treatment in addition to control was replicated three time. The plastic container covered with muslin cloth and tight with rubber bands and kept under laboratory conditions. The adult mortality and number of laid eggs /female was recorded after 2, 4, 6 and 8 days of treatment. The eggs removed and counted from banana peel by pin and placed in a petri dish having wet black cloth to calculate egg hatchability%. Mortality percentages were calculated and subjected to probit analysis according to Finney (1971). The concentration which cause 50% mortality (LC_{50}) and the time required for 50% mortality (LT_{50}), confidence limits and slope ± SE were calculated.

6. Statistical analysis:

Data were subjected to one-way ANOVA test (Duncan's Multiple Comparison Range Tests at 0.05% level). The means were separated through Tukey's HSD (Honest Significant Difference) test at a significance level of 0.05 probability. Percentage repellency (PR) data was calculated by applying this Schneider-Orelli's formula (Püntener, 1981).

$$\text{Corrected \%} = \frac{(\text{Mortality \% in treated plot} - \text{Mortality \% in control plot})}{100 - \text{Mortality \% in control plot}} \times 100$$

Results and discussion

1. Phytochemical screening:

The phytochemical screening of ethanolic extracts showed the presence of different types of active constituents (Table, 1). Alkaloids, steroids, flavonoids, terpenoids, tannins and saponins were present in the *G. max L* ethanolic extract. Alkaloids, steroids and saponins were identified in *L. luteus* extracts. *C. arietinum* ethanolic extract contains alkaloids, steroids, flavonoids, terpenoids and tannins. The variability in active compounds in each extract is expected to affect their biological activities. Results of the present study is in accordance with many previous studies. Phytochemical analysis indicated the presence of flavonoids, alkaloids, steroids, and tannins in *L. luteus, C. arietinum* and *G. max* ethanolic extracts (Maknickiene *et al*., 2013; Mamta *et al*., 2013 and Kumaran and Citarasu 2015). The variability in active compounds and their concentrations in the extracts are expected to affect in the biological activities against pest (Elena *et al*., 2016).

Table (1): Phytochemical screening of various extracts of tested plants.

Experimental Plants	Phytochemical Constituents					
	Alkaloids	**Steroids**	**Terpenoids**	**Flavonoids**	**Tannins**	**Saponins**
Lupinus luteus	+	+	-	-	-	+
Glycine max	+	+	+	+	+	+
Cicer arietinum	+	+	+	+	+	-

2. Toxicity of tested plant extracts against *Bactrocera zonata* adults:

Toxicity of ethanolic extracts of three legume plants *L. luteus, G. max* and *C. arietinum* seeds against the *B. zonata* adults after 6 and 8 days of treatment are presented in Table (2). Ethanolic extracts of *G. max* and *C. arietinum* seeds (LC_{50} = 366.2 and 433.9 mg L^{-1}, respectively) were more toxic than *L. luteus* seeds ethanolic extract (LC_{50} = 627.5 mg L^{-1}) after 8 days of exposure. According to confidence limits, the toxicity of ethanolic extracts of both *G. max* and *C. arietinum* seeds against *B. zonata* adults after 8 days of exposure was comparable. Toxicity of the three tested plant extracts against *B. zonata* adults is time dependent (Table, 2). According to the LT_{50} values of the tested extracts, *G. max* and *C. arietinum* act more fast against *B. zonata* adults with LT_{50} values 4.5 and 5.2 days compared to *L. luteus* extract with LT_{50} value 15.5 days at 500 mg / L (Table, 3). Botanical insecticides are good alternatives to chemical insecticides and approved to be effective in insect control (Rehman *et al.*, 2009b).

The over use of chemical pesticides causes many environmental and health problems. Therefore, It is recorded that, *Lupinus* species are a source of alkaloids, which provide the plants with protection from adverse weather conditions, microorganisms, fungi, insects, and herbivores (Rybiński *et al.*, 2018). The ethanolic extract of marine algae *Callyspongia crassa* and *Grayella cyathophora* achieved considerable toxicity against *B. zonata*

under laboratory conditions (Elnagar *et al*., 2018). Acetone and water extracts of *Acacia auriculiformis* A. Cunn. bark significantly prolonged the larval period and total developmental period, decreased percentage pupation, percentage emergence, oviposition and egg hatching of *Bactrocera cucurbitae* (Coquillett) in a dose dependent manner (Kaur *et al*., 2010). Siddiqi *et al*. (2011) reported that the acetone extract of *Curcuma longa* achieved high mortality percentages to *B. zonata* and caused high inhibition of pupa formation.

Table (2): Insecticidal effect of different concentrations of extracts on adult of *Bactrocera zonata.*

Extracts		Exposure period			
		LC_{50}	Confidence limits	Slope±SD	Chi^2 Tabulated
Lupinus luteus	6days	712.5	670.8-755.3	7.3±0.7	3.8
	8days	627.5	592.9– 659.3	7.5±0.6	3.8
Cicer arietinum	6days	483.8	410.4-536.4	4.4±0.7	3.8
	8days	433.9	373.1-473.6	7.6±1.3	3.8
Glycine max	6days	442.6	328.3-514.9	3.4±0.7	3.8
	8days	366.2	254.4-436.2	4.3±0.9	3.8

Table (3): LT_{50} values for adult of *Bactrocera zonata* exposed to *plants* extract at concentration of 500 mg / L.

Extracts	LT_{50}	Confidence limits	Slope±SD	Chi^2 Tabulated	C
Glycine max	4.5	4 - 5	2.7±0.2	9.5	0.8
Cicer arietinum	5.2	3.8-6.6	3.2±0.3	9.5	0.8
Lupinus luteus	15.5	11.4-20.4	3.5±0.3	9.5	0.8

2. **Ovicidal activity and effects on some biological aspects of tested plant extracts against *Bactrocera zonata*:**

Effects of the three legume plants *L. luteus* , *G. max* and *C. arietinum* seeds ethanolic extracts on the *B. zonata* egg hatchability are presented in Table (4). It is clear that, *C. arietinum* seeds ethanolic extract had the higher activity where the egg hatchability reached to 0% at concentration of 1000 mg / L compared to 88.5% in control. The egg hatchability reached to 69.8 and 58.4% when the *B. zonata* eggs where treated by *L. luteus* L. or *G. max* L. at 1000 mg / L compared to 88.5%in control. The ovicidal activity of the tested three plant ethanolic extracts is concentration dependent. Similar type of response in insects caused by plant extracts has also been reported from other laboratories. Sharaby (1988) reported pronounced reduction in egg production and egg viability when *Phthorimaea operculella* were exposed to the vapours arising from paper treated with 220 µl of *Citrus sinensis*.

The number of laid eggs / female was highly affected when adults of *B. zonata* where exposed to the ethanolic extracts of the three tested plant seeds (Table, 4). *Glycine max* L and *C. arietinum* seeds ethanolic extracts were the most potent with 2.0 and 0.0 egg laid / female at 1000 mg / L, respectively compared to 49.0 egg / female in control. The tested ethanolic extracts of the three tested plant seeds reduced the adult longevity of *B. zonata* and the effect was concentration dependent (Table, 4). The adult longevity reached to 6.7days when adults was exposed to *C. arietinum* seeds ethanolic extract at 1000 mg / L compared to 59.3 days in control.

Table (4): Effects of the tested plants extracts on egg hatchability, number of egg/female and longevity (days) for *Bactrocera zonata*.

Treatments	Concentration (mg/L)	Egg hatchability(%) ±SD	Number of egg/female ±SD	Time of exposure(days) ±SD
Control	**0.0**	88.5±9.1^{a}	49.0±8.1^{a}	59.3±1.2^{a}
Lupinus luteus	**500**	76.5±8.2^{b}	12.3±0.4 ^{b}	32.0±3.5^{b}
	750	71.2±5.7^{b}	11.25±1.3^{b}	12.7±1.2^{c}
	1000	69.8±1.6^{bc}	5.0±3.0^{cd}	9.3±2.3^{de}
Glycine max	**500**	75.0±8.3^{b}	5.5±3.5^{c}	11.3±1.2^{cd}
	750	65.5±1.7^{cd}	2.0±0^{cd}	8.0±0^{ef}
	1000	58.4±8.3^{d}	2.0±1.0^{cd}	7.3±1.2^{ef}
Cicer arietinum	**500**	33.3±0^{e}	1.1±0.2^{cd}	11.3±1.2^{cd}
	750	33.3±0^{e}	1.0±0^{cd}	7.3±1.2^{ef}
	1000	0.0^{f}	0.0^{d}	6.7±2.3^{f}

Means with the same letters in the same column are not significantly different according to L.S.D. test at 0.05 level of probability.

Insecticidal properties of any plant extracts depend on the active constitutes. Saponins have strong detrimental effects on insects, causing mortality, growth retardation and decreased fecundity (De Geyter *et al.*, 2007). In addition, lower food intake of insects fed on saponin-containing plant extracts was recorded (Taylor *et al.*, 2004 and Golawska *et al.*, 2006). According to Ishaaya (1986) saponins slow down the passage of food through the insect gut. Perhaps they reduce the digestibility of the food by inhibiting the secretion of digestive enzymes (Proteases and amylases) (Golawska *et al.*, 2006). Shimada (2006) reported tannins to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Steroidal compounds are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Hence, the presence of these compounds in the tested plant extracts corroborates the insecticidal activities observed.

The systemic insecticides are not the preferred choice for fruit flies control in fruit crops. Furthermore, the insecticides having contact action remained insufficient to give successful control of fruit flies, unless targeting the fruit fly adults in abandoned areas and vegetation. This behavior of flies suggests that, such control strategies may be useful as an integrated pest management (IPM) approach. Therefore, plant extract formulations affecting the oviposition have an added advantage over synthetic insecticides and can be a tool in integrated pest management programs for the control of fruit flies (Khattak *et al*., 2006).

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Abstracts are required for all the manuscripts. They should be typed in one paragraph and limited to max.150- 200 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords: Most important words of paper. Should be from 4 to 6 words.

Text: Main text should contain (1) an Introduction (2) a Material and Methods (3) a Results (4) a Discussion (5) a Conclusion (6) a References

Text formatting: Use a normal, plain font (e.g., 14 Point Times Roman) for text.

Abbreviations should be defined at first mention and used consistently thereafter. Authors should adhere to the rules governing scientific nomenclature to the International Code of Zoological Nomenclature. All biotica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names including authors (and Order: Family) when the English term is first used in the main text, with the exception of common domestic plants and animals. Scientific names should be as follows: In the Title only give the Latin name but No authority or (Order: Family); **i**n the Abstract all Latin names should be accompanied with the correct authority and with (Order: Family)**;** in addition, at the first mention in the body of the text - and only then - these data should be given; authority, the order, family, should also go in the Key Words list.

Footnotes

Footnotes on the title page are not given reference symbols. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data).

References

The list of References should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Cite references in the text by name and year in parentheses. Some examples:

• Negotiation research spans many disciplines (Abd-Rabou, 1996).

• This result was later contradicted (Simmons and Abd-Rabou, 2009).

• This effect has been widely studied (Abd-Rabou ,1998; Simmons *et al.*, 2002; Evans and Abd-Rabou, 2005and Abd-Rabou *et al.*, 2005).

List style

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal article

Abd-Rabou, S. (1998): The efficacy of indigenous parasitoids in the biological control of *Siphoninus phillyreae* (Homoptera: Aleyrodidae) on pomegranate in Egypt. Pan-Pacific Entomolgists, 74 (3): 169-173.

Evans and Abd-Rabou (2005): Two new species and additional records of Egyptian Aphelinidae. Zootaxa,833:1-7.

Simmons, A. and **Abd-Rabou, S. (2006):** Whitefly populations in vegetables crops with different fertilizers. 52nd Annual meeting of the South Carolina Entomological Society, Mc Cormick, Sc., October 19-20.

Abd-Rabou, **S. and Simmons, A. M. (2012):** *Bemisia tabaci* (Hemiptera: Aleyrodidae) whitefly as a pest in Egypt. Advances In Agricultral Research In Egypt, 10 (1): 1-82.

Figures Line-drawing should be clear and of high quality. Cite all figures in numerical order in the manuscript.

Tables The title should be self-explanatory and include enough information so that each table is intelligible without reference to the text or other tables. The title should summarize the information presented in the table without repeating the subheadings. Subheadings should be brief, nonstandard ones can be explained in footnotes. Cite tables in numerical order in the manuscript. Information presented in a table should agree with that in the text.