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Effect of treated cucumber by cytokinin hormone on the infestation of *Aphis gossypii* (Hemiptera: Aphididae) and *Tetranychus urtiace* (Acari: Tetranychidae) under glasshouse

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Cucumber, cytokinin hormone, *Aphis gossypii*, *Tetranychus urtiace* and glasshouse.

Abstract:

Cucumber (*Cucumis sativus* L.) is considered as one of the most important fresh consumed vegetables in Egypt and it is used to cultivate under open field conditions and greenhouse. The aim of this research work is to study effect of treated cucumber plants *C. sativus* by different concentrations of cytokinin hormone CKs (25 ppm, 45ppm and 65 ppm) on the infestation by *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Tetranychus urtiace* Koch. (Acari: Tetranychidae) at two locations in Giza and Qalubiya Governorates during 2018 season under glasshouse conditions. Also, this research work was carried out to study effect of treated cucumber plants by the same concentrations of the same hormone on the morphological characteristics and internal components of treated cucumber plants. Results obtained showed that cucumber plants treated with small concentration of CKs (25 ppm) were lower infestation by both *A. gossypii* and *T. urticae* compared to control. While cucumber plants treated with medium concentration of CKs (45ppm) were had no significant differences in the infestation by both *A. gossypii* and *T. urticae* compared to control. On the other hand cucumber plants treated with high concentration of CKs (65ppm) were higher infestation by both two pests compared to control. Also, results obtained showed that treated cucumber plants with small concentration of CKs improved morphological characteristics and internal components of these plants compared to control, while treated cucumber plants with medium concentration of the hormone had no significant differences on the morphological characteristics and internal components of these plants compared to control. Treated cucumber plants with high concentration of the same hormone had badly effect on the morphological characteristics and internal components of these plants compared to control.

Introduction

Cucumber (*Cucumis sativus* L.) considers one of the most important vegetables crops in Egypt and all over the world which cultivated in the open field and under glasshouse conditions. Also, its cultivated area increased gradually during the last years, especially in the new reclaimed

areas for purposes local consumption and exportation to the foreign markets (Hanafy, 2004). Cucumber crop infested with large scale of different insects such as *Aphis gossypii* Glover (Hemiptera: Aphididae). It is considered one of the most damaging insects infesting vegetables crops either in the open field or under greenhouse conditions (Adriaan *et al.*, 2013). Also, *A. gossypii* beside its effects on leaves and fruits, transmit cucumber mosaic virus (CMV). It causes a serious disease of narrow-leafed lup in (Deborah *et al.*, 2012). Marabi *et al*. (2017) stated that aphids cause sporadic yield losses due to direct feeding damage.They reported that the aphid *A. gossypii* a harmful pest on most vegetables crops and causes direct damage by reducing plant vigor and indirect damage by honeydew secretion and transmission of several viruses. Red spider mite, *Tetranychus urticae* Koch. (Acari:Tetranychidae) also is considerd as one of the most important pests infesting cucumber plants both in the open field and under glasshouse conditions. *T. urticae* is a species of plant-feeding mite generally considered to be a serious pest on cucumber plants and other vegetables crops. It is the most widely known member of the family Tetranychidae, it is a serious pest on cucumber plants under glasshouse conditions (Derek, 2013).

Cytokinin hormone (CKs) is considered one of the most famous and important phytohormon. There are more studies showed the important role of this hormone for growth regulators plants and its role for change morphological and physiological plant adjectives when used by different concentrations. Marcel (2015) reported that cytokinins are plant hormones that have among many other functions on the morphological and physiological characteristics on different plants. Also, Giron *et al.* (2013) stated that phytohormone cytokinins play important roles in regulating plant growth and defense against many dangerous pests. In India, Srivastava and Srikant (2015) studied effect of cytokinin hormone on photosynthesis, alkaloid and other parameters in *Papaver somniferum* L. and studied the influence of different foliar application of cytokinin hormone on growth, CO2, exchange rate, total chlorophyll, plant height and weight and fresh and dry weight of the leaves and shoots.

The aim of this research work is to study effect of treated cucumber plants var. *Cucumis sativus* L. by different concentrations of cytokinin hormone CKs (25 ppm, 45 ppm and 65 ppm) on the infestation by *A. gossypii* and *T. urtiace* at two locations, Giza Governorate and Qalubiya Governorate during 2018 season under glasshouse conditions. As well as , this research work was carried out to study effect of treated cucumber plants by the same concentrations of the same hormone on the morphological characteristics and internal components of treated cucumber plants.

Materials and methods

1. Experimental design:

This study was conducted on cucumber plants (*C. sativus*) at two locations , Giza and Qalubiya Governorates during 2018 season. Plants were cultivated at both locations at the same time in a timely manner for the cultivation of cucumber (early summer planting) during period (February – April). At both locations we used two glasshouses, each glasshouse divided into four parts, three parts for the three treatments (Three concentrations of CKs) and the fourth part left as control. In the first treatment we immersion cucumber seedlings in low concentration of CKs (25ppm) for period 24 hour before cultivated. In the second treatment we immersion seedlings in medium concentration of CKs (45ppm) for period 24 hour before cultivated. In the third treatment we immersion seedlings in high concentration of CKs (65ppm) for period 24 hour before cultivated. Lastly, in the fourth treatment we did not immersion cucumber seedlings in any hormone before cultivated, this treatment used as control. These

seedlings cultivated under glasshouse conditions at both two locations at the same time. Then it was conducted all agricultural operations in a manner quite similar at two locations. The normal and recommended agricultural practices were applied, also no chemical control against insects were used during the whole experimental period. An artificial infestation with *A. gossypii* and *T. urticae* were done at the first and second glasshouses, respectively at the same time in the two locations. It is proven accurate observations of the infestation by the two pests number in all plants biweekly. Directly counting was done biweekly during the seasons at both locations allover plants.

2.Laboratory design:

Laboratory studies were carried out to study effect of treated cucumber plants by different concentrations of cytokinin hormone (CKs) on the morphological characteristics of treated cucumber plants such as root length (cm), shoot length (cm) and plant height (cm) and comparing these characteristics with control plants , did not treat with any hormone. Also, these laboratory experiments were carried out to study effect of treated cucumber plants by the same concentrations of cytokinin hormone (CKs) on the internal components of treated cucumber plants such as total protein (mg/g), total sugar (mg/g), starch (mg/g), amino acids (mg/g) and total phenols (mg/g), and comparing these concentrations with control plants did not treat with any hormone.

3. Determination of protein banding pattern:

3.1.Total protein extraction:

Total proteins were extracted from 0.5 kg fresh tissue of cucumber leaves. The tissues were ground in liquid nitrogen with a mortar and pestle. Then few mls of tris buffer extraction were added (1:2, tissue: buffer). The medium of extraction contained tris-HCL buffer (0.1mM tris, pH 7.5, 4m M B-mercapto ethanol, 0.1m M EDTA-Na_2, 10m M KCl and 10m M $MgCl_2$). The crude homogenate was centrifuged at 10.000xg for 20min. The supernatant was used for gel analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970).

3.2. Loading on a gel:

3.2.1. Gel preparation:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% acrylamide and 0.8% bis-acrylamide running gel consisting of 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. Stacking gel (10 mm) was made using 4.5% acrylamide containing 0.8% bis-acrylamide in 0.125 M Tris–HCl (pH6.8) and 0.1% SDS. The electrophoresis buffer contained 0.025 M Tris-HCl, 0.19 glycine and 0.1% SDS. The samples were homogenized in 0.12M Tris-HCl (pH 6.8), 0.4 SDS, 10 B-mercaptoethanol, 0.02% bromophenol bule and 20% glycerol. The samples were then heated for 3min. in a boiling water bath before centrifugation. The gel was run under cooling at 90v for the first 15min., then 120v the next 0.5 hour and finally 150v for the remaining 1.5 hour (Sheri *et al*., 2000).

3.2.2. Sample loading:

A known volume of protein sample was applied to each well by micropipette. Control wells were loaded with standard protein marker.

3.2.3. Electrophoresis conditions:

The running buffer was poured into pre-cooled (4°C) running tank. The running buffer was added in the upper tank just before running, so that the gel was completely covered. The electrodes were connected to power supply adjusted at 100 v until the bromophenol blue dye entered the resolving gel, and then increased to 250v until the bromophenol blue dye reaches the bottom of the resolving gel.

3.2.4.Gel analysis:

Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio–Rad Quantity one Software version 4.0.3.

4. Statistical analysis:

In these experiments, effect of treated cucumber plants by different concentrations of (CKs) on the infestation by *A. gossypii* and *T. urticae* and effect of treated cucumber plants by different concentrations of (CKs) on the morphological characteristics and the internal components of cucumber plants were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988).

Results and discussion

1. **Effect of treated cucumber plants by different concentrations of (CKs) on the infestation by *Aphis gossypii* and *Tetranychus urtiace*.**
2. **In Giza Governorate :**

Data tabulated in Table (1) show the population fluctuation of *A.gossypii* and *T. urticae* on cucumber plants which treated by different concentrations of citokinin hormone (CKs) in Giza Governorate during 2018 season. Data showed that cucumber plants which treated by low concentration of CKs (25ppm) were lower infestation by both *A. gossypii* and *T. urticae* compared to control, cucumber plants which treated by medium concentration of CKs (45ppm) were had no significant differences in the infestation by the two pests compared to control and cucumber plants which treated by high concentration of CKs were higher infestation by both the two pests compared to control.Whereas mean number of aphid in control was (37.9 aphid/leaf), mean number in the first treatment (low concentration of CKs) was (18.4 aphid/leaf), mean number in the second treatment (medium concentration of CKs) was (35.6 aphid/leaf) and mean number of aphid in the third treatment (high concentration of CKs) was (40.6 aphid/leaf). Also, as the same trend, the mean number of *T. urticae* in control was (24.5 pest/leaf), mean number in the first treatment was (13.2 pest/leaf), the mean number in the second treatment was (22.6 pest/leaf) and the mean number in the third treatment was (28.9 pest/leaf).

Table (1): Population fluctuation of *Aphis gossypii* and *Tetranychus urtiace* on cucumber plants which treated bydifferent concentrations of cytokinin hormone (CKs) in Giza Governorate during 2018 season.

Date	*Aphis gossypii*				*Tetranychus urtiace*			
	25 ppm	**45 ppm**	**65 ppm**	**Control**	**25 ppm**	**45 ppm**	**65 ppm**	**Control**
1/2/2018	10.7 ^{c}	26.5 ^{a}	29.8 ^{b}	28.3 ^{a}	6.3^{b}	15.7^{a}	19.3 ^{b}	17.2^{a}
8/2/2018	12.6 ^{c}	28.2 ^{a}	33.4 ^{c}	31.2 ^{a}	8.4 ^{c}	18.9^{a}	21.7 ^{b}	19.4^{a}
15/2/2018	14.2^{b}	31.7 ^{b}	35.1^{c}	33.7 ^{a}	10.8 ^{c}	19.2^{a}	24.3 ^{b}	21.5^{a}
22/2/2018	17.4 ^{c}	33.1 ^{a}	37.7 ^{b}	35.6 ^{a}	12.3^{b}	21.6^{a}	28.5^{c}	22.3^{a}
1/3/2018	19.7 ^{c}	35.9 ^{b}	39.8 ^{b}	38.8 ^{a}	13.2 ^{c}	23.8^{a}	31.1 ^{c}	24.5 ^{a}
8/3/2018	21.4^{b}	38.2 ^{b}	41.7^{c}	40.2 ^{a}	15.8^{b}	24.7^{a}	33.8 ^{b}	25.9 ^{a}
15/3/2018	23.1 ^{c}	40.8 ^{a}	43.8^{b}	42.4 ^{a}	17.2 ^{c}	25.5^{b}	35.2^{c}	27.7 ^{a}
22/3/2018	24.7 ^{c}	41.5 ^{a}	45.9 ^{b}	43.4 ^{a}	18.3 ^{c}	27.9^{b}	36.4^{c}	29.5 ^{a}
29/3/2018	25.3^{c}	43.2 ^{a}	48.7^{c}	45.5 ^{a}	20.5 ^{c}	29.1^{a}	37.5 ^{b}	31.3 ^{a}
5/4/2018	22.6 ^{c}	40.3 ^{b}	45.4 ^{b}	41.8 ^{a}	17.7^{b}	25.9^{a}	30.1 ^{b}	28.7 ^{a}
12/4/2018	18.2 ^{c}	37.2 ^{a}	43.5 ^{b}	39.2 ^{a}	13.8 ^{c}	22.3^{b}	28.4 ^{b}	25.5 ^{a}
19/4/2018	15.8^{b}	34.2 ^{b}	42.6^{b}	37.9 ^{a}	10.1 ^{c}	20.5^{b}	26.3 ^{b}	23.4 ^{a}
26/4/2018	13.2^{c}	31.5 ^{a}	39.8 ^{c}	34.3 ^{a}	7.3^{c}	19.1^{a}	23.8^{c}	21.3 ^{a}
Total	**238.9**	**462.3**	**527.2**	**492.3**	**171.7**	**294.2**	**376.4**	**318.2**
Mean	**18.4**	**35.6**	**40.6**	**37.9**	**13.2**	**22.6**	**28.9**	**24.5**
F(0.05)	**732.25**				**845.63**			
LSD	**1.025**				**1.032**			

Means within columns bearing different subscripts are significantly different (P< 0.05)

1.2. In Qalubiya Governorate:

Data tabulated in Table (2) showed the population fluctuation of *A.gossypii* and *T. urticae* on cucumber plants which treated by different concentrations of citokinin hormone (CKs) in Qalubiya Governorate during 2018 season.

Table (2): Population fluctuation of *Aphis gossypii* and *Tetranychus urtiace* on cucumber plants which treated by different concentrations of cytokinin hormone (CKs) in Qalubiya Governorate during 2018 season

Date	*Aphis gossypii*				*Tetranychus urtiace*			
	25 ppm	**45 ppm**	**65 ppm**	**Control**	**25 ppm**	**45 ppm**	**65 ppm**	**Control**
1/2/2018	9.7 ^{c}	24.7 ^{a}	29.6 ^{c}	26.3 ^{a}	5.3^{c}	13.5 ^{a}	19.5 ^{b}	15.7^{a}
8/2/2018	10.6 ^{c}	27.5 ^{b}	31.5 ^{b}	29.2 ^{a}	7.4 ^{c}	15.7 ^{b}	20.3 ^{c}	17.3^{a}
15/2/2018	11.2 ^{b}	29.3 ^{a}	33.7 ^{c}	30.7 ^{a}	8.8 ^{c}	17.5 ^{b}	21.3 ^{b}	19.5^{a}
22/2/2018	13.4 ^{c}	31.2 ^{a}	35.9 ^{b}	33.6 ^{a}	10.2^{b}	20.6 ^{a}	23.5^{c}	21.3^{a}
1/3/2018	15.2 ^{c}	33.7 ^{b}	37.5 ^{b}	36.8 ^{a}	11.5 ^{c}	21.8 ^{a}	26.6 ^{b}	23.5 ^{a}
8/3/2018	17.5 ^{b}	35.9 ^{a}	39.8 ^{c}	38.2 ^{a}	13.7^{b}	23.1 ^{b}	28.8 ^{b}	25.4 ^{a}
15/3/2018	18.4 ^{c}	37.3 ^{a}	41.5 ^{c}	39.4 ^{a}	15.2 ^{c}	25.7 ^{a}	30.2^{c}	27.2 ^{a}
22/3/2018	20.3 ^{c}	38.7 ^{b}	43.8 ^{c}	41.4 ^{a}	17.3 ^{c}	27.5 ^{a}	33.4^{c}	28.5 ^{a}
29/3/2018	22.5 ^{b}	39.5 ^{b}	45.7 ^{c}	43.5 ^{a}	19.5 ^{c}	28.3 ^{a}	35.5 ^{b}	29.3 ^{a}
5/4/2018	18.6 ^{c}	37.4 ^{a}	43.1 ^{b}	39.8 ^{a}	16.7^{b}	24.9 ^{a}	30.2 ^{c}	26.7 ^{a}
12/4/2018	14.2 ^{c}	35.8 ^{b}	41.5 ^{b}	36.2 ^{a}	12.8 ^{c}	21.3 ^{b}	28.1 ^{c}	23.5 ^{a}
19/4/2018	12.8 ^{b}	33.2 ^{a}	38.6 ^{c}	34.9 ^{a}	8.1 ^{c}	19.5 ^{a}	26.3 ^{b}	20.4 ^{a}
26/4/2018	10.2 ^{c}	30.2 ^{a}	35.8 ^{b}	32.3 ^{a}	5.3^{b}	15.1 ^{a}	23.8^{c}	17.3 ^{a}
Total	**194.6**	**434.4**	**498.0**	**462.3**	**151.8**	**274.5**	**347.5**	**295.6**
Mean	**15.0**	**33.4**	**38.3**	**35.5**	**11.7**	**21.1**	**26.7**	**22.7**
F(0.05)	**645.32**				**765.21**			
LSD	**1.043**				**1.035**			

Means within columns bearing different subscripts are significantly different (P< 0.05)

Data showed that cucumber plants treated by low concentration of CKs (25ppm) were lower infestation by both *A. gossypii* and *T. urticae* compared to control, cucumber plants treated by medium concentration of CKs (45ppm) were had no significant differences in the infestation by both the two pests compared to control and cucumber plants treated with high concentration of CKs were higher infestation by both two pests compared to control. Whereas mean number of aphid in control was (35.5 aphid/leaf), the mean number in the first treatment (low concentration of CKs) was (15.0 aphid/leaf), the mean number in the second treatment (medium concentration of CKs) was (33.4 aphid/leaf) and the mean number of aphid in the third treatment (high concentration of CKs) was (38.3 aphid/leaf). Also, as the same trend, the mean number of *T. urticae* in the control was (22.7 pest/leaf), the mean number in the first treatment was (11.7 pest/leaf), the mean number in the second treatment was (21.1 pest/leaf) and the mean number in the third treatment was (26.7 pest/leaf).

These results agreement with those obtained by Heba (2013) in Egypt who reported that the plants *Zea mays* treatment with low concentration of triacontanol hormone TRIA (35 ppm.) was low infestation with *Euprepocnemis plorans plorans* (Charpentier) (Orthoptera. : Acrididae) comparing to control. The plants treated with high concentration of the same hormone (50 ppm.) were high infestation with the same insect comparing to control. Also, Gupta *et al.* (2009) reported the role of CKs in pest control and reported that plants treated with low concentration of CKs were less infestation by insects than control plants. Singh and Bhattacharya (2001) recorded an efficient role of CKs in reduction of survivorship and developmental parameters of larvae of *Spilarctia oblique* Walker

(Lepidoptera: Arctiidae) upon feeding on diets containing CKs, referring to insecticidal activity of CKs. From the entire last, it was suggested the incorporation of CKs in the Integrated Pest Management (IPM) modules for pest control.

3. Effect of cytokinin hormone (CKs) on the morphological characteristics and internal components of cucumber plants:

Data tabulated in Table (3) show the effect of treated cucumber plants by different concentrations of cytokinin hormone (CKs) on the morphological characteristics and internal components of these plants.

Table (3): Effect of treated cucumber plants by different concentrations of cytokinin hormone (CKs) on the morphological characteristics and internal components of these plants during 2018 season

Adjective	25 ppm	45 ppm	65 ppm	Control
Root length (cm)	110.76^{c}	97.32^{a}	82.42^{b}	95.25^{a}
Shoot length (cm)	155.43^{c}	140.25^{a}	125.28^{a}	135.21^{a}
Plant height (cm)	266.19^{c}	237.57^{b}	207.70^{b}	230.46^{a}
Total protein (mg/g)	19.75^{c}	16.58^{a}	11.25^{a}	15.47^{a}
Total sugars (mg/g)	32.84^{c}	27.35^{a}	21.46^{b}	25.73^{a}
Strach (mg/g)	45.65^{c}	37.46^{a}	30.78^{b}	35.86^{a}
Amino acids (mg/g)	15.13^{c}	10.63^{b}	6.67^{a}	9.75^{a}
Total phenol (mg/g)	13.65^{c}	8.64^{a}	4.35^{b}	7.86^{a}

Means within columns bearing different subscripts are significantly different ($P< 0.05$)

Data showed that cucumber plants which treated with low concentration of CKs (25ppm) improved morphological characteristics of treated plants such as (root length, shoot length and plant height) and internal components of cucumber plants such as (total protein, total sugars, starch, amino acids and total phenols) compared to control, cucumber plants which treated with medium concentration of CKs (45ppm) were had no significant differences in both morphological characteristics and internal components of these plants compared to control, and cucumber plants which treated with high concentration of CKs had badly effect of the morphological characteristics and internal components of cucumber plants compared to control.

These results agreement with those obtained by Kumaravelu *et al.* (2000) in India who reported that the morphological characteristics such as (root length, shoot length, plant height and other morphological characteristics) and physiological characteristics such as (total protein, total sugars, starch, total phenol and other physiological characteristics) were improved when the treated plants with small and medium concentrations of cytokinin hormone (CKs) and became better than control. These characteristics were worse than control when treated plants with high concentration of (CKs). Shukla *et al.* (2013) in Netherlands studied effect of cytokinin hormone (CKs) at lower concentrations on growth, plant hormones and artemisinin yield in *Artemisia annua* L. and found when treated plants with (CKs) produced a statistically significant positive effect on artemisinin level as well as on plant height, leaf and herbage yield , but these adjectives decreased when treated plants with higher concentrations of (CKs). Also these results agreements with those obtained by Eriksen *et al.* (2015) in Oslo (Nerweg) who reported that when treated tomato and maize plants with cytokinin

(CKs) caused a significant increase in the dry weight of the tomato plants, leaf area and dry weight measurements of tomato leaves at different stages of development. Richard and Stanley (1981) in Michigan (United States) reported that cytokinin (CKs) increased fresh and dry weight and total reducible nitrogen (total N) of rice (*Oryza sativa* L.) seedlings.

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Effect of magnetic field on some biological and physiological aspects of the glassy clover snail *Monacha cartusiana* (Stylommatophora: Hygromiidae)

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Abstract :

The present work was carried out to investigate the effect of magnetic field (MF) on the biological and physiological responses of *Monacha cartusiana* (Müller) (Stylommatophora: Hygromiidae) and land snail one of the serious agricultural pests in Egypt. Snails exposed to magnetic field (5 similar magnet piece each one with a magnetic power 18 milli–tesla).The obtained results showed reduction in the percentage of egg hatchability (fertility) which recorded 62.16% for magnetic field and 91.01% for control groups and mean incubation period of *M. cartusiana* eggs for magnetic field was (35.5 days) compared to control (23.5 days). Also, result showed that magnetic field caused significant increase in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the hemolymph of tested land snail. While, exhibited significant decrease in the activity of alkaline phosphatase (ALP), acid phosphatase (ACP) and Lactate oxidase (LO) enzymes. On the other hand, the levels of total protein, total lipid and cholesterol content were significant decrease after exposure *M. cartusiana* to magnetic field.

Introduction

Land gastropods have greatly increased in economic importance and they are considered a group of the most serious pests attacking agricultural crops around the world (Barker, 2002). They cause great damage to vegetables, field crops, orchard trees as well as ornamental and medical plants (Abed, 2011 and Lokma, 2013). The glassy clover snail *Monacha cartusiana* (Müller) (Stylommatophora: Hygromiidae) causes damage to vegetables and field crops (El-Deeb *et al*., 2003). Magnetism and using magnetic field seems to be promising physical method in pest control (Hussein *et al*., 2014). Changes and alteration of the main components such as protein and lipids as well as the enzyme activity only appears under such stresses that pest exposed to stresses may be physical factors e.g. temperature, different types of waves e.g. gamma rays (Hussein *et al.,* 1999) and electro-magnetic waves (Hussein *et al.,* 2014).

The magnetic fields effects on chordates, fishes behavior (Krylov *et al*., 2013), orientation of reptiles and birds migration (Schneider *et al*.,1994), some mammals development and growth like mice (Sathon *et al*., 1996), orientation and metabolismof snails (Brown and Webb, 1960) and insect

orientation, development, behavior (Kandil *et al.*, 2018). Magnetic fields can induce changes in enzyme activity (Chen *et al*., 2009), the synthesis and release of neurohormons (Perić-Mataruga *et al*., 2008) and influence on nucleic acids and protein synthesis (Schmitz *et al*., 2004).

The aim of this work was to investigate the effect of magnetic field (MF) on some biological aspects such as egg hatchability and incubation period of land snail *M .cartusiana*. Also, to determine some physiological effect in hemolymph of *M. cartusiana*. The investigated biochemical parameters were the activity of vital enzymes such as aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and Lactate oxidase (LO) as well as total protein (TP), total lipid (TL) and cholesterol content.

Materials and methods

1. Collection and adaptation of snails:

Adults of the land snail *M. cartusiana* were handily collected from infested Egyptian clover and lettuce fields from Dakahlia Governorate. Healthy individuals were kept in a pot (50× 30 × 25cm), containing moist clay of about 7- 10cm height and were covered with muslin to prevent snails from escaping (Baker and Hawke, 1991). Snails were fed on fresh leaves of lettuce for 14 days to be a laboratory acclimatized. Dead and unhealthy snails were removed and only healthy ones with the same shell diameter were used in the experiments. Laboratory conditions at 25± 2ºC and 75± 5% soil moisture.

2. The procedures of the experiment:

The magnetic field was created in each treated pots by adjusting and fixing 5 similar magnet pieces in the four main directions of the pot in addition to the 5th piece in the central point. Each magnet piece with a magnetic power 18 milli– tesla (m.t.) measurements were carried out using Teslameter apparatus (Faculty of Engineering / Menoufia University). Each pot (15 cm diameter) contained five adult land snails*, M. cartusiana* and replicated thouree times, while control pots were with the same diameter and contain five snails without magnets pieces.The pots of control were in the same laboratory but far 1 meter from the magnets pots (treatment).Fresh food and moisture were supplied as required.The soil of each pot was examined daily (Staikou and Lazaridou- Dimitriadou, 1990) to search new clutches of eggs.

3. Incubation period and hatchability:

Newly deposited clutches of eggs laid under laboratory conditions were collected by a fine hair brush. Date of egg lying was estimated.Each batch of eggs was placed in pots containing 5 g of sterile moist soiland covered with black cover.The eggs were examined daily to record the date of hatching and incubation period. Percentage of egghatchability was calculated according to the following equation:

$$\text{Percentage of hatchability} = \frac{\text{No.of hatching eggs}}{\text{The total No. of eggs}} \times 100$$

4. Biochemical analysis:

4.1. Samples preparation:

Samples wee prepared according to El-Gohary and Genena (2011). Shells of tested snails were removed by making a cut around the whorls in a continuous manner starting at the aperture opening using bone scissors. Snails tissues were dissected out and all tissues of treated and control groups were homogenized in distilled water. The homogenates were centrifuged at 3000 rpm for 20 min. at 5^{o}C in refrigerated centrifuge. The deposits were discarded and the supernatants were kept in a deep freezer till use to determine the activities of biochemical parameters, such as aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), Lactate oxidase (LO) enzymes as well as total protein, total lipid and cholesterol content in hemolymph of control and treated snails.

4.2. Biochemical measurements:

- Aminotransferases (AST and ALT) activities were estimated by the method of **Reitman and Frankel (1957)**.
- Alkalinephosphatase (ALP) activity was estimated according to the method of Deutsche Gesellschaft für Klinische Chemie (DGKC) (1972).While, Acidphosphatase (ACP) activity according to the method of Kind and King (1954).
- Lactate oxidase (LO) was measured according to Babson and Babson (1973).
- Total proteinwas determined according to themethod of Bradford (1976), total lipid according to Frings *et al*. (1972) and total cholesterol according to Ellefson and Caraway (1976).

5. Statistical analysis:

Data were calculated analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less was considered significant. All statistical analysis was done with Cohort Software (2004).

Results and discussion

1. Effect of magnetic field on the total number of laid eggs and its hatchability of *Monacha cartusiana*:

Data in Table (1) showed reduction in the total number of eggs laid and number of hatching eggs resulted from exposed *M. cartusiana* to magnetic field. Results recorded 148 and 267 eggs for total number of laid eggs and number of hatching eggs were 92 and 243 eggs for magnetic field and control groups, respectively. Therefore, the percentage of hatchability (fertility) was 62.16% for magnetic field and 91.01% for control groups.

Table (1): Effect of magnetic fieldon the total number of laid eggs and its hatchability of *Monacha cartusiana*.

Treatment	Total No. of laid eggs	No. of hatching eggs	Percentage of hatchability
Magnetic field	148	92	62.16
Control	267	243	91.01

2 .Effect of magnetic field on the incubation period of *Monacha cartusiana* eggs:

The tabulated results in Table (2) clear that,incubation period of *M. cartusiana* which exposed to magnetic field were ranged between (25 and 46 days) and (16 and 31 days) for magnetic field and control groups, respectively. So, mean incubation period of *M. cartusiana* eggs for magnetic field was (35.5 days) compared to control (23.5 days). Levin and Ernst (1995) observed that a 30 mT static magnetic field applied to sea urchin eggs produced alterations in the time of cell division and induced two developmental abnormalities, exogastrulation and collapsed embryos. Also, delays hatching relative tocontrol groups. Maciej *et al*. (2011) found that direct exposure of eggs of the two subspecies, *H. aspersa maxima* and *H. aspersa aspersa* to direct magnetic field or alternating electromagnetic field of 5–10 μT has a negative effect compared to the control group. The effect of alternating field on the survival rate and growth rate of *H. aspersa* is positive or neutral, while the influence of direct field is more negative compared to the control group. Hussein *et al.* (2014) Showed that there was a linear negative relationship between the force of the magnetic field and the hatchability percentage in *Sitotroga cerralella* hatching eggs decreased from 90% in the control to 22% with the magnetic field.

Table (2): Effect of magnetic field on the incubation period and egg hatchability of *Monacha cartusiana* eggs.

Treatment	Av. No. of hatched eggs after (days)											Range (in days)		Mean
	16	19	22	25	28	31	34	37	40	43	46	Min	Max	
Magnetic	-	-	-	14.3	19.7	24.3	34.9	46.7	51.3	56.6	60.3	25	46	35.5
Control	17.9	31.1	42.7	68.2	84.3	86.7	-	-	-	-	-	16	31	23.5

3. Effect of magnetic field on some biochemical parameters in hemolymph of *Monacha cartusiana:*

3.1.Effect on aspartate aminotransferase (AST) and alanine aminotransferase (ALT):

Data in Table (3) and Figure (1) indicated the effect of magnetic field on the activity of AST and ALT enzymes in the land snail *M. cartusiana*. Results showed that magnetic field caused significant increases in the enzymes activity 131.45 U/L and 141.35 U/L for AST and ALT, respectively than control group 90.55U/L and 51.05 U/L. Tiwari and Singh (2005) found induction transamination in different tissues of the fresh water snail *Lymnaea acuminate* Lamarck (Gastropoda: Lymnaeidae) after sublethal exposure to the *Euphorbia tirucalli* latex extract. Significant changes in AST and ALT activities in the land snails pointed out to the functional disorder of the liver (Arfat *et al*., 2014).

3.2.Effect on alkaline, acid phosphatase and lactate oxidase enzymes:

Data in Table (3) and Figure (1) illustrated the effect of magnetic field on the activity of alkaline, acidphosphatase andlactate oxidase enzymes in *M.cartusiana* snail. Results revealed that ALP, ACP and LO activity significantly decreased in snails exposure to magnetic field where recorded 229.65 U/L; 1.56 U/L and 1.05 mg/dL for ALP, ACP and LO respectively, comparing with 368.5 U/L, 4.45 U/L and 2.42 mg/dL in control group. Ljiljana *et al*. (2010) found that a significant decrease in acid phosphatase activity after exposed *Helix pomati*a land snail to alternating magnetic field (ELF-MF) compared to the control group. El-Bolkiny *et al*. (2000) reported that DDC molluscicides caused significant decrease in the activity of lactate oxidase (LO)is an enzyme known to activate vitellogenesis and responsible for the egg formation and production in schistosomiasis snails by inhibition of the egg laying capacity. Reduction of ALP activity may be related to thecessation of protein synthesis dueto the effect of the toxin on the general metabolism of the animal (Henderson and Triebskorn, 2002).

Table (3): Effect of magnetic field on aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate oxidase (LO) enzymesactivity in *Monacha cartusiana.*

Treatment	Parameters				
	Aspartate transaminase (U/L)	Alanine transaminase (U/L)	Alkaline phosphatase (U/L)	Acid phosphatase (U/L)	Lactate oxidase (mg/dl)
Magnetic field	131.45^{a} ±2.61	141.35^{a} ±1.36	229.65^{b} ±1.01	1.56^{b} ±0.26	1.05^{b} ±0.09
Control	90.55^{b} ±2.59	51.05^{b}±1.70	368.5^{a}±3.18	4.45^{a}±0.32	2.42^{a}±0.17
LSD 0.05	10.15	6.05	9.25	1.13	0.54

Each value is the mean ± SE. Values followed by the same letter (s) in a column are not significantly different according to Duncan′ s test at level 0.05

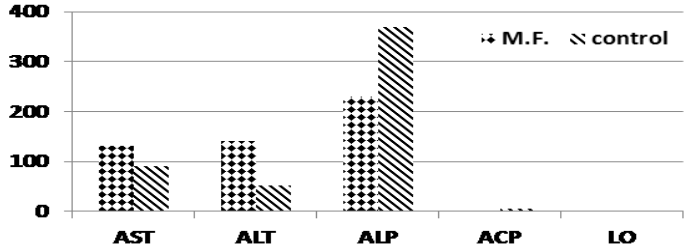

Figure (1):Effect of magnetic field on aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate oxidase (LO) activity in *Monacha cartusiana*.

3.3. Effect on total protein, total lipid and cholesterol:

Data presented in Table (4) and Figure (2) showed significant decrease in the levels of total protein, total lipid and cholesterol content. Result was recorded 1.6 g/dL, 1.76 mg/dL and 20.73 mg/dL for total protein, total lipid and cholesterol compared with control group 3.35 g/dL , 3.41 mg/dL and 46.05 mg/dL,respectively.These results agree with those reported by Thompson (1988) and Bielefeld (1991) they demonstrated that a depletion of hemolymph glycogen and lipids in *B. glabraia* snails caused inhibition of egg production and degenerative changes in its hermaphourodite gland. Gaber *et al*. (2007) reported that the depression in total lipid may be due to decline in lipid synthesizing capacity and / or due to an increase in the hydrolysis of hepatic lipid to combat the stress conditions. Hussein *et al*. (2015) investigated the effect of magnetic field of some insectsresults showed that each of body weight and growth rate as well as the physiological aspects was affected with the magnetic field.

Table (4): Effect of magnetic field on total proteins, total lipids and cholesterol content in *Monacha cartusiana*.

Treatment	Parameters		
	Total Protein (g/dl)	Total Lipid (mg/dl)	Cholesterol (mg/dl)
Magnetic field	1.6^{b} ±0.17	1.76^{b} ±0.16	20.73^{b} ±1.62
Control	3.35^{a} ±0.26	3.41^{a} ±0.11	46.05^{a} ±1.93
LSD 0.05	0.87	0.53	6.99

Each value is the mean ± SE. Values followed by the same letter (s) in a column are not significantly different according to Duncan′ s test at level 0.05

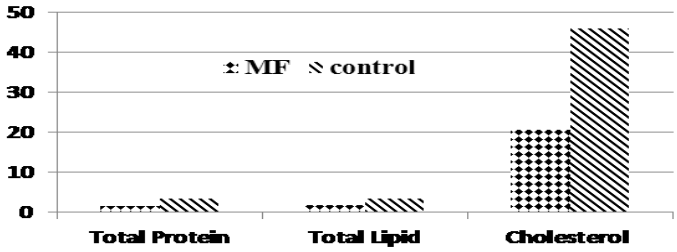

Figure (2): Effect of magnetic field on total proteins, total lipids andcholesterol content in *Monacha cartusiana*.

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Record of the beet fly *Pegomyiah yoscyami* (Diptera: Anthomyidae) and relation with its larval – pupal parasitoid *Opius nitidulator* (Hymenoptera: Braconidae) in Kafr EL-Sheikh Governorate

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Abstract:

All previous researches since 1981 (where sugar beet cultivation began in Egypt) till now - mentioned that the beet fly is *Pegomyia mixta* Vill. (Diptera: Anthomyidae) infested sugar beet fields at Kafr El-Sheikh Governorate. But, this work demonstrates that there is another sibling species of beet fly *Pegomyia hyoscyami* (Panzer) (Diptera: Anthomyidae). It was reared on sugar beet plants. The current study was carried out at Al-Nataf Village, Kafr El-Sheikh Governorate during two successive seasons 2017 / 2018 and 2018/ 2019 to assessment the role of the parasitoid; *Opius nitidulator* Nees (Hymenoptera: Braconidae) attacking larval stage of *P. hyoscyami* by collected 15 blotches (one blotch contain 2 to 5 larvae) per sample/ month and the correlation coefficient values between *P. hyoscyami* populations and its larval – pupal parasitoid *O. nitidulator* was carried out.The obtained data showed that the individuals of *P. hyoscyami* form 98.25% and 98.08% out of the total number of flies in both seasons, respectively. Whereas, the larval-pupal parasitoid, *O. nitidulator* constitute 100 and 100% out of the total populations in the two seasons. Statistical analysis indicated that a highly positive significant correlation between *P. hyoscyami* and its parasitoid. Values of "r" were 0.993 and 0.937 in both seasons, respectively.

Introduction

Sugar beet *Beta vulgaris L.* (Family: Chenopodiaceae) is a main source of sugar in Egypt and allover the world. In Egypt, the total area cultivated with sugar beet crop reached about 600000 feddans in 2018/2019 season, from which about 25% was cultivated in Kafr El-Sheikh Governorate (Ministry of Agriculture and Land Reclamation, 2018). The sugar beet fly *Pegomyia mixta* Vill. (Diptera: Anthomyidae) is one of the most prevalent and destructive insect of sugar beet crop which causing a substantial reduction in sugar content (40.50%) and root weight (31.50%) (Metwally *et al*., 1987). Many reviewers surveyed this insect pest on sugar beet cultivation since 1981 (where sugar beet cultivation began in Egypt) till now in Kafr El-Sheikh Governorate (Abo-Saied, 1987;

Abou-Attia, 1999; Mesbah, 2000; Bazazo, 2010; El- Mahalawy, 2011; Bazazo *et al.*, 2015 and El-Dessouki, 2019).

Opius nitidulator Nees (Hymenoptera : Braconidae) was recorded as internal larval parasitoid on beet fly attacking the full grown larvae before pupation and one adult parasitoid emerges individually from the host pupa (Ewais, 1990; Hassanein *et al*., 1993; Awadalla, 1997; El-Serwy, 2008; Bazazo, 2010 and Bazazo *et al*., 2017). Mousa (2005) indicated that braconid endoparasitoid was a main factor in regulating the population density of beet fly in sugar beet fields.

This current investigation in the first study in Kafr El-Sheikh Governorate to identify this new record species of the beet fly *P. hyoscyami*. Also, this study showed the correlation coefficient values between *P. hyoscyami* populations and its larval–pupal parasitoid, *O. nitidulator*.

Materials and Methods

The current study was conducted at Al-Nataf village, Kafr El-Sheikh Governorate during two successive seasons 2017/2018 and 2018/2019. About half feddan was cultivated with sugar beet variety (Pyramids) on 20th October in both seasons. Recommended agricultural practices of sugarbeet cultivation were achieved, and application of insecticides was excluded thouroughout the whole season.

1. The percentages of parasitism and emerged fly adults:

Fifteen blotches (one blotch contain 2 to 5 larvae) per sample every month from late December to early May in two seasons were randomly collected and transferred to the laboratory and kept in five petri dishes (9 cm), containing filter papers, until pupation. Newly pupae were put in other petri dishes till adult stage emergence (fly or parasitoid). The numbers of pupae, parasitoid and fly adult were recorded and the percentages of parasitism were calculated. Sampling started on 20th December and repeated every month for the first season till 14th May and 21st December until 10th May for the second season.

2. Identification of insect samples:

The adult of fly and its parasitoid were taken by a file brush and put in small vials containing alcohol 70%, after that these samples transferred to Insect Identification Unit – Plant Protection Research Institute – Giza – for identification by aid Prof. Dr. Ayman Ibrahim.

3. Statistical analysis:

Simple correlation coefficient values between *P. hyoscyami* and its parasitoid, *O. nitidulator* during the two seasons were calculated according to Snedecor and Cochouran (1989).

Results and discussion

1. Recording the percentages of emerged fly adults and parasitism by *Opius nitidulator:*

Data in Tables (1 and 2) indicated that the percentages of parasitism caused by *O. nitidulator* on sugar beet fly *P. hyoscyami* ranged between 16.67 to 40.90% and 0.00 to 52.38% in 2017/2018 and 2018/2019 seasons, respectively. While, the average of parasitism during the whole season were 36.36% and 36.25% in both seasons, respectively. Also, the percentages of emerged fly adults, *P. hyoscyami* ranged between 59.10 to 83.33% and 47.62 to 100.00% in 2017/2018 and 2018/2019 seasons, respectively. Whereas, the average of emerged fly adults during the whole season were 63.64% and 63.75% in two seasons, respectively.

Table (1): Percentages of emerged fly adults and parasitism during 2017/2018 season, per 15 blotches per sample/monthly.

Date of examination	No. of pupae	No. of parasitoid	% of parasitism	No. of fly adults	% of emerged fly adults
20/12/2017	6	1	16.67	5	83.33
21/1/2018	9	3	33.33	6	66.67
22/2/2018	10	3	30.00	7	70.00
24/3/2018	15	6	40.00	9	60.00
25/4/2018	22	9	40.90	13	59.10
14/5/2018	26	10	38.46	16	61.54
Total	88	32	36.36	56	63.64

Table (2): Percentages of emerged fly adults and parasitism during 2018/2019 season, per 15 blotches per sample/monthly.

Date of examination	No. of pupae	No. of parasitoid	% of parasitism	No. of fly adults	% of emerged fly adults
21/12/2018	4	0	0.00	4	100.00
23/1/2019	6	2	33.33	4	66.67
25/2/2019	14	4	28.57	10	71.43
22/3/2019	16	5	31.25	11	68.75
26/4/2019	19	7	36.84	12	63.16
10/5/2019	21	11	52.38	10	47.62
Total	80	29	36.25	51	63.75

These results demonstrated that the beet fly *P. hyoscyami* is a new record insect species on sugar beet plants in Kafr El-Sheikh Governorate. As the authors aware, this study is the first investigation on this insect species and very little literature is known about it in sugar beet fields at Kafr El-Sheikh Governorate. Anonymous (2019) reported that *P. hyoscyami,* the beet leaf miner or spinach leaf miner is a grey fly about (0.24 inch) long (Figure,1). It lays egg masses on the undersides of leaves of beet plants. Every egg masses contain (2 to 5 eggs) develop into larvae that burrow into the leaf hollowing out large patches of the leaf between leaf surfaces, often damage large parts of the leaf Figure (2). Saiko (1976) showed that *P. hyoscyami* is a sibling species to *P. betae* and *P.mixta.* Angelova (2007) demonstrated that *P. hyoscyami* is found in Egypt, and *O. nitidulator* an important parasitoid to its larvae.

Figure (1): Larvae and adult of *Pegomyia hyoscyami.*

Figure (2): Infection symptoms of *Pegomyia hyoscyami.*

2. Correlation coefficient values between *Pegomyia hyoscyami* and its parasitoid *Opius nitidulator* during 2017/2018 and 2018/2019 seasons:

Correlation coefficient values were calculated considering the record of the percentages of emerged fly adults and percentages of parasitism were found in Tables (1 and 2). Data presented in Table (3) indicated that a highly positive significant correlation between *P. hyoscyami* and its parasitoid, *O. nitidulator* in two seasons. Values of "r" were 0.993 and 0.937 in 2017/2018 and 2018/2019 seasons, respectively.

These results showed that *O. nitidulator* as a main factor in regulating the population numbers of *P. hyoscyami* in sugar beet fields at Kafr El-Sheikh Governorate.

Table (3): Correlation coefficient values between *Pegomyia hyoscyami* and its parasitoid *Opius nitidulator.*

Seasons	*Pegomyia hyoscyami* X *Opius nitidulator*	
	"r" value	Status of significant
2017/2018	0.993**	Highly significant, P < 0.01
2018/2019	0.937**	Highly significant, P < 0.01

3.Identification of insects by insect identification unit, Agricultural Research Center:

The results illustrated in Table (4) showed that the individuals of *O. hyoscyami* form 98.25% and 98.08% out of the total numbers in two seasons, respectively. Whereas, the larval – pupal parasitoid, *O. nitidualtor* constitute 100% out of the total individuals in two seasons according to Insect Identification Unit–Plant Protection Research Institute, Agricultural Research center.

Table (4): Identification of insect specimens, during 2017/2018 and 2018/2019 seasons.

Seasons	Total of pupae	Parasitoid			Flies adult		
		No.	Species	%	No.	Species	%
2017/2018	88	32	*Opius nitidulator*	100%	57	*Pegomyia hyoscyami* (56)	98.25
						Pegomyia mixta. (1)	1.75
2018/2019	80	29	*Opius nitidulator*	100%	52	*Pegomyiah yoscyami* (51)	98.08
						Pegomyia mixta (1)	1.92

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Effect of the infestation by *Aphis nerii* and *Tetranychus urticae* on the vase live period of jasmine flowers under glasshouse

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Abstract:

This study was carried out to study effect of infested jasmine flowers (*Jasmine* spp. Family Oleaceae) by *Aphis nerii* (Boyer) (Hemiptera:Aphididae) and *Tetranychus urticae* Koch. (Acari: Tetranychidae) on the vase life period (the flowers life after picking) of jasmine flowers under glasshouse conditions at two locations, El-Orman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during successive season 2018. This is because vase life period is very important parameter in cut flowers and there are many factors affected on it. Therefore this study divided into two parts, first part studied effect of infested jasmine flowers by *A. nerii* and *T.urticae* on the vase life period of jasmine flowers after picking. Second part studied effect of infested jasmine flowers by the same pests on the internal components of these flowers which correlated with vase life period such as total sugar and total protein. Results showed that the infestation by *A. nerii* reduced the vase life period of jasmine flowers after picking more the infestation by*T. urticae* compared to control (which non infested by the same pests). Also results showed that the infestation by *A. Nerii* reduced total suger and total protein at the infested jasmine flowers more than the infestation by *T.urticae* compared to control. Lastly, results showed that the infestation by *A. nerii* and *T.urticae* changed the number and arrange of the protein banding patterns (amino acids) of infested jasmine flowers petals compared to control.

Introduction

Jasmine flowers (*Jasmine* spp. Fam. Oleaceae) is considered one of the most important and popular cut flowers in Egypt and allover the world which cultivated in the open field and under greenhouse conditions. A jasmine flower is a very popular flower around the world especially in the tropics because of its unique fragrance. Also, its cultivated area increased gradually during the last years, especially in the new reclaimed areas for purposes local consumption and exportation to the foreign markets, this beside its uses in different medicinal purposes.The human love to the jasmine flowers due to their beautiful colors, style of flowers, smiles

and tolerant the inferable weather factors (Fishman *et al*. , 2015).

Jasmine flowers infested with large scale of insects belong to many orders and families such as *Aphis nerii* (Boyer) (Hemiptera: Aphididae) and *Tetranychus urticae* Koch. (Acari: Tetranychidae) which are consider important pests of jasmine flowers and many other flowers. Jaskiewicz (2012) who reported that the strong infestation by *A.nerii* resulted in the deformation of stems, leaves and flowers of jasmine plants. Derek (2015) in Australia who reported that *A. nerii* and *T. urticae* are serious pests on jasmine flowers, andthey feed mainly on the young leaves and developing flower-buds of jasmine flowers.

This study was carried out to study the effect of infested jasmine flowers by *A.nerii* and *T.urticae* on the vase life period of jasmine flowers under glasshouse conditions at two locations, El-Orman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during successive season 2018.

Materials and methods

1. Experimental design:

This study was conducted on jasmine flowers grown in two locations El-Orman Garden (Giza Governorate) and International Garden (Alexandria Governorate) under glasshouse conditions during successive seasons 2018. The glasshouse in each garden with an area of 27x45 m of each one was divided into three parts, first part left as control, second part had artificially infestation by *A. nerii* and the third part had artificially infestation by *T. urticae*. Each part contains 5 plots (3x5 m^2) for each, and each part isolated completely from others. Jasmine seedlings were planted in glasshouse conditions at the same time on November (the planting time of jasmine plants). All agricultural operations of irrigation and fertilization and others are completely identical in the two glasshouses were done without application of any insecticide.

Artificially infestation was done by *A.nerii* in the second part and by *T.urticae* in the third part with careful observation of the mean numbers of these pests during plant growth period and especially during the flowering stage from February-August. At the end of the first growing season, 100 flowers were collected from each part at the two locations. At both of two glasshouses all postharvest treatments were identical but conducted separately. Until the arrival of the flowers for the final stage, a stage put flowers in Wares glass (vase) where each group is divided into five containers respective 20 flower per each one (vase) and in the presence of water only without adding any other materials prolong or reduce the period of the existence or the life of flowers in glassware. With taking into account the complete separation between the containers and control containers with daily monitoring of the status of flowers in both of the two glasshouses.

2. Effect ofinfestation by *Aphis nerii* and *Tetranychus urticae* on the internal components of jasmine flowers:

These experiments was carried out to study effect of infestation by *A.nerii* and *T.urticae* on the vase life period of jasmine flowers thourough study effect of the infestation by the same pests on the internal components of jasmine flowers specifically two elements (total sugars and total protein) which have strong correlated with the vase life period.

3. Determination of protein banding pattern:

3.1. Total protein extraction:

Total proteins were extracted from 0.5 kg fresh tissue of jasmine flowers. The tissues were ground in liquid nitrogen with a mortar and pestle. Then few mls of tris buffer extraction were added (1:2, tissue: buffer). The medium of extraction contained tris-HCL buffer (0.1mM tris, pH 7.5, 4mM B-mercaptoethanol, 0.1mM EDTA-Na_2, 10mM KCl and 10mM $MgCl_2$). The crude homogenate was centrifuged at 10.000xg for

20min. The supernatant was used for gel analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of **Laemmli (1970).**

3.2. Loading on a gel:

3.2.1. Gel preparation:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 12.5% acrylamide and 0.8% bis acrylamide running gel consisting of 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. Stacking gel (10 mm) was made using 4.5% acrylamide containing 0.8% bis-acrylamide in 0.125 M Tris–HCl (pH6.8) and 0.1% SDS. The electrophoresis buffer contained 0.025 M Tris-HCl, 0.19 glycine and 0.1% SDS. The samples were homogenized in 0.12M Tris-HCl (pH 6.8), 0.4 SDS, 10 B-mercaptoethanol, 0.02% bromophenolbule and 20% glycerol. The samples were then heated for 3min. in a boiling water bath before centrifugation. The gel was run under cooling at 90v for the first 15min, then 120v the next 0.5 hour and finally 150v for the remaining 1.5hour. **(Sheri *et al*., 2000).**

3.2.2.Sample loading:

A known volume of protein sample was applied to each well by micropipette. Control wells were loaded with standard protein marker.

3.2.3.Electrophoresis conditions:

The running buffer was poured into pre-cooled (4°C) running tank. The running buffer was added in the upper tank just before running, so that the gel was completely covered. The electrodes were connected to power supply adjusted at 100 v until the bromophenol blue dye entered the resolving gel, and then increased to 250v until the bromophenol blue dye reachs the bottom of the resolving gel.

3.2.4.Gel Staining and distaining:

After the completion of the run, gel was placed in staining solution consisting of 1g of Coomassie Brilliant bule–R-250; 455 ml methanol; 90ml glacial acetic acid and completed to 1L with deionized distilled water. The gel was destained with 200ml destaining solution (100ml glacial acetic acid, 400ml methanol and completed to 1L by distilled water) and agitated gently on shaker. The destaining solution was changed several times until the gel background was clear.

3.2.5.Gel analysis:

Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio–Rad Quantity one Software version 4.0.3.

4.Statistical analysis:

In the experiments,effect of the infestation by *A.nerii* and*T.urticae* on the vase life period of the jasmine flowers and effect of the infestation by the same pests on the total soluble sugar and total protein of the Jasmine flowers were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988).The sugar and protein were analyzed by High Pressure Liquid Chouromatograph (HPLC).

Results and discussion

1. Effect of the infestation by *Aphis nerii* and*Tetranychus urticae* on the vase life period of jasmine flowers:

Data tabulated in Table (1) show means of life time (vase life period) of the jasmine flowers which infested by *A. nerii* and *T. urticae* compared to control (the flowers non infested) for the five varieties (colors) of jasmine flowers (yellow, red, pink, blue and white) at the two examined locations. Data obtained showed the means of vase life period of jasmine flowers in control which did not infested by any pests ranged from 9.7 to 12.3 days for the five varieties (colors) of jasmine flowers, means of vase life period of jasmine flowers which infested by*A. nerii* ranged from 4.9 to 6.8 days, while means of vase life period of jasmine flowers which infested by *T. urticae* ranged from 5.3 to 7.8 days.

Table (1): Effect of insect infestation by *Aphis nerii* and *Tetranychus urticae* on the vase life period of jasmine flowers after picking compared to control.

Jasmine	Vase life period per days				
	Aphis nerii	*Tetranychus urticae*	Control	F(0.05)	LSD
Yellow	6.3[c]	7.2 [b]	11.5[a]	215.44	1.052
Red	6.8[c]	7.5 [b]	10.3[a]	283.17	1.043
Pink	5.3[b]	6.5 [c]	9.7[a]	334.21	1.024
Blue	4.9[c]	5.3 [c]	10.2[a]	251.96	1.052
White	5.9 [c]	7.8 [b]	12.3 [a]	253.15	1.037

Means within columns bearing different subscripts are significantly different (P< 0.05)

Statistical analysis showed that high significant differences between the vase life period of jasmine flowers which infested by *A. nerii* and *T. urticae* compared to non-infested flowers (control) at both the five examined varieties of jasmine flowers. Whereas F (0.05) value and LSD value for the five examined varieties of jasmine were (215.44, 1.052), (283.17, 1.043), (334.21, 1.024) (251.96, 1.052) and (253.15, 1.037), respectively.

These results agreement with those obtained by Jaskiewicz (2012) in Poland who reported the effect of *A. nerii* feeding on the flowering of jasmine and reported that *A. nerii* when found in greater numbers caused deformation of the leaf blades, shorting of shoots and petioles, as well as deformation of the flowers. Miles (2015) in Australia reported that in warm weather *T. urticae* walks off buds of jasmine during a "critical period " coinciding with the opening of the sepals, and studied showed this behavior of pest feeding affected on the vase life period of these flowers after picking. Also, results obtained agreement with those obtained by Stone (2012) who studied effect of infested jasmine flowers by thouree species of aphids on the vase life period of these flowers and estimated the damage on these flowers as a result of infestation by these insects.

2. Effect of the insect infestation by *Aphis nerii* and *Tetranychus urticae* on the internal components of jasmine flowers:

2.1. Effect of the insect infestation by*Aphis nerii* and *Tetranychus urticae* on the total soluable sugar:

Data tabulated in Table (2) show the total soluble sugar content in different varieties (colors) of jasmine flowers after infestation by *A. nerii* and *T. urticae* compared to control. Whereas total soluble sugar content at the five varieties of jasmine flowers (yellow, red, pink, blue and white) which infested by *A. nerii* were 23.52, 25.16, 23.45, 21.52 and 22.37 (mg/g) respectively, total soluble sugar content at the five varieties of jasmine flowers which infested by *T. urticae* were 29.32, 30.54, 29.75, 27.15 and 26.32 (mg/g), respectively,while total soluble sugar content at the five varietiesof jasmine flowers in control which non infested by any pests were 33.25, 34.28, 32.25, 30.57 and 31.25 (mg/g), respectively. Generally, the infestation by *A. nerii* reduced total soluble sugar in all varieties of jasmine flowers more than the infestation by *T. urticae* compared to control. Statistical analysis in (Table, 2) showed high significant differences between the total soluble sugar in different jasmine varieties which infested by *A. nerii* and *T. urticae* compared to control, whereas F(0.05) value and LSD value for the five examined varieties of jasmine were (325.32, 1.045), (243.45, 1.034), (325.52, 1.052), (234.21, 1.032) and (352.15, 1.025), respectively.

Table (2): Determination of total soluble sugar (mg/g) in different colors of jasmine flowers infested by *Aphis nerii* and *Tetranychus urticae* compared to control.

Color	Determination of total soluble sugar (mg/g)				
	Aphis nerii	*Tetranychus urticae*	Control	F(0.05)	LSD
Yellow	23.52[c]	29.32[b]	33.25[a]	325.32	1.045
Red	25.16[b]	30.54[c]	34.28[a]	243.45	1.034
Pink	23.45[c]	29.75[b]	32.25[a]	325.52	1.052
Blue	21.52[c]	27.15[b]	30.57[a]	234.21	1.032
White	22.37[b]	26.32[b]	31.25[a]	352.15	1.025

Means within columns bearing different subscripts are significantly different (P< 0.05)

2.2. Effect of insect infestation by *Aphis nerii* and *Tetranychus urticae* on total protein:

Data tabulated in Table (3) showed the total protein content in different varieties (colors) of jasmine flowers after infestation by *A. nerii* and *T. urticae* compared to control. Whereas total protein content at the five varieties of jasmine flowers (yellow, red, pink, blue and white) which infested by *A.nerii* were 16.32, 15.28, 13.45, 18.72 and 17.25 (mg/g), respectively, total protein content at the five varieties of jasmine flowers which infested by *T. urticae* were 20.15, 19.31, 18.23, 21.14 and 20.35 (mg/g), respectively, while total protein content at the five varieties of jasmine flowers in control which non infested by any pests were 26.35, 27.42, 24.11, 27.35 and 26.44 (mg/g), respectively. Generally, the infestation by *A. nerii* reduced total protein in all varieties of jasmine flowers more than the infestation by *T. urticae* compared to control.

Table (3): Determination of total protein (mg/g) in different colors of jasmine flowers infested by *Aphis nerii* and *Tetranychus urticae* compared to control.

Color	Determination of total protein (mg/g)				
	Aphis nerii	*Tetranychus urticae*	Control	F(0.05)	LSD
Yellow	16.32[c]	20.15[c]	26.35[a]	265.33	1.033
Red	15.28[c]	19.31[b]	27.42[a]	322.15	1.052
Pink	13.45[b]	18.23[b]	24.11[a]	215.28	1.032
Blue	18.72[c]	21.14[c]	27.35[a]	234.15	1.044
White	17.25[c]	20.35[b]	26.44[a]	370.12	1.035

Means within columns bearing different subscripts are significantly different (P< 0.05)

Statistical analysis in (Table, 3) show high significant differences between the total proteins in different jasmine varieties which infested by *A. nerii* and *T. urticae* compared to control whereas F(0.05) value and LSD value for the five examined varieties of jasmine were (265.33, 1.033), (322.15, 1.052), (215.28, 1.032), (234.15, 1.044) and (370.12, 1.035), respectively.

2.3. Change in protein banding patterns:

Data tabulated in Table (4) showed the changes in protein banding patterns (amino acids) of infested jasmine flowers petals by *A. nerii* and *T. urticae* compared to control (non infested flowers). Also showed that the infestation by *A. nerii* and *T. urticae* affected on the number and arrange of the protein banding patterns (amino acids) of infested jasmine flowers.The obtained results are agreement with those obtained by Galeotti *et al.* (2014) who studied effect of the infestation by rose aphid *M. rosa e*on the interior components of jasmine flowers, they found that the total protein in the jasmine petals reduced as result to the infestation by aphid. Peng and Miles (2017) studied the changes in the internal components of jasmine flowers such as protein, sugar and vitamins which changed as result of infestation by aphid. Becker and Apel (2016) reported that the decrease in total protein may be due to the decrease in carbohydrate content which acts as a carbon source in protein synthesis in jasmine flowers due to the infestation by *T. urticae.*

Table (4): Change induced by infestation with *Aphis nerii* and *Tetranychus urticae* in the protein banding pattern (amino acids) of jasmine flowers.

No of band	M.wt. (kDa)	Marker (M)	Control	*Aphis nerii*	*Tetranychus urticae*
1	199.0	Glycen	_	_	_
2	115.0	Alanen	+	_	+
3	89.0	Valen	+	_	_
4	77.0	Liocen	+	_	+
5	65.0	Isoliocen	+	_	+
6	51.0	Brolen	+	+	+
7	44.0	Venilalanen	+	+	+
8	31.9	Treptovan	+	_	_
9	33.0	Methionen	+	+	+
10	31.0	Aspartek acid	+	+	+
11	30.7	Glotamic acid	+	+	_
12	25.0	Laycen	_	_	_
13	22.0	Argnen	_	_	_
14	25.4	Hesteden	+	_	+
15	19.9	Seren	+	+	_
16	12.7	Sestayn	_	_	+
17	11.14	Asparagen	+	+	+
18	11.2	Glotamen	+	+	_
Total	_	18	14	8	10

M.wt. : Molecular weights kDa : Kilo Dalton

Also, the obtained results are agreement with those obtained by Nichols (2010) in France who studied the quantitative changes in soluble sugars (glucose, fructose and sucrose) of jasminepetals as a result of infestation by thouree species of aphids and estimated the damage. Decheva *et al.* (2011) in Bulgaria investigated the changes in thetotal sugar (glucose, fructose, and sucrose), starch, free amino acid and protein in buds of jasmine flowers, the level of 12 free amino acids identified decreased as result of the infestation by two species of aphids.

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Effectiveness and biochemical effects of neem against different species of grasshoppers (Orthoptera: Acrididae)

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Abstract:

Efficacy of neem was tested against 3rd, 4th and 5th nymphal instars of different species of grasshoppers at El-Baharia Oasis Western Desert of western Egypt by using micron Ulva sprayer (ULVA+). Mortality percentages were calculated after 2, 4, 6, 8, 10 and 12 days post treatment. The results showed that there is no mortality in the check (untreated after 2, 4, 6, 8, 10 and 12 days). Data cleared that the percentages of mortality of nymphal instars of grasshoppers were 10, 15, 25, 30, 65, 88 % after 2, 4, 6, 8, 10 and 12 days post treatment, respectively. The effect of neem on ALP, AST and ALT activity were tested. ALP activity showed that significant decreased between treatment insect, 5.09, 11.6 and 14.22(U) and control 12.9, 14.4 and 16.2 (U) after 2, 4 and 6 days posttreatment. AST activity was significant increased between treatment insects 68, 57 and 49 U/gm body weight and control 22, 28 and 34 U/gm body weight after 2, 4 and 6 days posttreatment. Also ALT activity was significant increased between treatment insects 73, 76 and 69 U/gm body weight and control 19, 26 and 37 U/gm body weight after 2, 4 and 6 days after treatment.The efficacy of neem in all treatments can be useful for development safe elements for an IPM strategy to grasshoppers.

Introduction

Locust and grasshoppers (Orthoptera: Acrididae) are considered one group of the serious agricultural pests that cause considerable damage to different crops and pasture grasses in Africa and Asia particularly during outbreaks (Showler, 1993). Several species of grasshoppers such as; *Euprepocnemis plorans plorans*, *Hetracris annulosa*, *Acrotylus insubricus*, *Chourotogonus homalodemis*, *Acrididella nasuta*, *Catantops axillaris* and *Aiolopus strepens* are considered among the most were found to attack the agricultural crops in Egypt and many parts of the world. Also, locust and grasshoppers generally have very high reproductive rates and are able to respond to unfavourable climatic conditions with rapid population increase (Bateman *et al*., 1993). 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 neem

tree (*Azadirchta indica* A. Juss), from the Meliaceae (mahogany) family, known as Indian lilac, has long been recognized for its properties both against insects and in improving health (Barrek *et al*., 2004). The effect of antifeedant (Neem) and IGRs (Cascade) and their mixture at different concentrations on nymphal instar of the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) by feeding technique. The biochemical effects of neem and cascade on the mortality percentages, malformations and some biochemical changes were studied by Soltan (2014). Abdelbagi *et al*. (2019) investigated the potential of the systemic growth regulator effects of various neem seeds products against immature stages of desert locust infesting potted millet plants in Sudan. All neem seed product induced significant systemic antifeedant activity, ranging from 52 to 99% against the immature.

The present study aimed at studying the effect of neem against some different species of grasshoppers and some biochemical effects alkaline phosphatease (ALP), aspartate transferase (AST) and alanine transaminase (ALT) 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. **Chemical used (Neem):** Neem Force 0.15 % EC (Azaderachtin) ([22,23-3h$_2$] dihydroazadirachtin) at the rate 1 litre/ha.
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 10x25 = 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, there was no alive individual. Mortality counts were calculated after 2, 4, 6, 8, 10 and 12 days posttreatment 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 3rd, 4th and 5th 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/hour.
- **Swath width:**3m according to wind velocity.
- **Weather conditions at applications:**

Wind: 4–6 m/sec, measured by anemometer.

Temperature: 33^{o}C ± 2 ^{o}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.

3. Collection of haemlymph: according to the technique was followed as described by Amin (1998).

4. Alkaline phosphatase determinations:
Alkaline phosphatase (ALP) was determined according to the method described by Powell and Smith (1954).

5. Transaminase determination:

Aspartate transferase (AST) and alanine transaminase (ALT) were determined colormetrically according to the method of Reitman and Frankle (1957).

Statistical analysis:
Data were analyzed using general linear model procedures (SAS, 1995).

Results and discussion

1. Effectiveness of neem against different species of grasshoppers *:*

The effect of neem was tested under field conditions against 3rd, 4th and 5th different nymphal instars of the grasshoppers by using ULVA+ spraying equipment after 2, 4, 6, 8, 10 and 12 days post- treatment. Data in Table (1) showed that the efficacy of neem against nymphal instars of grasshoppers after 2, 4, 6, 8, 10 and 12 days posttreatment. Results showed that there is no mortality in the check (untreated after the same intervals dates). Data cleared that the percentages of mortality of nymphal instars of grasshoppers were 10, 15, 25 ,30, 65 and 88% after 2, 4, 6, 8 , 10 and 12 days posttreatment, respectively. The present result in this concern agreed with (Abdelbagi *et al*., 2019; Schmutterer and Feres, 1990; Soltan, 2014 and Nicol and Schmutterer, 1991).

Table (1): Mortality percentage of neem against nymphal instars of the grasshoppers, after 2, 4, 6, 8, 10 and 12days post treatment in the field.

Days after treatment	Neemmortality %	Controlmortality %
2	10	0
4	15	0
6	25	0
8	30	0
10	65	0
12	88	0

2.Biochemical effects of neem on alkaline phosphatase (ALP) aspartate transferase (AST) and alanine transaminase (ALT)activity to nymphal instars grasshoppers:

Data in Table (2) showed that, the effects of neem on ALP, AST and ALT activity. Neem show significant decreased that ALP activity between treatment insect 5.09, 11.6 and 14.22(U) and control 12.9, 14.4 and 16.2 (U) after 2, 4 and 6 days after treatment. And AST activity was significant increased between treatment insects 68, 57 and 49 U/gm body weight and control 22, 28 and 34 U/gm body weight after 2, 4 and 6 days after Neem treatment. Also ALT activity was significant increased between treatment insects 73, 76 and 69 U/gm body weight and control 19, 26 and 37 U/gm body weight after 2, 4 and 6 days after Neem treatment. These results agree with those obtained by (Abdel-Aal, 2002; Assar *et al*., 2012; El-Sheikh, 2002 and Soltan, 2014).

Table (2): The effect of neem on alkaline phosphatase (ALP) (U), aspartate transferase (AST) and alanine transaminase (ALT) (U/gm body weight) activity of nymphal instars grasshoppers.

Days	ALP (U)		AST (U/gm body weight)		ALT (U/gm body weight)	
	Treatment insects	Control insects	Treatment insects	Control insects	Treatment insects	Control insects
2	5.09^{c}	12.9^{a}	68^{a}	22^{c}	73^{a}	19^{c}
4	11.6^{b}	14.4^{a}	57^{a}	28^{c}	76^{a}	26^{c}
6	14.22^{b}	16.2^{a}	49^{b}	34^{c}	69^{b}	37^{c}
LSD	9.6	7.83	47.2	38.5	35.6	29.1

F:Measurement of distance between individual distributions.
Means with the same letter are not significantly different.
Means with the different letter are significantly different and a=*, b=, c***.**

Acid and alkaline phosphatases have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Tsumuki and Kanehisa, 1984). Sridhara and Bahat (1963) stated that the increase of both phosphatase enzymes during development is reflected in increase or decrease in acid-soluble phosphorus content. Transamiase enzymes were considered as key enzymes in the formation of non essential amino acids, which is formed inside the body not taken outside in metabolism of nitrogen waste and gluconensis (Mordue and Goldworthy, 1973).Azmi *et al*. (1998) stated that the transaminases (ALT and AST) enzymes help in the production of energy and serve as a strategic link between the carbohydrates and protein metabolism and are known to be altered during various physiological and pathological conditions.The efficacy of neem in all treatments can be useful for development safe elements for an IPM strategy to grasshoppers.

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Occurrence and population dynamics of the true spider and pests associated with wheat plants in Qalubiya and Beni–Suif Governorates

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Spiders, incidence, frequency, ecology, crops, aphid, thrips, *Tetranychus* and Egypt.

Abstract:

Spiders associated with wheat plants were recorded in Qalubiya and Beni-Suef Governorates in Egypt, during the period from January to May 2016 and 2017 seasons. Results revealed the occurrence of fifteen spider species belonging to eleven families, namely, Agelenidae, Araneidae, Dictynidae, Filistatidae, Linyphiidae, Lycosidae, Cheracanthiidae, Philodromidae, Salticidae, Theridiidae and Uloboridae. Agelenidae, Araneidae, Dictynidae, Linyphiidae, Lycosidae, Salticidae and Theridiidaefamilies were the most dominant with frequency percentage of 100% on wheat in two Governorates. Population density of spider families in Qalubiya Governorate reached the highest peaks on wheat in early March by 42 and in early May 44 individuals during 2016, whereas, it has one peak in early March recorded 49 individuals during 2017. However, the population density of spider families in Beni-Suef have two peaks in early March by 48 and in early May 51 individuals during first year, while in the second year the peaks in mid February by 50 and in mid March recorded 53 individuals. The two spotted spider mite *Tetranychus urticae* Koch. (Acari : Tetranychidae) and *Aphis gossypii* Glover (Hemiptera: Aphididae) has one annul peak in March, while the *Frankliniella tritici* (Fitch) (Thysanoptera : Thripidae) have one annul peak on mid of May in the two Governorates. The results suggested that the true spider can play an important role for control pests on wheat plant in the two Governorates.

Introduction

Wheat (*Triticum aestivum* L.) is a convenient, nutritious and economical source of food. It provides about 20% world food calories and food for nearly 40% of the world's population. The cereal is grown on 23% global cultivated area is for great importance in bread, diet, pharmaceutics and other industry but also important product of international trade for worldwide market (Istvan, 2006). Aphids a serious pest of wheat and other field crops is an important sucking pest of various field crops, fruits and vegetables. Aphid attacks started in 1st week of January in all plots and increased with the vegetative growth of plant and reached at peak level in the 3rd week of March and after that its population started to decrease and population of bio-control agents was low at

start but maximum during the March when aphid population was at its peak level (Aheer *et al.*, 2008 and Ullah *et al.*, 2014)

Spiders may play an important role in controlling field populations of economic agricultural pests infesting some important drops. True spider sare world wide distributed and occupy many ecological environments thourough agroecosystems. Taxonomists documented about 117 families, 4128 genera and 48288 species(World Spider Catalog, 2019). The majority of spider species are polyphagous generalist predators (Pekár *et al.*, 2011).

Spiders are important mortality agents of horticultural/agricultural pests such as aphids, leafhoppers, planthoppers, fleahoppers, mites and other soft bodies insect like lepidopterous larvae as well as some flying insect (Khan and Misra, 2004). Several spider species from various families Araneidae, Dictynidae, Lycosidae, Cheracanthiidae, Salticidae, Thomisidae and Theridiidae have been commonly found associated with different crops, weeds, ornamental plants and fruit trees (Ghabbour*et al.*, 1999; Sallam, 2002; Habashy *et al.*, 2005; El-Gepaly *et al.*, 2018 and Abo-Zaed *et al.*, 2019).

Ecological parameters and taxonomic importance of different species of spiders from fruit gardens, cotton fields, citrus and guava fruit gardens were investigated by many researchers (EI-Hennawy, 1992; Sallam, 1996; Mohafez, 2004; Maqsood, 2011 and El-Gepaly *et al.*, 2018). The aim of the present work was to investigate the occurrence and population density of the spiders and pests associated with different pests infesting wheat crop in Qalubiya and Beni-Suif Governorates from early January to mid May during 2016-2017.

Materials and methods

A detailed study was conducted at Qaha Station, Plant Protection Research Institute, Qalubiya Governorate and Sids Station, Plant Protection Research Institute Beni-Suif Governorate on wheat crop during two successive years 2016 and 2017.

1. Collected spider:

Spiders were collected by two methods:

1.1. Plants shaking methods:

The spiders on wheat foliage were collected by shaking the plants on a cloth or a shake sheet. This method is referred as the drop cloth method. Then wheat plants were shocked over the shaking white cloth (1m*1m) biweekly during the period from early January to mid May.

1.2. Pitfall trap methods:

Samples of the soil spiders fauna were collected from the study are by pit-fall method described by Uetz and Unzicker (1976) and Southwood and Henderson (2000). Ten traps were distributed in the experimental wheat area (1/2 feddan). In this study, the number of spiders trapped in primarily depends on their location activity. The traps were used in each sampling date in different plots, according to (Habashy *et al.*, 2005). The spiders after then brought to the laboratory for identification. Samples were conducted biweekly during the surveying period. The surveyed spiders were kept in glass vials containing 75% ethyl alcohol and droplets of glycerin.

2. Identification of true spider:

Identification of adult females is depending on shape of eyes and epigyneal plate of female or on the palp in case of male (Sallam, 2002). Identification of specimens followed the descriptions of (Petrunkevitch, 1939; Kaston, 1978 and Jocqué and Dippenaar-Schoeman, 2007). Characteristers of obtained families, genera and species were presented. In some cases, identification was possible only to the genus level.

$$\text{Population density (P.D.)} = \frac{\text{No. of individuals of species}}{\text{No of sample containing this species}}$$

$$\text{Frequency of occurrence (F.O. \%)} = \frac{\text{No of sample containing a species} \times 100}{\text{No of collected sample}}$$

3. Population dynamics of pests:

Thirty leaves were collected from 10 wheat plants from January to mid May during two seasons 2016-2017. Samples were collected in a polyethylene bags and

transferred to laboratory. To kill insects, piece of cotton moistened with chloroform put in each sample and left for 15 minutes. The sample was emptied in Petri dish and cleaned from plant residues. Then it was examined under stereomicroscope to separate and count the major insect pests. This process was performed at weekly intervals thouroughout the entire period of investigation. Specimens were mounted for light microscopy according to the procedure detailed by Kosztarab and Kozár (1988). While the spider mite individuals were counted by direct examination under stereo–microscope andcleared in Nesbitt solution for about 15 minutes after that, mounted on microscope slides in Hoyer's medium was used to set most mites on the slides (Jeppson *et al.*, 1975).

4. Statistical analysis:

Data were subjected to the statistical analysis. Monthly average temperatures (°C) and average relative humidity (RH.%) 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 spider and pests by using SAS statistical software (SAS Institute, 2003).

Results and discussion

1. Occurrence and frequencies of spider families associated with wheat during 2016-2017 seasons at Qaha in Qalubiya Governorate:

The obtained data in Table (1) showed that, the collected spiders were 15 different spider species belonging to 15 genera under 11 different families. The families as shown in Table (1) were Agelenidae, Araneidae, Dictynidae, Filistatidae, Linyphiidae, Lycosidae, Cheracanthiidae, Philodromidae, Salticidae, Theridiidae and Uloboridae. The highest abundant families in this study were observed in family Salticidae, including thouree different species. The salticid spiders were; *Plexippus paykulli* (Audouin), *Memenorus* sp. and *Euophourys granulate* Denis. Two species of the family Theridiidae were *Theridion melanosticum* (Cambridge) and *Enophognatha deserta* Lerey and Amitai., two species of the family Lycosidae were *Lycosa* sp. and *Pardosa* sp., while the other families contain one species , Agelenidae (*Tegenaria* sp.), Araneidae (*Neoscona* sp.), Dictynidae (*Dictyna* sp.), Filistatidae (*Filistata* sp.), Linyphiidae (*Bathyphantes* sp.), Cheracanthiidae (*Cheiracanthium inclusum* (O.P.Cambridge)), Philodromidae (*Thanatus albini* Audouin) and Uloboridae (*Uloborus walckenacrius* Latreille).

As shown in Table (1), the frequency of occurrence of the collected families, Agelenidae, Araneidae, Dictynidae, Filistatidae, Linyphiidae, Lycosidae, Cheracanthiidae, Philodromidae, Salticidae, Theridiidae and Uloboridae were 100, 100, 100, 95, 100, 100, 95, 85, 100, 100 and 65, respectively on wheat at Qaha in Qalubiya Governorate. The total numbers of spider were 645 individuals at Qaha in Qalubiya Governorate during 2016-2017 seasons. The family Salticidae was the highest abundant population of wheat plants during the seasons 2016-2017 at Qaha region and recorded 137 spider individuals, followed by Lycosidae recorded 93 individuals while, the lowest number of spiders were recorded from family Uloboridae 14 spider individuals Table (1). The highest population density of spider on wheat plant on family Salticidae were 6.85 followed by Lycosidae and Philodromidae were 4.65 and 4.18, respectively.

Table (1): Occurrence and frequencies of spider families associated with wheat during 2016-2017 seasons at Qaha in Qalubiya Governorates

Families and species	Total individuals of species	Total No. of samples containing species	Population density (P.D.)	Frequency of occurrence (F.O. %)
Agelenidae Koch, 1837	48	20	2.40	100
Tegenaria sp.	48	20	2.40	100
Araneidae Simon 1895	44	20	2.20	100
Neoscona sp.	44	20	2.20	100
Dictynidae Cambridge, 1871	40	20	2.00	100
Dictyna sp.	40	20	2.00	100
Filistatidae Ausserer, 1867	41	19	2.16	95
Filistata sp.	41	19	2.16	95
Linyphiidae Blackvall, 1859	56	20	2.80	100
Bathyphantes sp.	56	20	2.80	100
Lycosidae Sundevall, 1833	93	20	4.65	100
Lycosa sp.	49	20	2.45	100
Pardosa sp.	44	20	2.20	100
CheracanthiidaeWagner, 1887	47	19	2.47	95
Cheiracanthium inclusum (O.P.Cambridge)	47	19	2.47	95
Philodromidae (Thorell, 1870)	71	17	4.18	85
Thanatus albini Audouin, 1826	71	17	4.18	85
Salticidae Blackwall, 1841	137	20	6.85	100
Plexippus paykulli (Audouin, 1826)	48	19	2.53	95
Memenorus sp.	48	20	2.40	100
Euophourys granulate Denis, 1947	41	20	2.05	100
Theridiidae Sundevall, 1833	54	20	2.70	100
Theridion melanosticum (Cambridge, 1876)	36	16	2.25	80
Enophognatha deserta Lerey& Amitai, 1981	18	14	1.29	70
Uloboridae Cambridge	14	13	1.08	65
Uloborus walckenacrius Latreille, 1806	14	13	1.08	65
Total number	645			

2. Occurrence and frequencies of spider families associated with wheat during 2016-2017 seasons at Sids in Beni-Suef Governorate:

The obtained data in Table (2) showed that; the collected spiders at Sids in Beni-Suef Governorate were 15 different spider species belonging to 15 genera under 11 different families similar in Qalubiya Governorate. The highest abundant families in this study were observed in family Salticidae, including thouree different species recorded 179 individuals followed by Lycosidae recorded 109 individuals, while the lowest number 25 individuals was recorded in the Uloboridae during 2016-2017 seasons.

As shown in Table (2), the frequency of occurrence of the collected families, Agelenidae, Araneidae, Dictynidae, Filistatidae, Linyphiidae, Lycosidae, Cheracanthiidae, Philodromidae, Salticidae, Theridiidae and Uloboridae were 100, 100, 100, 100, 100, 100, 95, 90, 95, 95 and 65, respectively on wheat plants at Sids in Beni-Suef Governorate. The highest population density of spider on wheat plant on family Salticidae were 9.26 followed by Lycosidae and Uloboridae were 5.45 and 1.92, respectively.

Table (2): Occurrence and frequencies of spider families associated with wheat during 2016-2017 seasons at Sids in Beni-Suef Governorate.

Families and species	Total individuals of species	Total No. of samples containing species	Population density (P.D.)	Frequency of occurrence (F.O. %)
Agelenidae Koch, 1837	61	20	3.05	100
Tegenaria sp.	61	20	3.05	100
Araneidae Simon 1895	55	20	2.75	100
Neoscona sp.	55	20	2.75	100
Dictynidae Cambridge, 1871	45	20	2.25	100
Dictyna sp.	45	20	2.25	100
Filistatidae Ausserer, 1867	46	20	2.30	100
Filistata sp.	46	20	2.30	100
Linyphiidae Blackvall, 1859	62	20	3.10	100
Bathyphantes sp.	62	20	3.10	100
Lycosidae Sundevall, 1833	109	20	5.45	100
Lycosa sp.	59	19	3.11	95
Pardosa sp.	50	20	2.50	100
CheracanthiidaeWagner, 1887	75	19	3.95	95
Cheiracanthium inclusum (O.P.Cambridge)	75	19	3.95	95
Philodromidae (Thorell, 1870)	52	18	2.89	90
Thanatus albini Audouin, 1826	52	18	2.89	90
Salticidae Blackwall, 1841	176	19	9.26	95
Plexippus paykulli (Audouin, 1826)	72	19	3.79	95
Memenorus sp.	58	19	3.05	95
Euophourys granulate Denis, 1947	46	20	2.30	100
Theridiidae Sundevall, 1833	48	19	2.53	95
Theridion melanosticum (Cambridge, 1876)	29	18	1.61	90
Enophognatha deserta Lerey& Amitai, 1981	19	15	1.27	75
Uloboridae Cambridge	25	13	1.92	65
Uloborus walckenacrius Latreille, 1806	25	13	1.92	65
Total number	754			

The obtained results are in harmony with that conducted by Ghabbour *et al*. (1999) who surveyed spiders in 18 different crops in Menoufiya Governorate, using pitfall traps, and recorded 10 spider families on winter crops, where Lycosidae was the dominant family constituting about 80% followed by Linyphiidae, Philodromidae, Gnaphosidae and Tetragnathidae. Also these results agree with finding by Ghabbour *et al.*, 1999; Sallam, 2002; Habashy *et al.*, 2005; El-Gepaly *et al.*, 2018 and Abo-Zaed *et al.*, 2019.

The present investigation was an attempt to explore the relation between temperature and relative humidity and the population dynamics of certain common true spiders associated with wheat plantations during the cultivated seasons, 2016 and 2017 in Qalubiya and Beni-Suef Governorates.

3. Population density of different spider families associated with wheat:

3.1. In Qalubiya Governorate:

The different recorded population density, temperature and relative humidity data were obtained from Tables (3 and 4), the total number of spiders were 294 and 351 individuals during the first and second year, respectively in Qalubiya Governorate.The highest abundance of the collected spider was for the members of family Salticidae at Qaha region (63 individuals), followed by family Lycosidae (41 individuals) and the lowest number of members of family Uloboridae (4 spider individuals). The population of spider individuals was recorded with low numbers in early January and gradually increased in numbers reached its peak in early March recorded 42 individuals at average temperature and relative humidity were 20.7°C and 53.4%, after that, the population fluctuated and peaked in early May recorded 44 individuals at average temperature and relative humidity were 26.8°C and 41.6% at Qaha in Qalubiya 2016 season Table (3) and Figure (1).

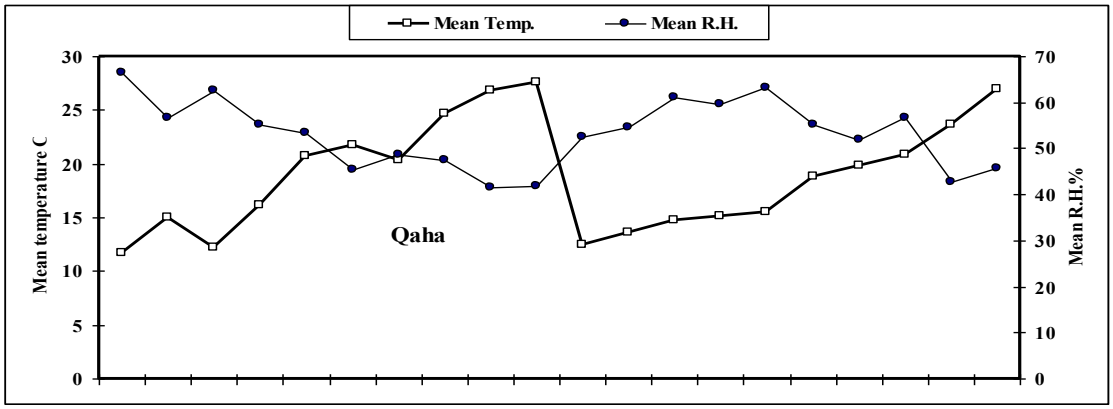
Similar results were observed in the second year 2017; members of the family Salticidae recorded the highest number were 74 individuals, while the lowest numbers were recorded in family Uloboridae 10 individuals. The population of spider individuals was recorded with low numbers in early January and gradually increased in numbers reached its peak in early March recorded 49 individuals at average temperature and relative humidity were 15.5 °C and 63.2 %, after that, the population gradually decreased till mid May (Table, 4). Statistical analysis present in the (Tables 3 and 4 and Figure, 1) showed that, the temperature was a significant positive correlation with the density of the population of spiders in the first year (0.66*), while non-significant negative correlation in the second year (-0.10). However, relative humidity had non-significant negative correlation with the spider population in the first year (-0.64) and positive in the second year (0.59).

Table (3):Population density of spider families associated with wheat during 2016 season at Qaha in Qalubiya Governorate.

Families	1/1	15/1	½	15/2	1/3	15/3	¼	15/4	1/5	15/5	Total
Agelenidae	2	2	2	2	3	3	4	2	2	2	**24**
Araneidae	2	1	3	3	4	4	3	2	1	1	**24**
Dictynidae	1	2	2	2	3	3	1	3	1	1	**19**
Filistatidae	2	3	2	2	3	4	2	1	2	1	**22**
Linyphiidae	3	3	2	3	4	4	2	2	3	3	**29**
Lycosidae	3	2	2	4	3	3	5	5	7	7	**41**
Cheracanthiidae	1	1	1	2	2	1	1	0	2	1	**12**
Philodromidae	0	0	4	4	7	1	0	1	5	3	**25**
Salticidae	3	4	5	4	9	7	5	2	12	12	**63**
Theridiidae	0	0	1	1	4	5	2	0	8	10	**31**
Uloboridae	1	1	0	0	0	0	0	0	1	1	**4**
Total	**18**	**19**	**24**	**27**	**42**	**35**	**25**	**18**	**44**	**42**	**294**
Mean Temp.	11.7	15.0	12.2	16.1	20.7	21.8	20.4	24.6	26.8	27.6	0.66*
Mean R.H.	66.3	56.6	62.7	55.1	53.4	45.5	48.5	47.6	41.6	41.7	-0.64

Table (4):Population density of spider families associated with wheat during 2017 season at Qaha in Qalubiya Governorate

Families	1/1	15/1	½	15/2	1/3	15/3	¼	15/4	1/5	15/5	Total
Agelenidae	1	1	2	2	4	4	3	3	2	2	**24**
Araneidae	1	1	2	2	2	3	4	3	1	1	**20**
Dictynidae	1	1	3	3	3	3	2	3	1	1	**21**
Filistatidae	2	2	2	2	2	2	3	3	0	1	**19**
Linyphiidae	1	1	2	3	5	5	3	3	3	1	**27**
Lycosidae	5	3	5	11	4	4	5	4	8	3	**52**
Cheracanthiidae	1	1	5	5	6	6	4	3	3	1	**35**
Philodromidae	1	2	8	3	13	10	1	2	3	3	**46**
Salticidae	3	4	5	8	8	8	17	13	5	3	**74**
Theridiidae	3	3	3	3	1	2	2	2	2	2	**23**
Uloboridae	1	1	1	1	1	0	1	2	1	1	**10**
Total	**20**	**20**	**38**	**43**	**49**	**47**	**45**	**41**	**29**	**19**	**351**
Mean Temp.	12.5	13.6	14.7	15.1	15.5	18.8	19.8	20.9	23.7	27.0	**-0.10**
Mean R.H.	52.4	54.6	61.2	59.5	63.2	55.2	52.0	56.7	42.6	45.7	**0.59**



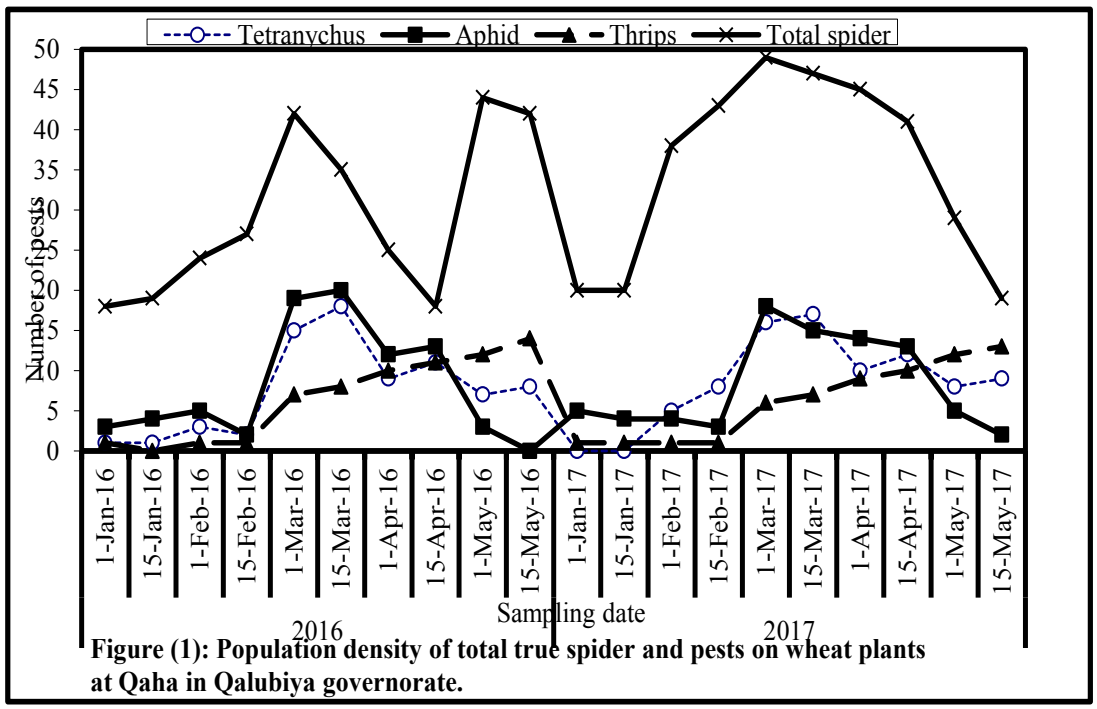


Figure (1): Population density of total true spider and pests on wheat plants at Qaha in Qalubiya governorate.

3.2. In Beni-Suef Governorate:

The results in Tables (5 and 6) and Figure (2) proved that, eleven spider families with annual total number of 349 and 405 individuals were collected from the wheat for 2016 and 2017, respectively (Tables, 5 and 6).

The highest abundance of the collected spider was for the members of family Salticidae at Sids region (89 individuals), and the lowest number of members of family Uloboridae (11 spider individuals). The population of spider individuals was recorded with low numbers in early January and gradually increased in numbers reached its peak in early March recorded 48 individuals at average temperature and relative humidity were 20.6°C and 51.6%, after that, the population fluctuated and peaked in early May recorded 51 individuals at average temperature and relative humidity were 26.9°C and 40.5% at Sids in Beni-Suef 2016 season (Table, 5 and Figure, 2).

The second year 2017; members of the family Salticidae recorded the highest number were 87 individuals, while the lowest numbers were recorded in family Uloboridae 14 individuals. The population of spider individuals was recorded with low numbers in early January and gradually increased in numbers reached its first peak in mid February recorded 50 individuals at average temperature and relative humidity were 15.1 °C and 58.2 %, whereas, the second peak was recorded in mid March was 53 individuals at average temperature and relative humidity were 18.9°C and 53.8% (Table, 6). Statistical analysis indicated that, the temperature was a highly significant positive correlation with the density of the population of spiders in the first year (0.77**), while non-significant negative correlation in the second year (-0.004). However, relative humidity had significant negative correlation with the spider population in the first year (-0.74*) and non-significant positive correlation in the second year (0.59)Tables (5 and 6) and Figure (2).

This result is accordance with Sallam (1996) and Hussein *et al.* (1998) observed that the five families Araneidae, Lycoside, Philodromidae, Salticidae and Theridiidae occurred in all the surveyed locations on citrus trees. Ghaobour *et al.* (1999) who found the shade of plants and the available humidity expressed as water requirement for each crop in addition to density of plants / acre directly affected abundance of activity density of soil fauna. Sallam (2002) who studied the influence of both temperature and the relative humidity on the population of the spiders in four locations in Egypt.

Table (5):Population density of spider families associated with wheat during 2016 season at Sids in Beni-Suef Governorate.

Families	1/1	15/1	½	15/2	1/3	15/3	¼	15/4	1/5	15/5	Total
Agelenidae	2	2	2	2	4	2	5	3	3	3	**28**
Araneidae	1	1	3	3	5	5	4	3	2	2	**29**
Dictynidae	1	1	1	1	4	4	1	4	2	2	**21**
Filistatidae	1	1	2	2	2	3	3	2	3	2	**21**
Linyphiidae	1	5	5	5	2	2	3	3	4	4	**34**
Lycosidae	3	4	1	3	5	5	5	7	6	6	**45**
Cheracanthiidae	2	2	0	1	10	5	1	1	6	4	**32**
Philodromidae	3	1	1	1	3	5	0	0	2	2	**18**
Salticidae	5	4	8	10	11	10	5	4	17	15	**89**
Theridiidae	1	1	3	2	2	3	2	1	3	3	**21**
Uloboridae	0	0	2	0	0	0	2	2	3	2	**11**
Total	**20**	**22**	**28**	**30**	**48**	**44**	**31**	**30**	**51**	**45**	**349**
Mean Temp.	11.0	15.5	12.2	16.3	20.6	22.0	20.5	24.7	26.9	27.6	**0.77****
Mean R.H.	64.0	54.4	61.4	53.6	51.9	44.2	46.5	46.3	40.5	40.3	**-0.74***

Table (6):Population density of spider families associated with wheat during 2017 season at Sids in Beni-Suef Governorate.

Families	1/1	15/1	1/2	15/2	1/3	15/3	1/4	15/4	1/5	15/5	Total
Agelenidae	2	2	3	3	6	5	4	2	3	3	**33**
Araneidae	2	2	3	2	3	4	2	5	2	1	**26**
Dictynidae	2	2	3	4	1	2	3	4	1	2	**24**
Filistatidae	3	2	2	3	2	4	2	4	1	2	**25**
Linyphiidae	2	1	2	2	6	5	5	3	1	1	**28**
Lycosidae	7	4	6	11	4	6	6	6	9	5	**64**
Cheracanthiidae	2	2	6	7	10	9	1	2	2	2	**43**
Philodromidae	2	1	8	4	3	4	2	3	2	5	**34**
Salticidae	4	6	7	8	7	8	21	18	4	4	**87**
Theridiidae	1	2	2	4	3	5	1	3	3	3	**27**
Uloboridae	1	2	2	2	1	1	0	0	3	2	**14**
Total	**28**	**26**	**44**	**50**	**46**	**53**	**49**	**48**	**31**	**30**	**405**
Mean Temp.	12.0	13.2	14.4	15.1	15.4	18.9	19.9	21.0	23.6	26.7	**-0.004**
Mean R.H.	51.0	53.1	59.6	58.2	61.8	53.8	50.6	55.9	41.3	44.5	**0.59**

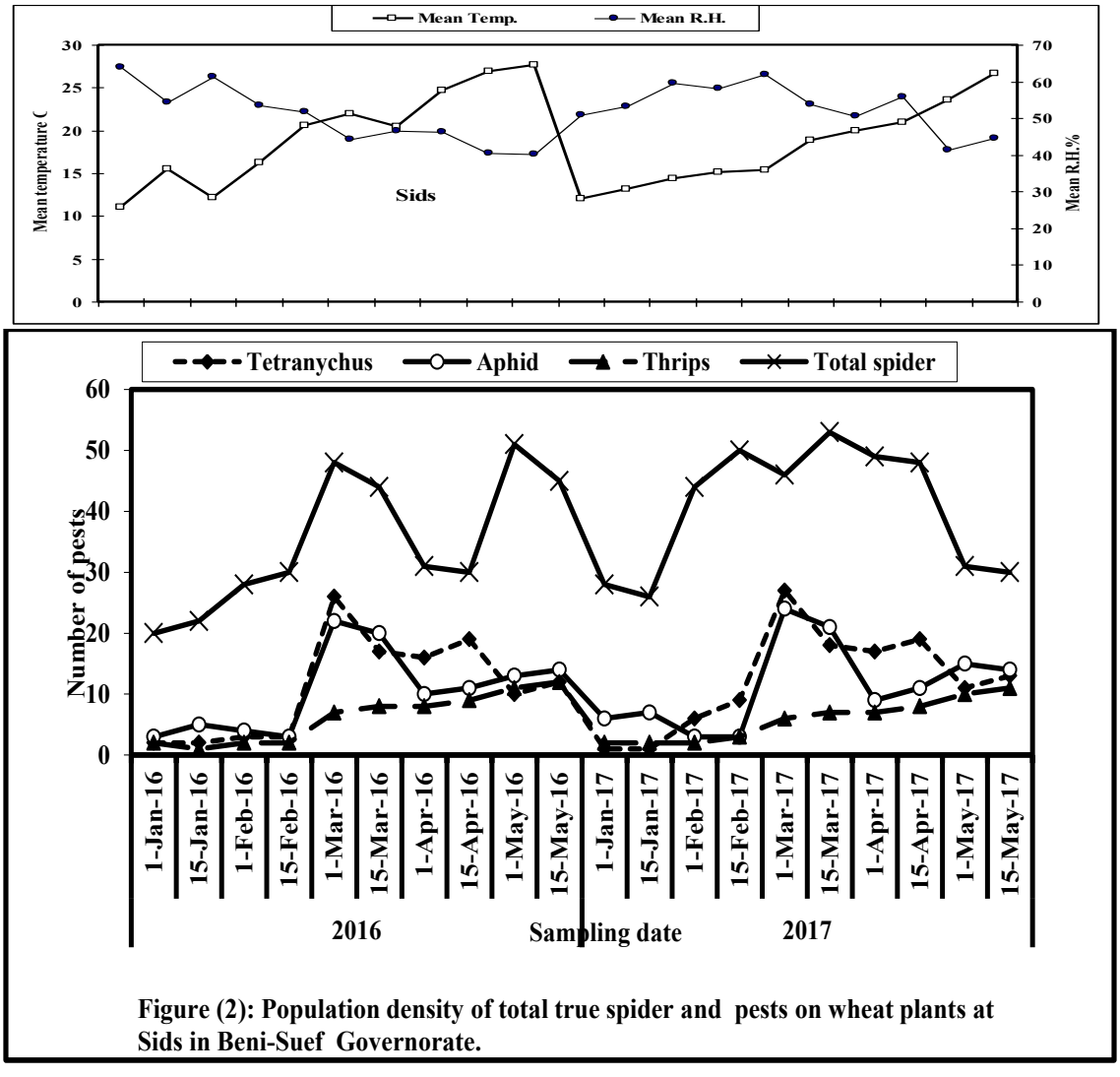


Figure (2): Population density of total true spider and pests on wheat plants at Sids in Beni-Suef Governorate.

4. Population density of mite and insect pests on wheat plants:

There are thouree pests were recorded on wheat in Qalubiya and Beni-Suef Governorates during two season 2016-2017: *T. urticae*, *A. gossypii* and *F.tritici.*

4.1. In Qalubiya Governorate:

As shown in Table (7) and Figure (1) indicated that, the incidence of *T. urticae* was available for two years 2016 and 2017. The mite occurrence was first observed during early January and came to peak in mid March recorded 18 and 17 individuals/ leaf during first and second season, respectively, after that the population decline till mid May (Figure,1).

Table (7):The correlation coefficient between temperatures, relative humidity, true spider and pest populations on wheat atQalubiya Governorate during 2016-2017.

Season	Pests	True spider	Mean Temperature	Mean RH
2016	***Tetranychus urticae***	**0.51**	**0.60**	**-0.64**
	Aphis gossypii	**0.12**	**0.17**	**-0.18**
	Frankliniella tritici	**0.59**	**0.95*** **	**-0.89*** **
2017	***Tetranychus urticae***	**0.74***	**0.40**	**0.14**
	Aphis gossypii	**0.74***	**-0.013**	**0.37**
	Frankliniella tritici	**0.03**	**0.95*** **	**-0.63**

The relationship between the true spider population and density of the tetranychid mite *T. urticae* was positively correlated (0.51 and 0.74*) during two successive years, respectively. The correlation analyses showed that mite occurrence had non-significant positive relationship with the average temperatures (0.60 and 0.40 in the two successive years. The relative humidity was non-significant negative correlated in the first year (-0.64) and non-significantly positively correlated (0.14) in the second year.

Data in Table (7) and Figure (1) indicated that, the population of *A. gossypii* was the first observed during early January in rear number and gradually increased in number and reach to a peak in mid March recorded 20 ind./ leaf in the first season, and in early March recorded 18 individuals/ leaf second season, after that the population decline till mid May. The relationship between the true spider population and density of the *A. gossypii* was positively correlated (0.12 and 0.74*) during two successive years, respectively. The correlation analyses showed that the population of Aphid had non-significant relationship with the average temperatures (0.17 and -0.013) in the two successive years, respectively. The relative humidity was non-significant negative correlated in the first year (-0.18) and non-significantly positively correlated (0.37) in the second year.

The aforementioned results in Table (7) and Figure (1) clarified that, the thripid insect *F. tritici* was recorded in fewer numbers in early January and gradually increased in number and reach its peak in mid May recorded 14 and 13 individuals/ leaf during the first and second seasons, respectively. The relationship between the true spider population and density of the *F. tritici* was positively correlated (0.59 and 0.03) during two successive years, respectively. The correlation analyses indicated that, the population of *F. tritici* had highly significant positive relationship with the temperatures (0.95*** and 0.95***) in the two successive years, respectively. While, the relative humidity had significant negative correlated in the first year (-0.89**) and non-significantly negatively correlated (-0.63) in the second year.

4.2. In Beni-Suef Governorate:

As shown in Table (8) and Figure (2), indicated that the population of *T. urticae* was first recorded in early January and reached a peak in early March recorded 26 and 27 individuals/ leaf during first and second season, respectively, after that the population decline till mid May (Fig.1). A similar trend was recorded on aphids, *A. gossypii* was the first observed during early January in rear number and gradually increased in number and reach to a peak in early March recorded 22 and 24 ind./ leaf in the first and second seasons, after that the population decline till mid May.The thrips, *F. tritici* was recorded in fewer numbers in early January and gradually increased in number and reach its peak in mid May recorded 12 and 11 Individual / leaf during the first and second seasons, respectively.

The relationship between the true spider population and density of *T. urticae, A. gossipii* and *F. tritici* was positively correlated during two successive years, respectively Table (8). These results indicated that the true spider can play an important role for control pests on wheat plant in the two Governorates.

These results agree with those of (Ibraheem *et al.*, 2007) who indicated that, highest mean thrips population (6.15/leaf) was recorded during 11/04/13 and 02/05/13 whereas, the minimum population of thourips (1.85/leaf) was recorded on 17/04/13.The two spotted spider mite recorded 3-4 peaks on the tested cultivars during the two seasons with highest peak of 26.0 mites/ leaf on Sakha 93 at early-April during the first season. During the second season, the highest peak of 7.0 mites/ leaf was recorded at the end of March on Gemiza 9.

Table (8):The correlation coefficient between temperatures, relative humidity, true spider and pest populations on wheat at Beni-Suef Governorate during 2016-2017.

Season	Pests	True spider	Mean Temperature	Mean RH
2016	***Tetranychus urticae***	0.62	0.62	-0.54
	Aphis gossypii	0.83**	0.66*	-0.63
	Frankliniella tritici	0.78**	0.94***	-0.89
2017	***Tetranychus urticae***	0.63	0.41	0.23
	Aphis gossypii	0.18	0.38	-0.07
	Frankliniella tritici	0.037	0.96***	-0.60

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Impact of two formulation types to emamectin benzoate on physicochemical properties and bioefficacy against *Sesamia critica* (Lepidoptera: Noctuidae) infesting *Zea mays* plants

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Abstract:

The corn *Zea mays* is economically one of the most important and widely grown as a stable food in many parts of the world, corn consumed directly by humans, production ethanol, starch ,syrup and animal feed. Corn infested with many insects from it, greater sugar cane borer *Sesamia critica* Lederer (Lepidoptera: Noctuidae) is considered the most serious insect pest infesting corn in Egypt. The objective of this study was to study the impact of formulation types of emamectin benzoate 5% on mean number of larvae, reduction percentages of *S. critica* after first and second spray during two successive seasons and physicochemical properties to insecticide. The field experiment was conducted at Agricultural Research station "Qaha" in Qalubiya Governorate. Corn were seedling at 20th May during two successive seasons 2018 and 2019, respectively. The results showed that larvae population decreased gradually by time from first day till 10th day from spray in case of Hypnose and Absolute but living larva population in case of control were increased by time. Hypnose was more efficacies on living larvae population compared with Absolute. Especially, reduction percentage, the data illustrated that, Hypnose recorded the highest reduction % followed by Absolute. The Hypnose categorized in the first rank followed by Absolute in the second rank with reduction % were 91.5 and 28.0 reduction %, respectively after 10 days. On the other hand , impact of two formulation types on physicochemical properties, the results showed that, pH in case of Hypnose and Absolute recorded 7.9 and 7.7 near to equalized or to slightly alkaline , but surface tension lowered to 33.7 and 34.4 dynes/cm, causing increasing wetting to treatment surface of plants, but density were equalized . The foam recorded 3 ml with Absolute and non foam in case of Hypnose, especially suspensibility % recorded 100 % suspensibility in case Hypnose formulation type (5% SG).

Introduction

The corn Zea *mays* is economically one of the most important and widely grown as a stable food in many parts of the world, corn consumed directly by humans, production ethanol, starch ,syrup and animal feed. Corn infested with many insects from it, greater sugar cane borer *Sesamia critica* Lederer (Lepidoptera: Noctuidae) is considered the

most serious insect pest infesting corn in Egypt. Used many insecticides, from it biopesticides offer more sustainable solutions to pest control than synthetic alternatives (Shaalan *et al.*, 2005 and Akdeniz and Özmen, 2011). Selective insecticides with modes of action different from those of broad spectrum neurotoxin insecticides are highly desirable in integrated pest management (IPM) programs. Among these insecticides are insect growth regulators (IGR,s) that affect the ability of insects to grow and mature normally. IGR,s have been developed due to their high activity and selectivity against insects with inherently low toxicity to non target wildlife. As a result of their mode of action, a subtle effect of these compounds is likely to pose a greater effect to immature stages than to adults of a number of insect species. An IGR, therefore, does not necessarily have to be toxic to its target, but may lead instead to various abnormalities that impair insect survival (Siddall, 1976). Most of the compounds that belong to the IGR class are not stomach or neurotoxins, but have a unique mode of action that disrupts the molting process or cuticle formation in insects or interferes with the hormonal balance of insects. They are characteristically slow with acting against a narrow range of sensitive stages of the insects life cycle harmful effect against target pests. Insect growth regulators may come from a blend of synthetic chemicals or from other natural sources, such as plants (Tunaz, 2004). Insecticides plant extracts could inhibition some enzymes (Chun and Zhang, 2003). All high doses of insecticides based on changes that lead to deformities and abnormal growth of wings and not molting to the next stages and finally die after 24-48 hours from treatment (Kodandaram *et al.,* 2008). Feeding behavior of plant feeding insect may change when they feed on the pesticide by which plant was treated, especially when exposure to a lethal dose; so change their metabolism in both digested or undigested food in their bodies leading to changes in the continuing growth, deformation and maturation (Srinivasa and Rao, 2003). The concept of using feeding attractants and stimulants in combination with a toxicant for adult control of noctuid pest has been documented (López *et al*., 2000). Emamectin benzoate belongs to the avermectin group of chemicals produced by the soil-dwelling actinomycete (NRRL 8165) alias, *Streptomyces avermitilis* (Burg *et al*., 1979). It possesses excellent insecticidal potency against neonates of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in foliar application with an LC90 value of 0.002 g/ml (White *et al.,*1997). Argentine *et al.* (2002) found that the LC90 values for emamectin benzozte ranged from 0.0050 to 0.0218 g/ml for six species of Lepidoptera. Dunbar *et al*. (1998) reported that emamectin benzoate was very effective in controlling *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and *H. zea* larvae at low active ingredient rates (0.0084-0.084 kg /ha). Jansson and Dybas (1996) reported that emamectin benzoate is stored as a reservoir in plant parenchyma tissues and this accounts for its long residual activity against several phytophagous insects. Jansson *et al.* (1996) reported that solid formulations of emamectin benzoate were as efficacious as the emulsifiable concentrate formulations in controlling *H. virescens* and beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) larvae.

Therefore the objective of this research work was to study the impact of formulation types of emamectin benzoate 5% on mean number of larvae , reduction percentages of *S. critica* after first and second spray and physicochemical properties to insecticide.

Material and methods

1. Experimental field and formulations of insecticide :

The field experiment was conducted at Agricultural Research Station "Qaha" in Qalubiya Governorate. Corn *Z. mays* were seedling at 20th May to study the impact of two formulation types to emamectin benzoate on larvae populations of *S. critica*

during two successive season 2018 and 2019 , respectively. Each treatment was repeated three times, on area about kirate (175 m^2) per each treatment. Treatment numbers was three treatments included control. When *Z. mays* plants infested with *S. critica* were sprayed two times by kanabsac sprayer motors during 20th June by spray solution contain emamectin benzoate , the period between two spraying 10th days .

1.1. The first formulation is Hypnose formulation type (5% SG**)** with a rate of 60gm, /feddan .

1.2. The second formulation is Absolute formulation type (5% ME.) with rate of 75 ml/ feddan.

1.3. Control was sprayed with ground water at 700 milimose.

The efficiency of treatments was determined by inspecting 30 randomly plants from each treatment then each sample was kept in a tightly closed paper bag and transferred to the laboratory in the same day for inspection under stereomicroscope at Qaha, Plant Protection Research Station Laboratory to count *S. critica* larvae. Inspection of plants was carried out before spraying and after 1,3,5,7 and 10 days from application, larvae livingly were accounted and recorded , the reduction percentage of larvae populations was calculated according to the equation of Henderson and Tilton (1955).

2. Physicochemical properties of insecticide at two formulations :

Emulsification stability and foaming were evaluated. Surface tension (dyne / cm), pH value , density and suspensibility % were measured using Tensiometer, respectively **(W.H.O. , 1973)**.

Results and discussions

1.Impact of formulation types on living larvae populations of *Sesamia critica* after spraying :

Data in Table (1) showed that the mean number of *S.critica* larvae during two successive seasons 2018 and 2019 , respectively after first and second spray and impact of two formulation types to emamectin benzoate on the mean number of *S. critica* larvae. From data in tabulated in Table (1), showed that larvae population decreased gradually by time from first day till 10th day from spray in case of Hypnose and Absolute but living larva population in case control were increased by time. Hypnose was more efficacy on living larvae population compared with Absolute, recorded 19th , 14th , 11th , 8th and 7th living larvae after 1,3,5,7and 10 days from spray. On the other hand, Absolute recorded 20th , 16th , 13th , 10th and 9th larvae in the same days aforementioned, respectively. Statistical analysis showed that there are significant differences between each treatments , except after first and ten days from spray indicated not significant .

Table (1): Impact of two formulation types to emamectin benzoate on populations of *Sesamia critica* larvae.

Treatments (trade, common and formulation types)			Pretreatment	Mean No. of larvae after first and second spray during 2018 and 2019 seasons					
Trade name	Common name	Formulations Types		1 day	3 day	5 day	7 day	10 day	residual
Hypnose	**Emamectin Benzoate**	**5% SG**	19 ab	19 a	14 b	11 b	8 c	7 b	11.8 b
Absolute	**Emamectin Benzoate**	**5% ME**	20 a	20 a	16 b	13 b	10 b	9 ab	13.6 b
Control	**Water**	----------	16 b	19 a	20 a	18 a	15 a	10 a	16.4 a
F	----------	----------	4.333	0.214	16.8	13.01	39.00	3.5	16.12
P	----------	----------	Ns	Ns	**	**	***	Ns	**
LSD	----------	----------	3.46	4.31	2.579	3.46	1.99	2.83	1.99

2.Impact of different formulations to emamectin benzoate for reducing the population of *Sesamia critica* larvae :

Data in Table (2) illustrated that, impact of two formulation types to emamectin benzoate. In respect to, initial effect of the tested formulations to emamectin benzoate on reduction percentage of *S. critica* larvae under field conditions showed that , Hypnose recorded the highest reduction % followed by Absolute , respectively. Hypnose and Absolute recorded reduction % similarity 15.7 and 15.78 % after 1st spray , but the results differed beginning 3 days till 10 days , recorded (41.05 and 36.00) , (48.5 and 42.2) , (53.08 and 46.00) and (91.05 and 28.0) after 3,5,7 and 10 days, respectively. The Hypnose categorized in first rank followed by Absolute in the second rank, with reduction % were 91.05 and 28.0 reduction %, respectively after 10 days **.**

Table (2): Impact of two formulation types to emamectin benzoate on reduction percentage of *Sesamia critica* populations.

Treatments (Trade, Common and Formulation Types)			Reduction percentage in larvae of *Sesamia critica* after first and second spray during 2018 and 2019 season				
Trade Name	**Common name**	**Formulation Types**	**1 day**	**3 day**	**5 day**	**7 day**	**10 day**
Hypnose	**Emamectin Benzoate**	**5% SG**	15.7 a	41.05a	48.5 a	53.08 a	91.05 a
Absolute	**Emamectin Benzoate**	**5% ME**	15.78 a	36.00 b	42.2 b	46.0 b	28.0 b
F	----------	----------	14.78	38.25	59.5	37.59	2.38
P	----------	----------	Ns	**	**	**	***
LSD	----------	----------	3.205	2.26	2.26	3.205	3.58

Statistical analysis illustrated that there are significant after 3, 5 and 7 days and highly significant differences after 10 days between Hypnose and Absolute. In fact Hypnose has constant influence till 10 days after 1st and 2nd spray during two successive seasons.

3. Impact of two formulation types on physicochemical properties to emamectin benzoate insecticide:

Data in Table (3) showed that, impact of two formulation types on physicochemical properties to emamectin benzoate insecticide such pH, surface tension (S.T.), density (Den.), foam and suspensibility % (Sus.%). The results from Table (3) it is obvious that , pH in case of Hypnose and Absolute 7.9 and 7.7 near to equalized or to slightly alkaline , due to retentions emamectin benzoate constant . In the same Table S.T. recorded 33.7 and 34.4 dyne/ cm , cause increasing wetting to treatment surface of plants, but Den. were equalized . On the other hand , foam record 3 ml with Absolute and non foam in case of Hypnose , especially Sus. % record 100 % suspensibility in case of Hypnose formulation type (5% SG). These results agree with Soliman, 1998 and 2004 and Soliman and Mohamed (2007).

Table (3): Impact of two formulation types on physicochemical properties to emamectin benzoate insecticide.

Treatments (Trade, common name and formulation types)			Physicochemical properties				
Trade Name	**Common Name**	**Formulations Types**	**PH**	**S.T** Dyn/ cm	**Den.** Gm/ cm	**Foam**	**Sus.%**
Hypnose	**Emamectin Benzoate**	**5% SG**	7.9	33.7	2	-	100
Absolute	**Emamectin Benzoate**	**5% ME**	7.7	34.4	2	3 ml	-

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Comparative toxicity study of different acaricides in laboratory preluding for field efficacy assessment against *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract:

Toxicity and field studies were carried out on spirodiclofen, pyridaben, abamectin and hexythiazox against the developing stages of susceptible strain (SS) and field strains (FS) of *Tetranychus urticae* Koch. (Acari: Tetranychidae). Abamectin had the highest resistance ratios levels of 6 and 4.1 fold in adult and developmental stage respectively. In adult stage, spirodiclofen and pyridaben had 1.2 and 1.1 fold, respectively. Developmental stage had equal folds of 1.1 for spirodiclofen, hexythiazox and pyridaben. In egg stage, hexythiazox, pyridaben and spirodiclofen had 1.3, 1.2 and 1 fold, respectively. High relative resistances based on LC_{50}s of abamectin were 357 and 171 fold for pyridaben and spirodeclofin, respectively in adult stage besides, 122, 9 and 7 folds for pyridaben, spirodeclofin and hexythiazox, respectively in developmental stage. The field experiments throughout 4 weeks post treatments on green bean crop in season of 2017 showed that the highest overall reduction percentages in *T. urticae* populations were occurred in spirodiclofen (55.90 %) and pyridaben (51.72 %) compared to abamectin (49.77 %). Meanwhile, season of 2018, abamectin had the highest overall reduction percentages of 56.00 % compared to spirodiclofen (51.84 %) and pyridaben (49.40 %). Hexythiazox had the lowest overall reduction percentages of 32.53 % and 36.17 % in seasons of 2017 and 2018, respectively. The residual control activity in both seasons showed that spirodiclofen had delayed initiating effect (7- 28 DATs) > pyridaben = abamectin (4 - 21 DATs) > hexythazox (4 - 7 DATs). Eventually, our study declared that spirodiclofen, pyridaben and hexythiazox were considered to be alternatives and complementary acaricides to abamectinin controlling different developmental stages of *T. urticae*.

Introduction

Two spotted spider mite (TSSM), *Tetranychus urticae* Koch. (Acari: Tetranychidae) is an important polyphagous pest that feed on over 1100 plant species including more than 150 crops (Bensoussan *et al*., 2016 and Seal, 2006). The stylet of *T. urticae* could penetrate the plant leaf causing damage to epidermal pavement cells or without damaging through a stomatal opening. The feeding duration may extend

more than 30 minutes that is enough to deplete a single mesophyll cell (Bensoussan *et al*., 2016). The accumulative effect of TSSM infestation coupled with favorable temperatures initiates serious economic losses in field grown strawberries as well as remarkable yield reductions and damages in cucumber eggplant, tomato and pepper in greenhouse and open field's worldwide (Nyoike and Liburd, 2012; Tehri *et al*., 2014; Van De Vrie *et al*., 1972 and El-Saiedy, 2015).

Spirodiclofen is a recent commercialized acaricide with a novel mode of action (of lipid synthesis inhibitor), against all developmental stages and female adults of tetranychid mite species. Spirodiclofen showed no cross-resistance in high resistance strains to at least one of organophosphates, mitochondrial electron transport inhibitors (METIs), hexythiazox and abamectin. Thus, it considered being a good alternative to these acaricides. Spirodiclofen achieved high and steady efficacy against the summer population of *Panonychus ulmi* (Koch.) (Acari: Tetranychidae) and TSSM in three vegetation seasons of cucumber (Rauch and Nauen, 2002; Marcic *et al*., 2011 and Farahani *et al*., 2018). Specifically, hexythiazox is entitled as unique ovicide with unknown mode of action to control mite growth via eggs and early developmental stages. It typically applies to bare-root plants and containerized, foliar, nonbearing and nursery canopies (United States Environmental Protection Agency (USEPA), 2007). Laboratory and field tests demonstrated that population of *P. ulmi* in an apple orchard in Pennsylvania had become highly resistant to hexythiazox during the mid-eighties of the twentieth century (Reissig and Hull, 1991). Pyridaben is a relative new selective and stoichmetric inhibitor of complex acaricide in the inner membrane of mitochondria that able to induce DNA damage and chromatin abnormalities in spermatozoa that leads to low in vitro fertilizing rate. It is used to control mites and some insects such as whitefly, aphid and thrips (Gomez *et al*., 2007 and Manas *et al*., 2013). Resistance of the *T. urticae* larvae to METIs-acaricides is an increasing problem. In a foliar spray bioassay trails, selected Japanese and English strains of *T. urticae* larvae showed a remakable resistance against pyridaben compared to the susceptible strain. Mutual crosses of homozygous and diploid females with hemizygous and haploid males in both susceptible and resistant strain revealed incompletely dominant inheritance of pyridaben resistance with slight differences between maternal and paternal inheritance. Increased oxidative metabolism of the METI-acaricides in the resistant strains could be partially suppressed in vivo by the monooxygenase inhibitor Piperonyl butoxide (Stumpf and Nauen, 2001). Abamectin belongs to avermectins which is a class of macrocyclic lactones group that produced directly by the actinomycete, *Streptomyces avermitilis* (Khalil and Abd El-Naby, 2018) or through semisynthetic modifications (Fisher and Mrozik, 1989 and Krieger, 2010). This ivermectin is known to include γ-aminobutyric acid blockade (Cohen *et al*., 2017). Some studies showed that collected *T. urticae* populations from strawberry field in Sao Paulo, Brazil recorded positive cross-resistance to abamectin and milbemectin because of the similarity in mode of action of these acaricides. This location had been frequently sprayed for about ten years and lasted by six times with abamectin before collecting these strains. Abamectin resistance seemed to be unstable in the absence of selection pressure within six months, which indicated by decreasing of resistance percentages in populations of resistant mites to levels equal or lower than 15% (Sato *et al*., 2005).

The present study investigates the toxicity of varied mode of action groups of acaricides; spirodiclofen, pyridaben, abamectin and hexythiazox against different development stages in both susceptible strain

(SS) and field strain (FS) of *T. urticae* . In addition, resistance ratio levels for each tested acaricide and relative resistance (RR) based on the most toxic acaricide were also employed. Accordingly, field studies were carried out to determine the overall reduction percentages and residual control activity for these acaricides.These field trials tried to compare the efficacy of lowest resistance acaricides as alternatives and complementary besides the other that possessing high resistance against *T. urticae* in field.

Materials and methods

1. Tested insecticides:

Table (1) showed that main group, sub-group, trade name, produced company and applied dosage of insecticides that used during the present study.

Table (1): The items of selected acaricides were demonstrated regarding to IRAC MoA classifications*(2019), trade name, produced company and applied dosage.

Common name	IRAC MoA Classification* 2019		Trade name (Formulation)	Produced company-origin	Doses $100L^{-1}$
	Main group	Sub-group			
Spirodiclofen	Inhibitors of Acetyl-CoA carboxylase	Tetranoic and tetramic acid derivatives	Infedor (24 % SC)	Bayer AG - Germany	30 ml
Pyridaben	Mitochondrial complex I electron transport inhibitors	METI acaricides and insecticides	Sanmite (20 % WP)	Nissan for chemical industries., Ltd - Japan	100 mg
Abamectin	Glutamate-gated chloride channel allosteric modulators	Avermictins	Gold (1.8% EC)	El-Helb pesticides&chemical Co - Egypt	40 ml
Hexythiazox	Mite growth inhibitor affecting chitin synthase 1	Hexythiazox	Prince (10% EC)		20 ml

***Insecticide resistance action committee – Mode of action classifications.**

2. Rearing of *Tetranychus urticae* colony:

The samples of TSSM were collected from castor oil leaves, *Ricinus communis*, free of insecticides treatments. The TSSM samples were adapted according to Singh and Clare (1993) at approximate conditions of 26 ± 2 ºC, ≈70 % RH. and 12:12 light/dark cycle on green bean seedling plants, *Phaseolus vulgaris* L., in plastic pots (20 cm diameter). Under these conditions, a new susceptible mass of TSSM strain was obtained after approximate 16 successive generations. These rearing methods were carried out in integrated Plant Protection laboratory, Alexandria, Egypt. On the other hand, field strain (FS) samples were collected randomly from the infested leaves of vegetative period of *P. vulgaris* plants from Ezbit-Mohseen Al-Kobra region during season of April, 2017. These collected samples of susceptible strain (SS) and FS were submitted to toxicity assay in laboratory.

3. Laboratory studies:

The toxicity of spirodeclofin, pyridaben, Abamectin, and hexythiazox were evaluated on eggs hatchings, developmental and adult stages of *T. urticae* according to susceptibility test methods of Insecticide Resistance Action Committee (IRAC) (2009).The toxicity evaluation was carried out on *T. uratica* SS versus to FS. Mortality percentages were corrected by using formula of Abbott (1925) and subjected to probit analysis (Finney, 1971). The resistance ratio levels between FS and SS for each tested acaricide were assigned. Moreover, RR based on LC_{50} valuesof abamectin (the most toxic acaricide) in FSs were assigned compared to the other tested acaricides.

4.Field trials:

Two field experiments were achieved within the second week of April in seasons of 2017 and 2018 on the vegetative period of green bean plant at Ezbit-Mohseen Al-Kobra, Alexandria. During the period of field assessments, agriculture processing followed the optimal agronomic procedures of green bean crop. All treatments were conducted in 45 m^2 micro-plots in a randomized complete block design with four replicates. Knapsack sprayer equipment (CP3) was used for spraying the selected acaricides at their recommended field rates (FRs). Control treatment was applied by water only. The spray solution volume was 3 liter per each micro-plot. Reduction percentages and residual control activity against *T. uratica* in all treated and untreated plots were calculated at 1, 4, 7, 14, 21, and 28 days post-treatment according to Henderson and Tilton's formula (1955).

4. Statistical analysis:

All the obtained results were subjected to analysis of variance (ANOVA). Means were determined for significance at 0.05 using LSD test using SAS software (2002).

Results and discussion

1. Toxicity assay and resistance assessments on different stages of *Tetranychus urticae* under laboratory conditions:

According to data in Table (2), the values of LC_{50}s after 4 days of exposure against adult stage of *T. urticae* treated with abamectin was 0.066 mg L^{-1}in FS compared to 0.011 mg L^{-1} inSS.Therefore, abamectin possessed the highest resistance ratio levels reached to 6 folds. Whereas, LC_{50}s of spirodiclofenin FS were 11.278 mg L^{-1}compared to 9.761 mg L^{-1} in SS had resistance ratio of 1.2 folds. In addition, LC_{50}s of pyridabenin FS were 23.561 mg L^{-1} compared to 20.951 mg L^{-1}in SS had 1.1 folds. In contrast, hexythiazox had no direct toxic effect on adult stage.

On the other hand, the LC_{50}s after 4 days of exposure against developmental stage (larvae, protonymph and deutonymph) of *T. urticae* treated with abamectin were 0.495 mg L^{-1}in FS compared to 0.121 mg L^{-1} inSS. Thus, abamectin had the highest resistance ratio reached 4.1folds. Meanwhile, the resistance ratios of spirodiclofen, hexythiazox and pyridaben had the same level of 1.1 fold. Where, values of LC_{50}s for spirodiclofenin FS were 4.303 mg L^{-1} compared to 3.927 mg L^{-1}in SS. Hexythiazox had 3.607 mg L^{-1}in FS compared to 3.313 mg L^{-1}in SS and pyridaben had 60.408 mg L^{-1}in FS compared to 57.663 mg L^{-1} in SS.

The LC_{50}s against eggs stage of *T. urticae* treated with hexythiazox were 4.934 mg L^{-1}in FS compared to 3.689 mg L^{-1}in SS. While, LC_{50}s of pyridabenwere 131.116 mg L^{-1}in FS compared to 112.189 mg L^{-1}in SS and spirodiclofen 0.625 mg L^{-1}in FS compared to 0.582 mg L^{-1} in SS. Subsequently, the resistance ratios of hexythiazox, pyridaben and spirodiclofen had non varying-folds of 1.3, 1.2 and 1.1 respectively. However, abamectin had no direct toxic effect on eggs stage.

Regarding to the LC_{50} values of abamectin in FSs of adult stage, the estimated RRs of the compared tested acaricides were 357 and 171 folds for pyridaben and spirodeclofin, respectively. The resistances of abamectinin FSs of developmental stage were 122, 9 and 7 folds for pyridaben, spirodeclofin and hexythiazox, respectively. However, abamectin had no toxic effect on the egg stage.

Table (2): Toxicity of the selected acaricides against different stages of susceptible and field strains of *Tetranychus urticae* under laboratory condition after 4 days of exposure.

Stages	Tested acaricides	Strain	LC_{50} (mg L^{-1})	Confidence limits (mg L^{-1})	Slope ± SE	x^2	*Df*	Resistance ratio	Relative resistance
Adult stage	Spirodiclofen	SS	9.761	8.898-10.659	2.870±0.197	10.464	5.0	1.2	171
		FS	11.278	10.388-12.151	3.434±0.240	8.346	5.0		
	Pyridaben	SS	20.951	19.931-21.958	5.131±0.373	4.370	5.0	1.1	357
		FS	23.561	22.419-24.667	5.772±0.442	1.587	5.0		
	Abamectin	SS	0.011	0.009-0.012	1.950±0.138	10.792	5.0	6.0	1
		FS	0.066	0.056-0.078	1.449±0.109	5.265	5.0		
	Hexythiazox	SS	-	-	-	-	-	-	-
		FS	-	-	-	-	-		
Developmental stage	Spirodiclofen	SS	3.927	3.730-4.134	4.816±0.322	3.434	5.0	1.1	9
		FS	4.303	4.097-4.508	5.348±0.351	2.028	5.0		
	Pyridaben	SS	57.663	55.040-60.370	5.238±0.384	7.356	5.0	1.1	122
		FS	60.408	57.748-62.962	5.843±0.436	6.464	5.0		
	Abamectin	SS	0.121	0.112-0.131	3.004±0.217	5.304	5.0	4.1	1
		FS	0.495	0.456-0.533	3.203±0.275	9.944	5.0		
	Hexythiazox	SS	3.313	3.034-3.603	2.922±0.208	9.332	5.0	1.1	7
		FS	3.607	3.304-3.924	2.942±0.201	10.513	5.0		
Eggs stage	Spirodiclofen	SS	0.582	0.524-0.647	2.378±0.170	0.807	5.0	1.1	-
		FS	0.625	0.563-0.695	2.378±0.172	10.726	5.0		
	Pyridaben	SS	112.189	105.208-119.078	4.029±0.304	10.617	5.0	1.2	-
		FS	131.116	125.199-36.712	6.086±0.429	10.712	5.0		
	Abamectin	SS	-	-	-	-	-	-	-
		FS	-	-	-	-	-		
	Hexythiazox	SS	3.689	3.542-3.842	5.496±0.389	12.063	5.0	1.3	-
		FS	4.934	4.692-5.167	5.414±0.381	4.263	5.0		

2. Field efficacy of tested acaricides against *Tetranychus urticae*:

The obtained results in season of 2017 (Table, 3) declared that spirodiclofen and pyridaben had the highest equal overall reduction percentages on *T. urticae* populations of 55.90 and 51.72 %, respectively compared to abamectin (49.77 %) and lasted with hexythiazox (32.53 %).

Table (3): Reduction (%) of *Tetranychus urticae* population after sequent days of exposure to selected acaricides under field conditions, season of 2017.

Tested acaricides	Population numbers* before treatment	Population numbers (Reduction %) after treatment at intervals (days)						Overall mean of population numbers (Reduction %)
		1	4	7	14	21	28	
Spirodiclofen	267.50	259.75	257.50	103.50	10.00	2.00	56.00	**136.61**
		$(1.84)^{i}$	$(2.03)^{i}$	$(58.74)^{f}$	$(95.72)^{bac}$	$(99.21)^{a}$	$(77.79)^{e}$	**$(55.90)^{a}$**
Pyridaben	255.00	247.75	26.50	6.25	3.50	188.75	236.50	**137.75**
		$(1.87)^{i}$	$(89.42)^{d}$	$(97.39)^{ba}$	$(98.43)^{ba}$	$(21.62)^{e}$	$(1.62)^{i}$	**$(51.72)^{a}$**
Abamectin	297.75	278.75	14.25	2.25	103.00	180.00	273.75	**164.25**
		$(5.07)^{i}$	$(95.13)^{bac}$	$(99.19)^{a}$	$(60.38)^{f}$	$(35.99)^{g}$	$(2.48)^{i}$	**$(49.77)^{ba}$**
Hexythiazox	264.75	255.00	16.25	21.75	223.75	243.00	246.00	**181.50**
		$(2.61)^{i}$	$(93.75)^{bdc}$	$(91.24)^{dc}$	$(3.19)^{i}$	$(2.81)^{i}$	$(1.44)^{i}$	**$(32.53)^{b}$**
Control	301.25	298.25	296.00	282.50	263.00	284.50	284.00	**287.07**

***Population numbers: numbers of adult individuals of *Tetranychus urticae* /10 leaves/plot.**

. Means of reduction percentages of population of mites with the same letter are not significantly different according to the $LSD_{0.05}$ for the interaction between treatments and days after treatments.

. Means of overall reduction percentages of population in the last column with the same letter are not significantly different according to the $LSD_{0.05}$.

The obtained results in season of 2018 (Table, 4) declared that abamectin had the highest overall reduction percentages of 56.00 % compared to spirodiclofen and pyridaben which had equal reduction percentages of 51.84 and 49.40 % respectively. Lastly, hexythiazox had the lowest overall reduction percentages of 36.17 %.

Table (4): Reduction (%) of *Tetranychus urticae* population after sequent days of exposure to selected acaricides under field conditions, season of 2018.

Tested acaricides	Population numbers* before treatment	Population numbers (Reduction %) after treatment at intervals (days)						Overall mean of population numbers (Reduction %)
		1	4	7	14	21	28	
Spirodiclofen	245.50	228.50	238.25	137.00	13.00	0.00	65.25	**132.50**
		(1.83)^{f}	(1.42)^{f}	(41.83)^{d}	(93.81)^{ba}	(100.00)^{a}	(72.15)^{c}	**(51.84)^{ba}**
Pyridaben	239.50	215.00	35.50	8.00	3.50	207.00	217.75	**132.32**
		(5.32)^{f}	(84.94)^{b}	(96.52)^{a}	(98.29)^{a}	(6.63)^{f}	(4.72)^{f}	**(49.40)^{ba}**
Abamectin	285.25	245.25	10.25	2.75	82.25	154.75	207.75	**141.18**
		(9.32)^{f}	(96.35)^{a}	(99.00)^{a}	(66.27)^{c}	(41.39)^{d}	(23.67)^{e}	**(56.00)^{a}**
Hexythiazox	252.00	233.00	25.50	15.75	172.50	219.75	227.00	**163.64**
		(2.48)^{f}	(89.72)^{ba}	(93.49)^{ba}	(19.92)^{e}	(5.80)^{f}	(5.60)^{f}	**(36.17)^{b}**
Control	289.25	274.25	284.75	277.50	247.25	267.75	276.00	**273.82**

***Population numbers: numbers of adult individuals of *Tetranychus urticae*/10 leaves/plot.**

- **Means of reduction percentages of population of mites with the same letter are not significantly different according to the $LSD_{0.05}$ = 10.49 for the interaction between treatments and days after treatments.**
- **Means of overall reduction percentages of population in the last column with the same letter are not significantly different according to the $LSD_{0.05}$ = 17.71**

3. Residual control activity of the tested acaricides:

Residual control activity of treatments in both seasons (Tables 3 and 4) were statistically estimated based on comparing the mean population numbers of *T. urticae* in treated plots along the intervals of DATs with their initial populations before treatments. The data in the two seasons showed that spirodiclofen had delayed initiations at the 7th day followed by high residual control activity periods that reached more than 28 DAT. pyridaben and abamectin had the same residual control activity periods from 4 to 21 DATs. Lastly, the residual control activities of hexythazox were limited from 4 up to 7 DATs.

Generally abamectin is one of the most widely common used acaricidein controlling mites in agriculture applications by farmers due to its rapid efficacy (Yorulmaz and Kaplan, 2014; Turan *et al*., 2016 and Cagatay *et al*., 2018). Certain reports declared that abamectin does not persist or accumulate in the environment and not toxic for non-target organisms (Khalil, 2013). Moreover, quite low abamectin residues in/on treated crops leadto minimal exposure during human consumption (Lasota and Dybas, 1990 and IRAC, 2019). High RR levels in *T. urticae* populations to abamectincollected from different locations representing vegetable greenhouses as well as open fieldswere monitored (Sato *et al*., 2005; Brown *et al*., 2017 and Cagatay *et al*., 2018). These facts lead our study to find out alternative low resistance acaricides to be employed and compatible beside abamectin in controlling program against TSSM in open fields. Subsequently, a lifeline could be realized for abamectin applications in crop protection.

Data of toxicity in this research showed an observed high resistance ratio levels of field and susceptible strains of TSSM to abamectin in adult and developmental stages.These observations were supported by the resistance in *T. urticae* and control failure's reports of abamectin manifested in

cotton fields of Midsouth state. Whereas, the resistance ratios of between fields and susceptible populations of TSSM in seasons of 2014 and 2015 ranged from 11.1 to 94.4 and 33.3 to 93.3 folds, respectively (Brown *et al*., 2017). Furthermore, high resistance levels to abamectin in three *T. urticae* populations collected from vegetable greenhouses in Antalya and Muğla, Turkey ranged between 223 and 404 folds compared to their susceptible populations (Cagatay *et al*., 2018).

Nevertheless, RR based on abamectin in this study showed that abamectin was more toxic in adult stages than pyridaben and spirodeclofin with 357 and 171 folds, respectively. While of toxic effect of abamectin in developmental stage transcend pyridaben, spirodeclofin and hexythiazox with 122, 9 and 7 folds, respectively. Eventually, abamectin had no toxic effect on egg stage. These results came in agree with values of RR based on abamectin in adults of *T. urticae*, presented in fenpyroximate (15 fold), spiromesifen (32 fold), chlorfenapyr (83 fold), propargite (198 fold), dicofol (376 fold) and hexythiazox (711 fold) (Kumari *et al*., 2017).

On the other hand, the obtained data of applied FRs of the tested acaricides in field trials showed that the overall reduction percentages within 4 weeks posttreatments were ordered as follows; spirodiclofen = pyridaben > abamectin > hexythiazox in season of 2017. However, varied data was recognized as abamectin > spirodiclofen = pyridaben > hexythiazox in season of 2018. Likewise, the efficacy of selective acaricides with their distinctive modes of action against the TSSM *T.urticae*, on strawberries in the potting trial from 1 to 3 weeks posttreatment, showed reductions in immature stages treated with spirodiclofen ranged from 61% to 91% and also pyridaben decreased the adults by 41% to 64% and the immatures up to 67%. Moreover, abamectin reduced the immatures by 59% within the first 2 weeks posttreatment (Niu *et al*., 2016). Therefore, the efficacy data of the tested acaricides may direct the usages of spirodiclofen and pyridaben as good alternatives beside abamectin in the insecticides resistance management (IRM) program against TSSM (Peshin *et al*., 2009).

Furthermore the field trial data in both seasons showed that spirodiclofen had delayed control activity periods (7- 28 DATs) > pyridaben = abamectin (4 - 21 DATs) > hexythazox (4 - 7 DATs). Variation of initial activity and residual control periods (lasting periods) of the tested acaricides in our field trials may be justified according to their action modes. Well known fact about abamectin that belongs to allosteric modulators of GABA chlorine channels shows its effects of paralyses, antifeeding and death to arthropods not before few days of treatment (Cagatay *et al*., 2014 and IRAC, 2019). In addition, spirodiclofen is inhibitor of acetyl-CoA carboxylase that cause desiccation followed by death in treated mites (IRAC, 2019). It has a long-lasting and stable acaricidal effect. Evaluation of spirodiclofen in Serbia on cucumber achieved high efficacy (91–98%) against *P.ulmi* and *T.urticae* with long-residual efficacy of (38–47 DAT) and (14–15DAT), in the 3rd and 1st evaluation, respectively. The trials carried out in several EU countries against *P. ulmi* on apple trees at low to medium initial infestation in summer season, showed efficacy higher than 90% throughout 26–32 DAT (Marcic *et al*., 2011 and Marcic, 2012). Pyridaben is mitochondrial complex I electron transport inhibitors. It provides residual control activity up to 45 days against all spiders and broad mites life (IRAC, 2019). Hexythiazox has slow-acting mite growth regulators that interfere with chitin synthesis and specific target site or proteins during the molting process of eggs, embryo and larval development. It possessed long residual control activity (28-45 days) (IRAC, 2019). Therefore, field trial data of residual control activities of spirodiclofen, pyridaben and hexythiazox considered them as good

alternatives beside abamectin in the insecticides resistance management (IRM) program against TSSM.

The toxicity studies showed observed high resistance ratio levels between FS and SS of TSSM to abamectin in adult and developmental stages. Nevertheless, RR based on abamectin showed that abamectin was more toxic in adult and developmental stages than the tested acaricides. On the other hand, field trial tests showed that the total population reduction of TSSM and residual control activities of the tested acaricides may direct spirodiclofen, pyridaben and hexythiazox as good low resistance alternatives and to be compatible beside abamectin in IRM program against specified developmental stages of TSSM.

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Comparative selectivity of acaricides to the predatory mites of *Phytoseiulus persimilis* and *Neoseiulus californicus* (Acari: Phytoseiidae)

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Abstract:

To address suitable acaricides, the selectivity and efficacy of five acaricides of bifenazate, cyflumetofen, pyridaben, pyridaben+clofentezine and spirodiclofen to two predacious mites *Phytoseiulus persimilis persimilis* (Athias-Henriot), *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and its prey *Tetranychus urticae* Koch. (Acari: Tetranychidae) were tested. The general selective toxicity ratio (G.S.T.R.) of five acarcides which combines the selectivity ratios of both LC_{50} and LC_{90} levels was estimated. Results revealed that both spirodiclofen and pyridaben +clofentezine had the highest toxicity on both predators, *P. persimilis* and *N. californicus*. The values were 1.81 and 1.69 ppm for *P. persimilis* and 2.69 and 1.66 ppm for *N. californicus* at LC_{50} and LC_{90} levels, respectively. However, cyflumetofen have the lowest toxicity values for each of *P. persimilis* and *N. californicus* with the same values of 0.001 ppm at LC_{90} levels. Based on the G.S.T.R., cyflumetofen, and bifenazate recorded harmless performances, whereas the other tested compounds recorded harm effects on the two used predators. The results of field studies showed that the greatest percent reductions of spider mite population on kidney bean plants were obtained by cyflumetofen (93.4 %) in China and pyridaben (89.3%) in Egypt. On other hand, pyridaben treatment gave the highest significantly percentage of reductions of 98.2 and 96.7 % against the two tested predators, respectively, under greenhouse conditions. However, the lowest percentage of reduction was obtained by the two treatments of bifenazate and cyflumetofen against the previous two predators with no difference effect between the two acaricides. It could be concluded that the recommended acaricides to control spider mites are bifenazate and cyflumetofen, based on the efficacy and toxicity with no or less harmful on associated predatory mites

Introduction

The two spotted spider mite *Tetranychus urticae* Koch. (Acari: Tetranychidae) is an important global agricultural pest. Its' high reproductive potential and short life cycle facilitate

rapid resistance development to many acaricides often after a few applications (Stumpf and Nauen, 2001 and Van Leeuwen *et al*., 2015). Although different strategies have been adopted for *T. urticae* management (McMurtry and Croft, 1997 and O'Neal *et al*., 2015), the application of pesticides remains essential for controlling them in many agro-ecosystems from reaching economic injury (O'Neal *et al*., 2015). If used properly, pesticides could suppress high populations of the two spotted spider mite. However, pesticides treatments are responsible for the reduction of associated predatory mites.

Predatory mites in the subclass Acari, family Phytoseiidae are often effective management components of agricultural systems (Van Lenteren and Woets, 1988). *Phytoseiulus persimilis persimilis* (Athias-Henriot) and *Neoseiulus californicus* McGregor (Acari: Tetranychidae) are widely used in biological programmes throughout the world (Cho *et al*., 1995 and McMurtry and Croft, 1997). However, several studies indicated that, despite the effectiveness of phytoseiid predators for biological control of spider mites on their host plants, the predators alone may not be able to maintain spider mite populations below an economic injury level for an extended period (Kim *et al.*, 2005). Currently, great efforts are directed towards reduction in the use of traditional pesticides and towards increase in the use of integrated pest management (IPM) techniques. However, the search for pesticides that are compatible with IPM programmes is an interesting approach.

Understanding the effects of chemicals and the impact of their residues on *T. urticae* and its associated predatory mites is necessary for pest management. The selectivity of pesticides against beneficial arthropods should also be considered. Selective insecticides have several advantages over broad-spectrum insecticides including shorter pre-harvest intervals due to their lower mammalian toxicity and greater compatibility with biological control because of their less harmful effects on natural enemies. Knowledge of miticides selectivity to predatory mites is important to their utilization in IPM programmes.

Integrated pest management (IPM) is already introduced as an effective tactics against the two spotted spider mites. Several factors bended this introduction. One of them is the side effect of pesticides on the predators. In this study, the efficacy, persistence and toxicity of five chemical acaricides commonly used to control spider mites in China was investigated. Subsequently, the purpose of present experiments were onducted to find out the most effective tested acaricides under field and greenhouse conditions against mites with no or less harmful on associated predatory mites.

The main aim of the present study is to clarify the toxicity or selectivity of five acaricides against the predator *P. persimilis, N. californicus* and its prey *T. urticae.*The results will be useful to set up a list of recommendeded registered acaricides as a general selective toxicity ratio for integrated mite management in both Egypt and China.

Materials and methods

1. Two spotted spider mite *Tetranychus urticae* and predator cultures:

Laboratory colonies each of the two spotted spider mite *T. urticae* and the two used predatory mites, *P. persimilis* and *N. californics* were maintained under 25 ± 1 °C, 65 ± 5% RH., and a photoperiod of 16:8 (L:D) h at the Laboratory of Predatory mites, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (IPP-CAAS), Beijing, China.

The strains of the predatory mites of *N. californicus* and *P. persimilis* were put on sub colony of each experimental unit. The experimental rearing unit each consisted of a 75 mm-diameter black plastic film placed on a piece of filter paper (90 mm diameter), both placed on a water saturated sponge (90 mm diameter × 60 mm height) laying in a 140 mm ×120 mm (diameter x height) plastic boxes. Each box was approximately half filled with water to isolate the rearing arena. These unites were maintained at 25ºC, 80% RH. and 16:8 L:D.

2. Miticidal formulations:

The five commercial pesticides of cyflumetofen 20%, bifenazate, (Acramite® 43%, pyridaben,(Damanling 15% EC); spirodiclofen (Envidor 240g/L SC) and pyridaben+clofentezine (5%+5%) were selected for trials because of their widespread use on fruits and vegetables in most conventional agriculture. Acaricides used with their active ingredient, trade names, mode of action, recommendeded dose and respective manufacturers are briefly shown in Table (1).

Table (1):General features and application doses of the acaricides used in the study.

Active ingredient	Trade name with formulation type	Mode of action	Application dose (mL/100 L water)	Source Supply
Cyflumetofen	Cyflumetofen 20% EC	Inhibit electron transport complex II, preventing the utilization of energy by cells.	30 mL	FMC China An Agricultural Sciences Company
Bifenazate	Acramite® 43%	Inhibit electron transport complex III, preventing the utilization of energy by cells.	35 mL	*Arysta Life Science*
Pyridaben	Pyridaben 15%	Inhibit electron transport complex I, preventing the utilization of energy by cells	150 mL	Jiangsu Huifeng Agrochemical Co., Ltd.
Pyridaben Clofentezine	Pyridaben Clofentezine SC 10% (5:5)	Incompletely defined mode of action leading to growth inhibition.	100 mL	Sichuan Guoguang Agrochemical Co., Ltd.
Spirodiclofen	Envidor® 240 SC	Inhibit acetyl coenzyme A carboxylase, part of the first step in lipid biosynthesis	30 mL	Bayer Crop Science

3. Toxicological experiments:

Direct spray technique was used to test the effect and selectivity of the tested acaricides against each of the adult females of the two-spotted spider mite*, T. urticae* and both the predators. One circular leaf disc each with 3 cm in diameter punched from the bean leaves were put in petri-dishes with 90 mm in diameter lined with water saturated cotton wool. Twenty-five adult females of the two-spotted spider mite at the same age (2 days) were transferred by aid of fine brush to the upper surface of each leaf disc to test the effect of the tested acaricides against themite.Serial concentrations for each tested acaricide were prepared in aqueous solution and four replicates for each concentration were used. The range of concentrations was chosen based on preliminary trials. Discs were sprayed with a constant amount of the toxicant solution determined by spraying pressure for three seconds by means of glass manual atomizer. Controls were sprayed only with distilled water, since distilled water was used to dilute the compounds.

On other hand, the same previous methods were followed with each of the two tested predatory mites. Ten adult females of each predatorwere transferred onto each leaf disc supplemented with *T. urticae* served

as the prey of the predatory mites. There were 5 replicates for each pesticide treatment and the control.

All treatments were incubated in growth chamber under constant conditions of 25±2°c and 65±5% R.H. and 16:8 hrs L:D. The mortality counts were estimated after 24 hrs of each treatment. The criterion for mortality was considered the failure of mites to respond positively by leg movement following light prodding with a fine brush. The percentage mortality in all treatments was corrected using Abbott's (1925) formula. The toxicity lines were statistically analyzed according to the method described by Finny (1977). Toxicity lines were statically analysis using a probit procedure for the subsequent experiments to assess the lethal LC_{50}, and LC_{90}.

4. Chemical control:

4.1. Greenhouse experiment:

Greenhouse trial was conducted in Institute of Plant Protection, Beijing, China to evaluate the impact of the five pesticides considered in glasshouse trial on each of *T. urticae*, *P. persimilis* and *N. californicus* populations in a more realistic situation. Seeds of local Chinese variety of kidney bean were sown directly in 100 pots 20-cm in diameter with the rate of 5 seeds per pots, filled with soil and peatmoss in a 2:1 ratio, respectively. The potted bean plants were grown in growth chamber conditions for one week until they reached the two true leaf stage. Then, the two-spotted spider mites *T. urticae* were released onto each plant and allowed to multiply for one week. Each of five pots were placed into an individual cage (120 by 60 by 60 cm). Cages were covered with nylon fabric. On other hand, four days before the pesticide applicationseither *P. persimilis* or *N. californicus*was released onto six cages for multiplication. The average release rate was approximately a 10:1 ratio (10 twospotted spider mite to one predatory mite). Cages were used to keep both the two-spotted spider mites and two associated predatory mites from dispersing between plants.

Experimental design was a completely randomized block with three groups (18 cages for each group). Each group was including six treatment (five treated and one untreated) with three replicates for each. Groups consisted of 1) only *T. urticae* without any predatory mites, 2) 10 *P. persimilis* adults released per infested plant, 3) 10 *N. californicus* adults released per infested plant.

Five acaricides were applied using a commercial hand sprayer until run-off for each group at the recommendeded dose. The control plants were sprayed with distilled water. The effect of five acaricides on *T. urticae, P. persimilis* and *N. californicus* each were evaluated 1, 3, 5, 7, 10, and 15 days after pesticide applications by counting phytoseiid motile forms. Data are reported as means and, efficacy was evaluated according to **Henderson and Tilton (1955).**

4.2. Field experiment:

The field experiment was in carried out in each of Ismailia Agriculture Research station, Egypt and Institute of Plant Protection, Beijing, China on kidney bean (*Phaseolus vulgaris* L.) at the same period from April 28th to 12th May in 2019. The field experiments were designed according to "Guidelines for field efficacy, pesticides against two spider mites, 2018 (Egypt)".

Bean seeds were sown directly in small pots (5 cm) with the rate of 2 seeds per pots, filled with soil and peatmoss in a 2:1 ratio, respectively. The pots were kept in growth chamber for one week, then bean seedlings were transferred to field. Bean seedlings were planted in plots 7.5 by 2.5 m consisting of five rows with 0.25-m row spacing. The plots were

arranged in a randomized complete block design with four replications and separated by an unplanted alley (1 m). All the normal agronomic practices were followed as usual throughout the experiment.

The experimental area was divided into six treatments including the control. Seven days before the test, each plot was infested with *T. urticae* with the same pieces of infested leaves and at the beginning of the trial, the beans in each plot was seriously infested. Each test plot was sprayed with the active ingredient at the recommendeded dose. The acaricides were sprayed with manual operated knapsack sprayer having 5 liters capacity fitted with hollow cone nozzle. The spray machine was clean completely before any acaricide was sprayed. The control plot was sprayed with water only. Forty leaves per treatment were picked up randomly for scouting of mites. Each treatment included four replicates. Only one spraying was applied. Samples were taken before treatment and then 1, 3, 5, 7 10, and 14 days after the application from treated and untreated plots. Representative samples were collected randomly after spraying with each replicate.

4.3. Statistical analysis:

Samples were examined and alive moving stages were counted recorded to one square inch of each leaf. Percentage of reduction was estimated according to the equation of Henderson and Tilton (1955).The numbers of motile forms of mites per plot were subjected to ANOVA and significant differences in means were identified by Tukey´s tests (0.05). The efficacy of acaricides was calculated by Henderson-Tilton's formula to estimate the percentage of reductions.

To calculate the general selective toxicity ratios of the tested acaricides, the method of Abd El-Aal *et al* .(1979) with slight modified by El-Adawy *et al*. (2000), was used as follow:

The linear equation of Finney is

$Y= a + b \log x$ (1)

Where Y= probit mortality, and x = concentration.

From above equation, LC90 and LC50 can be related

$6.28= a+b \log LC_{90}$ (2)

$5.00= a+b \log LC_{50}$ (3)

Where 6.23 and 5.00 values are the probit mortality of 90 and 50 %, respectively. By subtracting equation (3) from equation (2) and tacking the antilogarithm, Equation 4 is obtained:

$LC_{90}= LC_{50} x\ 10^{1.28/b}$ (4)

Assuming that three species, *T. urticae*, *P. persimilis,* and *N. californicus* are to be compared at LC_{90} the following equation results:

$LC_{90}(mite)/ LC_{90}(predator)= LC_{50}$ (mite) x $10^{1.28/b\ (mite)}$ / LC_{50} (predator) x $10^{1.28/b\ (predator)}$ (5)

Or

$LC_{90}(mite)/ LC_{90}(predator)= LC_{50}$ (mite)/ LC_{50} (predator) x $10^{1.28/b\ (mite)- 1.28/b\ (predator)}$ (6)

The selectivity ratio (s.r.) at LC_{90} level can be combined with LC_{50} in one parameter {general selective toxicity ratio (G.S.T.R.)} by employing the following equation:

G.S.T.R. = (experimental s.r.at LC50) x $10^{1.28\left(\frac{bp-bm}{bpxbm}\right)}$

Where:

G.S.T.R = general selective toxicity ratio,

s.r. = selectivity ratio

bp = slop of the toxicity line on the predator

bm = slop of the toxicity line on the mite

Results and discussion

Data in Table (2) showed that cyflumetofen was the most potent compound tested at LC_{50} (0.6 ppm) and LC_{90} (2.66 ppm) levels against the adult female of the mite *T. urticae*, followed discerningly bypyridaben (2.53 and 9.77 ppm), pyridaben+clofentezine (3.12 and

13.48 ppm), bifenazate (3.91 and 15.66 ppm) and spirodiclofen (10672.3 and 44567.48 ppm), respectively. However, pyridaben was the most potent compound tested at both levels of LC_{50} and LC_{90} against the adult female of the predatory mite of *P. persimilis,* followed discerningly bypyridaben+clofentezine, bifenazate, cyflumetofen, and spirodiclofenat LC_{90} level for the previous compound, respectively (Table, 3).

The same trend was observed with the predatory mite of *N. californicus* (Table, 4). The acaricides pyridaben+ clofentezine was the most impact compound tested at both levels of LC_{50} (1.16 ppm) and LC_{90} (8.11 ppm) against the adult female of the predator. While the lowest effective compound was spirodiclofen. The side effect of the tested five acaricides on the two predatory mites was shown in Table (5). The toxicity values varied from 0.001 to 1.81 for *P. persimilis* and 0.001 to 1.938 for *N. californicus* at LC_{50}. Whereas these values varied from 0.001 to 1.695 for *P. persimilis*and 0.001 to 1.486 for *N.californicus* at LC_{90}. The two acaricides of spirodiclofen and pyridaben+ clofentezine had the highest toxicity on the two predators of *P. persimilis* and *N. californicus* at the two former levels, respectively.

The values were 1.81 and 1.69 ppm for *P. persimilis* and 2.69 and 1.66 ppm for *N. californicus* at LC_{50} and LC_{90} levels, respectively. However, the acaricides of cyflumetofen have the lowest toxicity values at each of the levels for each of *P. persimilis and N. californicus* with the same values of0.001 ppm at LC_{90} and LC_{90} levels, respectively. The selectivity ratio of cyflumetofen, bifenazate, at LC_{50} and LC_{90} levels have values less than one for both tested two predators, which means these acaricides are safe to these predators; whereas the remaining acaricides have values greater than one at the same level. As regard to general selective toxicity ratios (Table, 5) it could be seen that all the tested acaricides have values greater than one except cyflumetofen and bifenazate.

In other findings, data (Table, 6) showed the effect of five tested chemical of acaricides on the populations of active stages of twospotted spider mite *T. urticae*on kidney bean under field conditions in China and Egypt. It revealed that all treatments reduced the mean numbers of mite population compared with untreated one. The highest percent reductions of mite's population in the whole period were obtained by the acaricide of cyflumetofen in China and pyridaben in Egypt. The obtained percentage reductions were 93.4 % by cyflumetofen, followed by pyridaben (91.3%), bifenazate (90.9%) and pyridaben+clofentezine (89.8%), with no significant difference among of these compounds. On other hand, the same trend was recorded in Egyptian field . Pyridaben application gave the highest percentage of reduction (89.3%), followed by cyflumetofen (88.7%), bifenazate (86.4%) and pyridaben+clofentezine (84.7%), with no significant difference among the compounds. However, the least percentage of reduction was obtained in spirodiclofen treatment in both field trails with an average reduction of 82.7% and 78.0% respectively. No phytotoxic symptoms were found on leaves or any other plant parts in any of the treated plots.

Results in Table (7) shows the effect of five tested compounds on the populations of the two predatory mites of the most common predators of the twospotted spider mite. The obtained results showed highly significant difference among the tested pesticides in their effect on the two predators of *P. persimilis* ($F=757.4$, $P<0.0001$) and *N. californicus* ($F=501.2$, $P<0.0001$) in the

whole period. Pyridaben treatment gave the highest percentage of reductions of 98.2 and 96.7 % for two predators, respectively, without significant difference effect for the treatments between pyridaben+clofentezine and spirodiclofen. However, the least percentage of reduction was obtained by the two treatments of bifenazate and cyflumetofen for the previous two predators with no difference effect between the two acaricides.

The foregoing results clearly show that existing of two selectivity ratios to each acaricide at LC_{50} and at LC_{90} levels maybe cause disturbance in our estimation for the tested compounds. The general selective toxicity ratio resulting from combining the two former levels LC_{50} and $LC_{90,}$ is more useful. It can be relied on as a sufficient parameter to determine the least toxic acaricide against the two predatory mites*P. persimilis* and *N. californicus*. Also, to recommended the suitable acaricides to control the two-spotted spider mite throughout integrated pest management which the predator is dominant.

Broadly effective pesticides used in pest control cause side effects on natural enemies as well as predator mites, *P. persimilis* and *N. californicus.* Identifying the side effects of the pesticides on natural enemies is of importance for developing integrated control methods. Therefore, it is necessary to searching for the suitable selective pesticides that have the lowest effect on the beneficials in integrated control programs. It is possible to use the pesticides identified as harmless or not very harmful for predatory mites in production areas. It is also thought that the development of resistance in pests will be reduced based on the decrease in pesticide use (Marcic, 2012).

Alternative management strategies include the use of predatory mites along with pesticides instead of using pesticides alone in production areas (Cloyd *et al*., 2006). To identify the most selective pesticides that could be used in pest biocontrol strategies, it is very important to know the side effects of these products on the most relevant natural enemies for each specific crop.

A similar pattern of toxicity against the two-spotted spider mite has been previously reported for spirodiclofen (Marcic, 2007 and Van Pottelberge *et al.,* 2009). The results showed that the field rate of spirodiclofen was very toxic (82-96% mortality) after 7 and 21days following treatment for *T. urticae* but not for the predatory mite *Amblyseius andersoni* Chant (Acari: Phytoseiidae) (Rhodes *et al.,* 2006). They also suggested that release of phytoseiid mites after applying bifenazate at half the recommendeded rate effectively could control *T. urticae* in strawberries. Pyridaben was tested against red spider mite on marigold plants and was found to give higher mortality of nymphs over the adults (Raymond *et al*., 2010). Lee and Kim (2015) evaluated effects of 9 acaricides to the predatory mite *N. californicus*. Cyenopyrafen, spirodiclofen, spiromesifen, acequinocyl, bifenazate, flufenoxuron and cyflumetofen exhibited low toxicity to adult females and nymphs of *N. californicus* and had little effect on the reproduction and hatching of eggs deposited by treated predators. Based on the results, the seven above-mentioned acaricides are appeared to be promising candidates for use in integrated mite management program where *N. californicus* is the major natural enemy.

In contrary, Salman and Turan (2017) revealed that acequinocly, etoxazole, bifenazate and milbemectin showed high levels of toxicity on the nymphs and adults of *P. persimilis* and *N. californicus* at seventh days after applying. Kim and Yoo (2002) reported that

bifenazate, acequinocyl, chlorfenapyr, flufenoxuron and fenbutatin oxide were very toxic against *P. persimilis* adults. In the field and greenhouse trails, there were fewer two spotted spider mites in the *N. californicus* treated plots compared with the *P. persimilis* treated ones. This suggests that *N. californicus* may be better for tolerance of the tested acaricides and environmental conditions than *P. persimilis*. In this study, it is also known that *P. persimilis* suppress quickly the twospotted spider mite populations under controlled conditions. Therefore, *P. persimilis* cannot survive in areas where its food is devoid. These results argued strongly that the use of various pesticides for the mite control should be carefully monitored in order to avoid deleterious side effects on the predator's complex that may result in a rapid increase of non-target insect species. Furthermore, field releases of the biological control agents such as *P. persimilis* and *N. californicus* could employ selective pesticides such as bifenazate and cyflumetofen.

Finally, results regarding side effect studies conducted with predatory mites in the pesticide lists to be updated in the future will make contribution to the early planning of an integrated mite management program. The identification of the side effects of the pesticides used will enable the use of preparations that are harmless or a little harmful to natural enemies. Thus, the feasibility of biological control will be facilitated, the use of excessive pesticide doses will be prevented, and the environment and the health of living individuals will be protected.

Table (2): Probit analysis for five acaricidal activities against adult female stage of the two spotted spider mite *Tetranychus urticae*.

Chemical Compound	Lethal concentrations [a] (95% Lower-Upper of confidence limits)		Slope [b] ± standard errors (SE)	X^2 (df; *P*-value) [c]	Heterogeneity
	LC_{50}	LC_{90}			
Bifenazate	3.91 (3.3-4.6)	15.66 (11.7-29.3)	2.12 ± 0.10	7.68 (5; *P*<0.001)	1.92
Cyflumetofen	0.60 (0.53-0.68)	2.66 (1.79-4.33)	1.98 ± 0.08	4.70 (5; *P*<0.02)	1.17
Pyridaben	2.53 (2.35-2.74)	9.77 (6.56-15.03)	2.19 ± 0.09	2.67 (5; *P*<0.01)	0.67
Pyridaben Clofentezine	3.12 (2.67-3.65)	13.48 (8.59-20.12)	2.01 ± 0.20	7.19 (5; *P*<0.001)	1.80
Spirodiclofen	10672.3 (10058.4-11323.50)	44567.48 (29542.1-70018.51)	2.06 ± 0.01	1.27 (5; *P*<0.003)	0.32

a = Delivered median lethal concentration (LC_{50}) expressed by infective propagules $ml^{-1}$and estimated by the logistic model. Mortality censored up to day 3 and 5 application. Control mortality averaged 1.1±0.2%.
b = Slope for mortality represents regression of proportion of larval mortality versus $\log_{10}$ of propagules ml^{-1}.
c = chi-squared goodness of fit test (X^2), degrees of freedom (df), and *P*-value represent the probability of slope.

Table (3): Probit analysis for five acaricidal activities against adult female stage of *Phytoseiulus persimilis*.

Chemical Compound	Lethal concentrations [a] (95% Lower-Upper of confidence limits)		Slope [b] ± standard errors (SE)	X^2 (df; *P*-value) [c]	Heterogeneity
	LC_{50}	LC_{90}			
Bifenazate	165.6 (133.8-205.1)	661.1 (432.0-987.5)	2.13 ± 0.12	7.69 (5; P<0.002)	1.92
Cyflumetofen	464.49 (373.1-578.4)	1944.55 (1388.9-3285.8)	2.06 ± 0.12	7.62 (5; P<0.001)	1.91
Pyridaben	1.51 (1.18-2.05)	7.21 (5.31-11.32)	1.94 ± 0.12	11.40 (5; P<0.001)	2.19
Pyridaben Clofentezine	2.05 (1.52-2.70)	12.87 (8.21-23.23)	1.60 ± 0.10	7.99 (5; P<0.001)	1.99
Spirodiclofen	6295.79 5147.82.40-7700.43)	32196.67 (29542.1-70018.51)	1.81 ± 0.11	5.24 (5; *P*<0.008)	1.31

Table (4): Probit analysis for five acaricidal activities against adult female stage of Neoseiulus californicus.

Chemical Compound	Lethal concentrations [a] (95% Lower-Upper of confidence limits)		Slope [b] ± standard errors (SE)	X^2 (df; *P*-value) [c]	Heterogeneity
	LC_{50}	LC_{90}			
Bifenazate	226.0 (186.7-273.6)	769.5 (536.1-1185.0)	2.41 ± 0.14	6.93 (5; *P*<0.001)	1.95
Cyflumetofen	546.75 (432.6-691.4)	2489.92 (1664.8-4160.3)	1.95 ± 0.12	7.79 (5; *P*<0.001)	1.64
Pyridaben	2.25 (1.75-2.86)	12.90 (7.51-20.84)	1.69 ± 0.11	6.55 (5; *P*<0.001)	1.74
Pyridaben Clofentezine	1.16 (0.87-1.51)	8.11 (5.07-14.91)	1.52 ± 0.10	6.97 (5; *P*<0.001)	1.22
Spirodiclofen	5506.17 (4510.68-29926.47)	29926.47 (21674.1-56704.08)	1.74 ± 0.11	4.88 (5; *P*<0.003)	1.95

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Table (5): Toxicity of five acaricides to the two spotted spider mite *Tetranychus urticae* and the two predators, *Phytoseiulus persimilis* and *Neoseiulus californicus*.

Compound	*Tetranychus urticae*			*Phytoseiulus persimilis*			Selective ratio at level		General selective toxicity ratio*	*Neoseiulus californicus*			Selective ratio at level[a]		General selective toxicity ratio*
	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}		LC_{50}	LC_{90}	Slope	LC_{50}	LC_{90}	
Bifenazate	3.9	15.7	2.12	165.6	661.1	2.13	0.024	0.024	0.023	226.0	769.5	2.41	0.017	0.020	0.015
Cyflumetofen	0.6	2.7	2.0	464.5	1944.6	2.1	0.001	0.001	0.001	546.8	2489.9	1.95	0.001	0.001	0.001
Pyridaben	2.5	9.8	2.2	1.51	7.21	1.9	1.675	1.359	2.005	2.3	12.9	1.69	1.100	0.760	1.648
Clofentezine	3.1	13.5	2.0	2.1	12.8	1.6	1.6	1.486	1.055	1.16	8.11	1.5	2.690	1.665	4.396
Spirodiclofen	10672.3	44567.5	2.1	6295.8	32196.7	1.8	1.81	1.695	1.384	5506.2	29926.5	1.74	1.938	1.489	2.591

***Values greater than 1 indicate unsafe to the predator.**

Table (6): Effect of five acaricides on the percentage reduction of the two spotted spider mite *Tetranychus urticae* infesting kidney bean plants during season 2019 under field conditions of China and Egypt.

Compound	Rate of application M/100 L.Water	Percentage reduction in mite's population at indicated days after treatment (Numbers of mite /inch^2 at indicated days after treatment)													
		China							Egypt						
		0*	3	5	7	10	15	Mean	0*	3	5	7	10	15	Mean
Bifenazate		(36.3)	100.0 (0.0)	100.0 (0.0)	90.5 (4.6)	84.5 (6.6)	79.3 (10.2)	90.9 (4.3)	(11.1)	100.0 (0.0)	91.4 (1.1)	82.5 (1.6)	81.2 (3.0)	77.0 (4.1)	86.4 (2.2)
Cyflumetofen		(51.0)	100.0 (0.0)	100.0 (0.0)	93.1 (4.7)	89.8 (6.1)	84.3 (10.9)	93.4 (4.3)	(12.7)	100.0 (0.0)	93.8 (0.9)	87.6 (2.1)	83.0 (3.1)	78.9 (4.3)	88.7 (2.1)
Pyridaben		(34.3)	100.0 (0.0)	100.0 (0.0)	92.0 (3.7)	83.6 (6.6)	81.2 (8.8)	91.3 (3.8)	(9.7)	100.0 (0.0)	92.4 (0.9)	86.9 (1.8)	85.8 (2.1)	81.3 (3.1)	89.3 (1.6)
Pyridaben Clofentezine		(40.8)	100.0 (0.0)	100.0 (0.0)	90.3 (5.3)	80.2 (9.5)	78.5 (11.9)	89.8 (5.3)	(8.9)	100.0 (0.0)	90.1 (1.1)	82.3 (2.3)	79.2 (2.9)	71.8 (4.4)	84.7 (2.1)
Spirodiclofen		(25.2)	100.0 (0.0)	92.5 (2.4)	84.9 (5.1)	71.0 (8.6)	68.5 (10.8)	82.7 (5.5)	(10.7)	100.0 (0.0)	88.3 (1.2)	77.3 (2.7)	68.0 (4.1)	63.7 (5.2)	78.0 (2.8)
Untreated		(40.6)	(34.0)	(51.3)	(54.4)	(47.7)	(55.2)		(10.7)	(9.8)	(12.3)	(14.3)	(15.4)	(17.2)	(10.7)

Note: *pretreatment. Number in brackets means .

Table (7): Effect of five acaricides on the percentage reduction of the two predatory mites of *Phytoseiulus persimilis* and *Neoseiulus californicus* **on kidney bean plants under greenhouse conditions.**

Compound	Rate of application M/100 L.water	Percentage reduction in mite's population at indicated days after treatment (Numbers of mite /inch^2 at indicated days after treatment)												
		Phytoseiulus persimilis						*Neoseiulus californicus*						
		0*	3	5	7	10	15	Mean	0*	3	5	7	10	15	Mean
Bifenazate	(36.3)	9.1 (0.9)	0.0 (1.8)	0.0 (2.0)	0.0 (2.5)	0.0 (2.4)	1.8 (1.9)	(11.1)	1.8 (1.0)	0.2 (1.5)	0.0 (1.8)	0.0 (1.2)	0.0 (1.3)	0.4 (1.4)
Cyflumetofen	(51.0)	10.3 (1.0)	7.6 (1.9)	0.0 (2.2)	0.0 (2.7)	0.0 (2.8)	3.6 (2.7)	(12.7)	2.9 (1.2)	0.2 (1.9)	0.0 (2.3)	0.0 (1.5)	0.0 (1.7)	0.6 (1.7)
Pyridaben	(34.3)	100.0 (0.0)	100.0 (0.0)	100 (0.0)	97.2 (0.1)	93.8 (0.2)	98.2 (0.1)	(9.7)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	92.3 (0.1)	91.1 (0.1)	96.7 (0.1)
Pyridaben Clofentezine	(40.8)	100.0 (0.0)	100.0 (0.0)	100 (0.0)	97.5 (0.1)	91.6 (0.3)	97.8 (0.1)	(8.9)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	94.7 (0.1)	81.2 (0.4)	95.2 (0.1)
Spirodiclofen	(25.2)	100.0 (0.0)	100 (0.0)	100 (0.0)	96.3 (0.1)	88.0 (0.3)	96.9 (0.1)	(10.7)	100.0 (0.0)	100.0 (0.0)	100.0 (0.0)	91.7 (0.2)	85.1 (0.4)	95.4 (0.1)
Untreated	(1.3)	(1.3)	(2.4)	(2.7)	(3.3)	(3.1)		(10.7)	(1.4)	(2.1)	(2.6)	(1.7)	(1.9)	(10.7)

***pretreatment. Number in brackets means .**

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Impact of certain pests on the response and productivity of okra cultivars in Sohag Governorate

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Abstract:

The present work was conducted at Shandweel Agricultural Research Station, Sohag Governorate during 2017 and 2018 okra growing seasons to determine the population trends and susceptibility degrees against the prevalent sap sucking arthropod pests inhabiting 4 okra cultivars. *Aphis gossypii* Glover (Hemiptera: Aphididae) , *Bemisia tabaci* (Gennadius) (Hemiptera:Aleyrodidae), *Empoasca discipiens* (Paoli) (Hemiptera: Cicadellidae) and *Tetranychus urticae* Koch. (Acari:Tetranychidae) were found to be the most dominant species on the tested okra cultivars. The highest populations of *A. gossypii*, *E. discipiens* and *T. urticae* were recorded during July, however, the highest populations of *B. tabaci* mature and immature stages were recorded during May and June. The tested okra cultivars showed varied susceptibility degrees against the studied pests. Except of, *A. gossypii*, white velvet okra cultivar showed some sort of resistance against *B. tabaci*, *E. discipiens* and *T. urticae*. It must be shed a light on the presentation of *A. gossypii* at obvious low numbers on balady green cultivar which showed some sort of resistance against this destructive insect pest. Under sprayed and unsprayed procedures, influence of the pest's infestation on some yielding characters was studied. Okra cultivars varied significantly in both seasons. Under natural infestation, the yield and great proportion of the yield components decreased significantly compared to managed infestation. golden coast okra cultivar gave the highest fresh fruit yield per feddan and followed by white velvet okra cultivar. Both cultivars harbored moderate levels of the above mentioned pests. However, white velvet recorded the lowest reduction for fresh fruit yield per feddan and followed by golden coast. So, it can be recommended using the later cultivars as a part of an integrated pest management program. Furthermore, these results could be helpful for varieties screening programs.

Introduction

Okra (*Abelmoschus esculentus* L., Malvaceae) is a warm season, annual vegetable and cash crop. It is a good source of vitamins, minerals and has a good caloric

value. Okra plants are subjected to be attacked by a variety of destructive sap sucking pests from seedlings until harvest. Amongst these pests; cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), whitefly *Bemisia tabaci* (Gennadius) (Hemiptera:Aleyrodidae), leafhopper *Empoasca discipiens* (Paoli) (Hemiptera: Cicadellidae) and two spotted spider mite *Tetranychus urticae* (Boisd.) (Acari:Tetranychidae). These arthropod pests were recorded as the most responsible for reduction in yield and hinder its quality (El-Khawas, 2005 and Saif Ullah and Aziz, 2012). Using tolerant or resistant and high yielding cultivars as an important component of integrated pest management (IPM) program of okra pests is meaningful because they are compatible with other control methods with no adverse side effects. The susceptibility of okra cultivars to pests has been studied by several authors (Amro *et al*., 2012; Abou Hatab and Elgendy, 2013; Allam *et al.*, 2014; Akbar and Khan, 2015 and Biswas *et al.*, 2016). Even with the importance of piercing sucking pests on okra cultivation, information on losses from these pests damage or management costs are still lacking. Also, the relationships between infestation and yield components of okra are not sufficiently studied. Therefore, the present study was conducted to determine the population trend of *A. gossypii*, *B. tabaci*, *E. discipiens* and *T.urticae* infesting four okra cultivars (White velvet, balady red, golden coast and balady green). Also, there response of these cultivars to pest's infestation under sprayed and unsprayed conditions was studied. Finally, the reduction in some yield components and yield income due to pest's infestation was also included.

Materials and methods

The present studies were carried out during the summer seasons of 2017 and 2018 at Experimental Farm of Shandweel Agricultural Research Station, Sohag Governorate, Egypt. Each experimental unit was 1/400 fedddan (10.5 m^2) including 5 rows, each of 3.5 m length and 70 cm width. Sowing was done on 15th April in both seasons by sowing three seeds per hill at 35 cm intervals in a randomized complete block design. Growing plants were thinned into one plant/ hill. Conventional agricultural practices were performed and insecticidal treatments were completely prevented.

1. Population trends of some piercing sucking pests infesting four okra cultivars:

Four okra cultivars (White velvet, balady red, golden coast and balady green) were cultivated in complete randomized block with three replicates. Sampling was started after emergence and continued until harvesting time. Each sample consisted of 10 leaves which picked up randomly from top, middle and lower canopy of okra plants at weekly intervals. The samples were kept into polyethylene bags and transferred to laboratory for examination using a stereomicroscope. The numbers of aphid, whitefly (adults and nymphs), leafhopper (adults and nymphs) and the two spotted spider mite (mobile stages) were counted and recorded. Population trends and peaks of each pest were determined.

2. Relative susceptibility of okra cultivars to infestation with certain piercing sucking pests:

The same 10 okra leaves were used to determine the relative susceptibility of the tested cultivars to the above mentioned pests. The pest's mean numbers were used to determine the relative susceptibility degree of the tested cultivars as described by Chiang and Talekar (1980) equation. Relative susceptibility degree was dependent on the general mean number of the pest ($\overline{X}$) and the standard deviation (SD). Cultivars that had mean numbers more than $\overline{X}$+2SD, were considered highly susceptible (HS), between $\overline{X}$ and $\overline{X}$+2SD, susceptible (S), between $\overline{X}$ and $\overline{X}$-1SD, low resistant (LR), between $\overline{X}$-1SD and $\overline{X}$-2SD, moderately resistant (MR) and less than $\overline{X}$-2SD, were considered

highly resistant (HR). Data were statistically analyzed by using F-test; means were compared according to Duncan's multiple range tests as described by Steel and Torrie (1982).

3. Response of four okra cultivars to pest's infestation under sprayed and unsprayed procedures:

To determine the effect of the selected piercing sucking pests on some vegetative and yield component, each of the above mentioned cultivars were sown in 6 plots. After germination, all the cultural practices were performed throughout the growing season uniformly in all plots. Piercing sucking pests were allowed to develop on three plots whereas the others were kept free from pests by spraying imidacloprid and abamectin three times. Ten plants were randomly taken from each plot to determine the following characters:

3.1.Fresh fruit yield characteristics:

3.1.1. Number of fresh fruits per plant:

Ten plants were randomly taken from each plot, the mean of the ten plants was used to determine the number of fresh fruits/ plant. The fruits were picked for fresh fruit at edible fruit maturity stage.

3.1.2. Fresh fruit yield. (Ton./fed.):

The average weight of fresh fruit / plot was calculated and multiplied by 400 to obtain fresh fruit yield /fed.

3.2. Seed yield and quality characteristics:

The following measurements were calculated

3.2.1. Number of seeds per dry fruitaverage (50 mature fruits from each plot).

3.2.2. 100- seeds weight (g).

3.2.3. Seed yield (kg/fed.).

4. Yield loss:

Loss in the yield of unsprayed plots was compared to the yield of sprayed plots and percent loss was calculated for each cultivar using the following formula:

Yield loss (%) = (sprayed plots yield −unsprayed plots yield)/ sprayed yield)×100

5. Statistical analysis:

Mean population of *A. gossypii*, *B. tabaci*, *E. discipiens* and *T. urticae* from unsprayed plots were analyzed by analysis of variance (ANOVA) to determine the susceptibility of four okra cultivars. However, the data of vegetative, some yield component and yield were analyzed by analysis of variance for sprayed and unsprayed plots. Differences in means were conducted using the least significant difference (LSD) procedure at P = 5% (Snedecor and Cochran, 1971). Comparisons within each okra cultivar between the sprayed and unsprayed were performed by using the t-test.

Results and discussion

1. Population trends of some piercing sucking pests infesting four okra cultivars:

1.1. *Aphis gossypii*:

Data illustrated in Figure (1) showed that aphid started to attack okra at May 2nd then increased to form three peaks on the four tested cultivars in both seasons. Three peaks were recorded on 30th May, 27thJune and 18th July in 2017 season and in 6th June, 4th July and 1st August in 2018 season for most of the tested cultivars.This finding is in agreement with the results of Abdel Hamed *et al.*(2011). Also, Akbar and Khan (2015) found that the population of *A. gossypii* peaked was recorded in June-July.

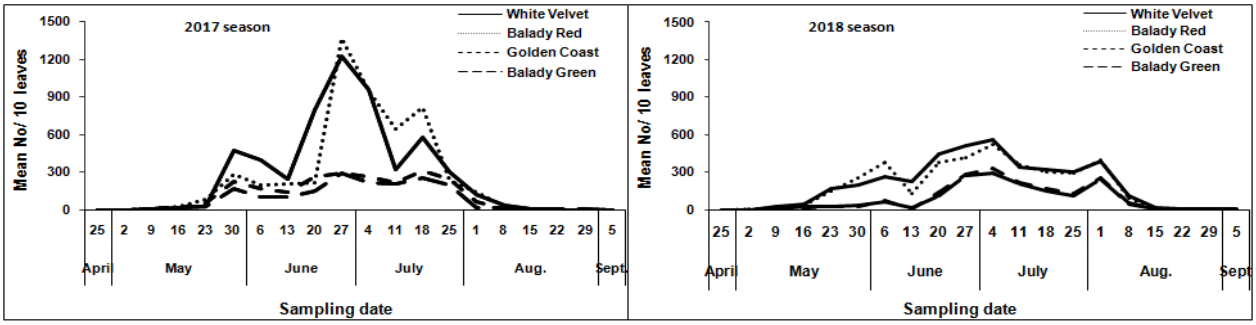

Figure (1): Population trend of *Aphis gossypii* on four okra cultivars in Sohag Governorate during 2017 and 2018 seasons.

1.2. *Bemisia tabaci*:

In respect to the mature stage, three peaks were detected on four tested cultivars in both growing seasons (Figure, 2). The peaks were recorded on 16th May, 6th June and 11st July in 2017 season and on 16th May, 13rd June and 18th July in 2018 season. In respect to the immature stage, two and three peaks were detected on the tested cultivars in 2017 and 2018 seasons, respectively (Figure, 3). The peaks were recorded on 23rd May and 20th June in 2017 season, and on 2nd and 30th May and 20th June in 2018 season. It is important to note that immature peaks were recorded 2-4 weeks before mature peaks.

The present results are in agreement with those obtained by Leite *et al.* (2005) who reported that, whitefly adult population increased from May to June, after the appearance of nymph population peaked in April. Also, Sahito *et al.* (2012) showed that *B. tabaci* attacked okra from germination till harvest and displayed three peaks in its population when the crop was sown on 20th March.

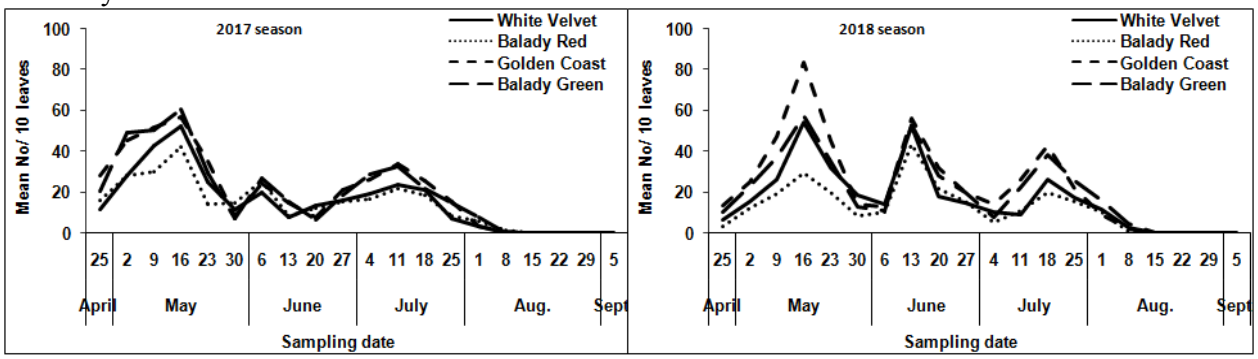

Figure (2): Population trend of *Bemisia tabaci* adults on four okra cultivars in Sohag Governorate during 2017 and 2018 seasons.

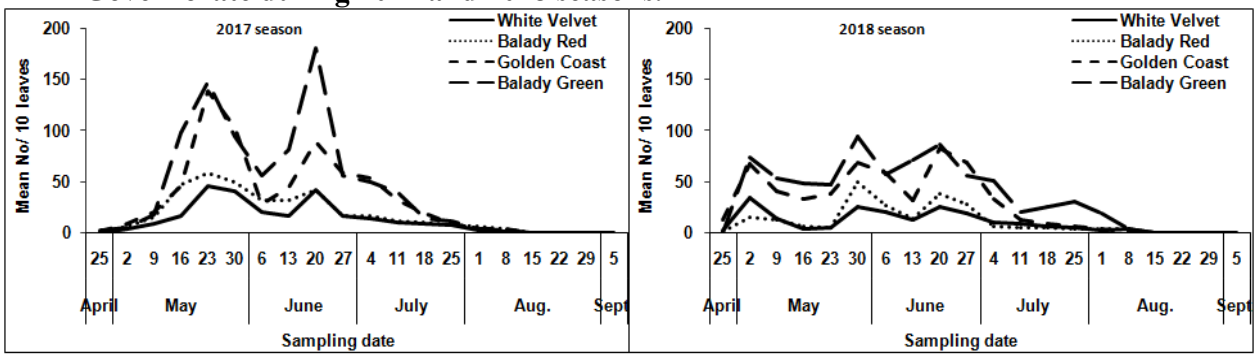

Figure (3): Population trend of *Bemisia tabaci* nymphs on four okra cultivars in Sohag Governorate during 2017 and 2018 seasons.

1.3. *Empoasca discipiens*:

Data illustrated in Figure (4) show the population density of *E. discipiens* (adults and nymphs) on four okra cultivars during 2017 and 2018 seasons. Four peaks were detected on the tested cultivars in both growing seasons. The peaks were recorded on 23rd May, 13th June, 4th and18thJuly in 2017 season, and on 6th and 27th June and 11th and 25thJuly in 2018 season. The previous results are in partial agreement with those obtained by Sahito *et al.* (2013), who found that the maximum and the minimum populations of *E. discipiens* were recorded in the start of June and during last the week of April, respectively. However, Javed *et al.* (2016) demonstrated that the *E.discipiens* population showed an increasing trend on all five okra varieties over 18 weeks, and maximum population was recorded in ambika variety during 17th week of data collection.

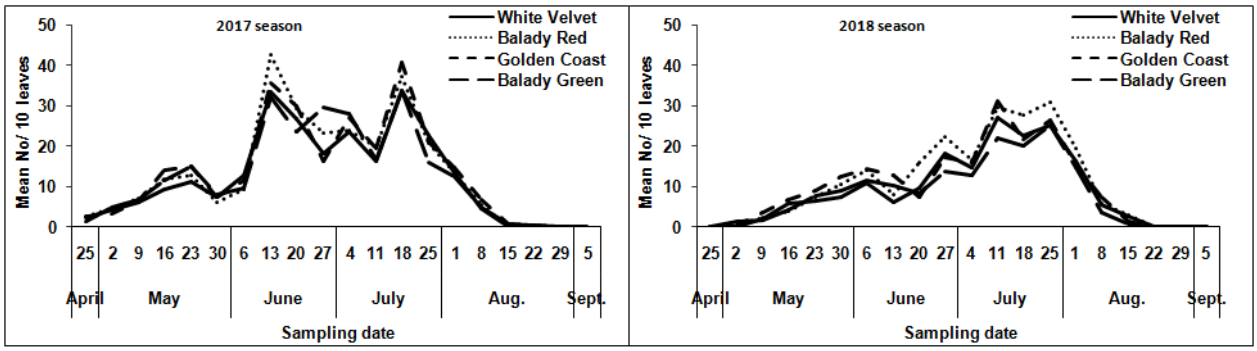

Figure (4): Population trend of *Empoasca discipiens* (adults and nymphs) on four okra cultivars in Sohag Governorate during 2017 and 2018 seasons.

1.4. *Tetranychus urticae*:

The population density of *T. urticae* mobile stages (adults and nymphs) on four okra cultivars during 2017 and 2018 seasons is graphically illustrated in Figure (5).Two peaks were detected for mobile stages on four tested cultivars in both growing seasons. The peaks were recorded on 23rd May and 27thJune in both seasons. In 2017 season, an additional peak was observed in 11thJuly for balady green cultivar. Our results are in partial agreement with those of Sahito *et al.* (2012), Amro *et al.* (2013) and Allam *et al.* (2014).

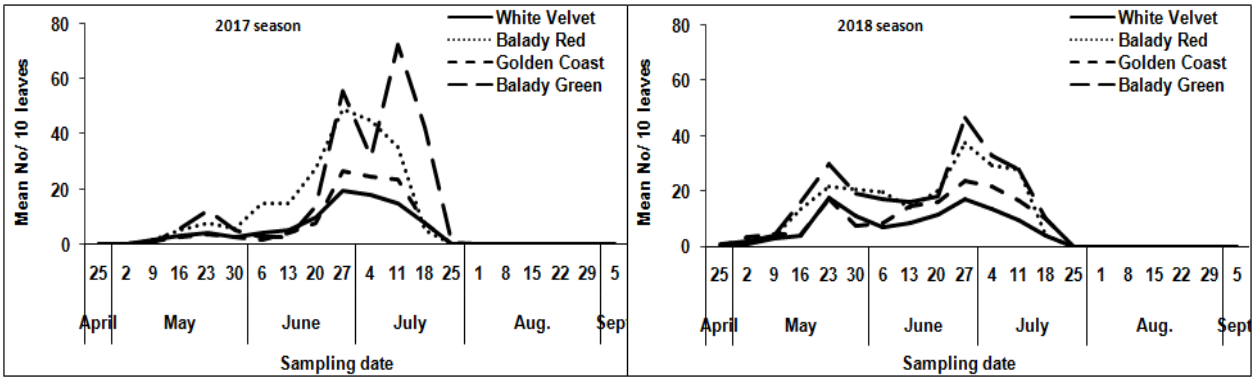

Figure (5): Population density of *Tetranychus urticae* (mobile stages) on four okra cultivars in Sohag Governorate during 2017 and 2018 seasons

According to the obtained results it can be conclude that assessment the population fluctuations of arthropod pests is the corner stone of managing insect pests associated with the crop. Determination the population fluctuation trends and peaks appearance dates of the prevalent arthropod species inhabiting okra plants was useful in pest's management in the area of study.

2. Relative susceptibility of okra cultivars to infestation with certain piercing sucking pests:

Data in Tables (1 and 2) present the susceptibility degrees of four okra cultivars to infestation with *A. gossypii*, *B. tabaci*, *E.discipiens* and *T.urticae* during 2017 and 2018 okra growing seasons.

2.1. *Aphis gossypii*:

Data presented in Table (1) revealed that *A. gossypii* was presented in so high numbers on okra leaves during 2017 growing season with an average of 278.93, 262.12 and 113.72 individuals / 10 leaves on white velvet, balady red, golden coast, respectively. Consequently these cultivars were appeared as susceptible (S) cultivars to this insect pest. It is important to note that balady green cultivars occupied the least numbers with an average of 91.02 individuals / 10 leaves and appeared as low resistant (LR) cultivar. Except of balady green cultivar which appeared as moderately resistant (MR) cultivar, similar results were recorded during the second season of study (Table, 2). It must be focusing on the presentation of *A. gossypii* at clearly low numbers on balady green cultivar which showed some sort of resistance against this destructive insect pest. Data revealed significant and high significant variations between the infestation of the tasted cultivars (F= 6.32* and 51.22**, respectively). In this approach, Abang *et al.* (2019) evaluated resistant in some okra accessions.They found that accession VI041210 was resistant to aphid infestation during the first season, while, VI057245 and gombo caféier were resistant during the second season.

2.2. *Bemisia tabaci*:

Concerning *B. tabaci*, data in Tables (1 and 2) revealed that golden coast and balady green occupied the highest numbers of its adults during 2017 and 2018 growing seasons and appeared as susceptible (S) cultivars. However, white velvet and balady red cultivars occupied lower numbers and showed some sort of resistance to *B.tabaci* adults and consequently appeared as low resistant (LR) and moderately resistant (MR) cultivars. Similar defense behavior was observed against *B.tabaci* nymphs by the golden coast and balady green okra cultivars. Significant and high significant variations between the infestations of the tasted cultivars were recorded during 2017 and 2018 growing seasons, respectively. This finding could be attributed to the antixenosis phenomenon presented by the latter cultivars toward *B.tabaci* adult's oviposition behavior and the antibiosis phenomenon against its immature stag as described by Knipling (1979). In the same approach, Chatterjee *et al.*(2019) stated that none of 15 okra genotypes were found completely free from the attack of whitefly. However, OH05 cultivar proved to be resistance against whitefly, while the variety samrat performed least.

2.3. *Empoasca discipiens*:

Although, the leafhopper *E.discipiens* was recorded in quietly low numbers on the 4 tested okra cultivars, white velvet and balady green showed some sort of resistance to this insect pest and occupied less numbers (12.30 and 12.78 individuals / 10 leaves during 2017 season and appeared as moderately resistant (MR) and low resistant (LR) cultivars, respectively. Similar results were obtained during 2018 season. Non significant and significant were recorded between the tested cultivars during 2017 and 2018 seasons, respectively (Tables, 1 and 2) . Similarly, Kadu *et al.* (2018) reported that none of the tested genotypes was found completely free from leafhopper infestation, although they significantly differed in their degree of pest number.

2.4. *Tetranychus urticae*:

The lowest mobile stages number of *T.urticae* was recorded on white velvet in both seasons, followed by golden coast in both seasons. However, the highest infestation was recorded on balady green followed by balady red. Mean numbers of 4.57, 10.57, 5.27 and 12.20 individuals/ 10

leaves were recorded on white velvet, balady red, golden coast and balady green cultivars, respectively, in 2017 season and 5.42, 10.70, 7.42 and 12.05 individuals/ 10 leaves were recorded on the previous cultivars, respectively, in 2018 season. Data revealed that the differences between four okra cultivars were high significant in both seasons. It can be note that cultivars occupied the lowest numbers appeared as moderately resistant (MR) and low resistant (LR) cultivars. However, the others appeared as susceptible (S) cultivars (Tables, 1 and 2). Allam *et al*. (2014) screened 8 okra varieties against *T. urticae*, and they found that the population of *T. urticae* varied significantly on the tested varieties. Also, the sensitivity of okra varieties varied according to months.

Except of, *A.gossypii*, white velvet okra cultivar showed some sort of resistance against *B. tabaci*, *E.discipiens* and *T.urticae*. Also, it must be shed a light on the presentation of *A. gossypii* at obvious low numbers on balady green cultivar which showed some sort of resistance against this destructive insect pest.

Table (1): Susceptibility of four okra cultivars to infestation by certain piercing sucking pests in Sohag Governorate during 2017 season.

Pest	Mean no./ 10 leaves and susceptibility degree				Mean ± SD	F. value	L.S.D. value
	White Velvet	Balady Red	Golden Coast	Balady Green			
Aphis gossypii	278.93 (**S**)	262.12 (**S**)	113.72 (**S**)	91.02 (**LR**)	186.45±847.67	6.32*	134.59
***Bemisia tabaci* adults**	15.20 (**LR**)	13.95 (**MR**)	20.00 (**S**)	19.60(**S**)	17.19±2.65	7.34*	3.91
***Bemisia tabaci* nymphs**	12.73 (**MR**)	17.75 (**LR**)	33.00 (**S**)	42.90 (**S**)	26.59±12.01	7.23*	17.86
Empoasca discipiens	12.30 (**MR**)	13.50 (**S**)	13.63(**S**)	12.78(**LR**)	13.05±0.54	2.80	N.S.
Tetranychus urticae	4.57 (**MR**)	10.57 (**S**)	5.27 (**LR**)	12.20 (**S**)	8.15±3.29	31.34*	2.35

(*): The F value is significant at P ≤ 0.05
S=Susceptible LR= Low Resistant MR= Moderately Resistant

Table (2): Susceptibility of four okra cultivars toinfestation by certain piercing sucking pests in Sohag Governorate during 2018 season.

Pest	Mean no./ 10 leaves and susceptibility degree				Mean ± SD	F. value	L.S.D. value
	White Velvet	Balady Red	Golden Coast	Balady Green			
Aphis gossypii	195.37 (**S**)	188.02 (**S**)	87.15 (**LR**)	81.40 (**MR**)	137.99±53.81	51.22**	30.04
***Bemisia tabaci* adults**	16.37 (**LR**)	12.18 (**LR**)	23.22 (**S**)	20.17 (**S**)	17.99±4.13	14.73**	4.31
***Bemisia tabaci* nymphs**	9.80 (**MR**)	11.20 (**LR**)	28.23 (**S**)	36.78 (**S**)	21.50±11.42	120.19**	4.16
Empoasca discipiens	9.02 (**LR**)	10.90 (**S**)	10.15 (**S**)	8.35 (**LR**)	9.61±0.99	8.98*	1.32
Tetranychus urticae	5.42 (**MR**)	10.70 (**S**)	7.42 (**LR**)	12.05 (**S**)	8.89±2.62	249.34**	0.66

(*): The F value is significant at P ≤ 0.05
S=Susceptible LR= Low Resistant MR= Moderately Resistant

3. Response of four okra cultivars to pest's infestation under sprayed and unsprayed procedures:

3.1. Fresh fruit yield characteristics:

The tested okra cultivars varied significantly under sprayed and unsprayed conditions in case of a number of fresh fruits per plant and fresh fruits yield per fedden in both seasons. It is clear that pest infestation affected on the response of okra cultivars (Table, 3). Also, the differences between sprayed and unsprayed plots were significant (Table, 4).

When plants left to natural infestation, the tested cultivars arranged into two significantly groups in both season, the highest included balady red (24.60 and 24.40 fresh fruits/ plant) and balady green (23.06

and 23.37 fresh fruits/ plant), while, the lowest one included white velvet (20.53 and 20.87 fresh fruits/ plant) and golden coast (20.47 and 20.93 fresh fruits/ plant) in the two seasons, respectively (Table, 3). Also, balady green and golden coast recorded the highest and the lowest mean number of fresh fruits/ plant, respectively under sprayed conditions in both seasons. On the other hand, balady red recorded the lowest reduction percentages, with 5.26 and 6.15% in 2017 and 2018 season, respectively, comparing with 28.50% and 31.81% in 2017 and 2018 seasons, respectively in balady green (Table, 4). Pest infestation reduced significantly number of fresh fruits per plant for all cultivars, except for balady red in 2017 season.

Golden coast recorded the highest weight of fresh fruit yield (Ton/fed.) under sprayed and unsprayed conditions in both seasons, followed insignificantly by balady green under sprayed conditions in 2017 season. However, the lowest weight of fresh fruit yield (Ton/fed.) recorded in balady green under unsprayed conditions in both seasons and in white velvet under sprayed one in both seasons, by insignificant difference with balady red in 2017 season. This behavior may due to pest infestation, balady green recorded 34.52% and 34.64% in the two seasons, respectively as the highest reduction, However, white velvet recorded 13.93% and 12.24% reduction as the lowest one in the two seasons, respectively (Table, 4).

These results were in harmony with Shannag *et al.* (2007) who demonstrated that aphid free cultivars varied considerably between each other in the number of pods and total pod weight per plant. Also, Jahangir *et al.* (2017) who tested five okra varieties against leafhopper and they found that the maximum fresh fruits yield per cultivated unit was recorded in green wonder variety (9074.997 kg/hectare) and the minimum was recorded in Sabzpari (7049.711 kg/hectare). Similarly, Rehmana *et al.* (2017) tested four okra varieties under field conditions against bollworm, whitefly and Jassid and they concluded that variety sada bahar resulted in maximum yield (1529.62 kg/ ha). Many authors found that the yield of fresh fruits increased in sprayed plots compared with infested one in regardless cultivar (Shannag *et al.*, 2007 and Samaila and Oaya, 2014).

Table (3): Number of fresh fruits per plant and fresh fruits yield per fed. of four okra cultivars under sprayed and unsprayed conditions in Sohag Governorate during 2017 and 2018 seasons.

Plant characteristics	Treatment	Season	Okra cultivars				F. value	L.S.D.
			White Velvet	Balady Red	Golden Coast	Balady Green		
No. of fresh fruits per plant	Unsprayed	2017	20.53	24.60	20.47	23.06	8.51	2.40
		2018	20.87	24.40	20.93	23.37	18.39	1.43
	Sprayed	2017	23.21	25.97	23.55	32.25	30.7	2.61
		2018	24.13	26.00	23.73	34.27	194.85	1.22
Fresh fruits yield (ton/fed.)	Unsprayed	2017	4.70	4.62	5.25	4.16	94.98	0.15
		2018	4.68	4.54	5.27	4.18	233.17	0.10
	Sprayed	2017	5.46	5.66	6.45	6.35	40.19	0.27
		2018	5.34	5.62	6.48	6.40	890.23	0.07

(*): The F value is significant at P ≤ 0.05

Table (4): Reduction percentages on number of fresh fruits per plant and fresh fruits yield per fed. of four okra cultivars caused by certain piercing sucking pests on in Sohag Governorate during 2017 and 2018 seasons.

Plant characters	Reduction%							
	2017 season				2018 season			
	White Velvet	Balady Red	Golden Coast	Balady Green	White Velvet	Balady Red	Golden Coast	Balady Green
No. fruits per plant	11.52*	5.26	13.09*	28.50*	13.54*	6.15*	11.8*	31.81*
Fresh fruits yield (ton/fed.)	13.93*	18.37*	18.56*	34.52*	12.24*	19.32*	18.77*	34.64*

(*): The difference between sprayed and unsprayed is significant at P ≤ 0.05

3.2. Seed yield and quality characteristics:

From the data in Table (5), it is evident that the four okra cultivars varied significantly under sprayed and unsprayed conditions in case of 100- seeds weight, number of seeds per dry fruit and seed yield per fed. in both seasons, however, in case of seed weight per plant, the differences between the previous cultivars were insignificant and significant in the two seasons, respectively. Also, it is clear that the differences between sprayed and unsprayed plots were significant in both seasons (Table, 6).

The highest weight of 100-seeds was recorded in balady green in both of sprayed (6.43 and 6.25 g) and unsprayed conditions (5.60 and 5.59 g), followed insignificantly by golden coast under unsprayed conditions in both seasons and by balady red under sprayed conditions in the 2017 season. While, the lowest weight of 100-seeds was recorded in white velvet in both of sprayed (4.49 and 4.48 g) and unsprayed conditions (3.77 and 3.68 g), followed insignificantly by golden coast under unsprayed conditions in both seasons and by balady red under sprayed conditions in the 2017 season. Golden coast proved insignificant difference between sprayed and unsprayed plots for weight of 100-seeds in both seasons of the study, this cultivar received the lowest loss of 4.41% and 1.91% in the two seasons, respectively (Table, 6). However, white velvet gave the highest reduction of 15.90% and 17.78% in the two seasons, respectively.

The highest number of seeds/ dry fruit was recorded in white velvet in regardless to pest infestation conditions with 85.33 and 86.22 seeds/ dry fruit under unsprayed conditions and with 88.73 and 87.17 seeds/ dry fruit in 2017 and 2018 seasons, respectively. No significant differences were found between the last one and golden coast in the first season under unsprayed conditions and balady green in both seasons under sprayed conditions. On the other hand, balady red recorded the lowest mean numbers of 52.83 and 56.72 seeds/ dry fruit under unsprayed conditions and 71.67 and 68.40 seeds/ dry fruit under sprayed conditions in the two seasons, respectively, followed insignificantly by golden coast in 2017 season. No significant differences were found between sprayed and unsprayed plots in case of white velvet and golden coast in both seasons, the two okra cultivars recorded 3.83% and 2.59%, respectively in 2017 season and 1.09% and 1.95%, respectively, in 2018 season (Table, 6). While, balady red and balady green affected significantly by pest infestation, the previous two cultivars recorded 26.28% and 28.15%, respectively, in 2017 season and 17.08% and 28.28%, respectively, in 2018 season.

No significant differences were found between the four tested cultivars under unsprayed and sprayed condition in the first season in case of seed yield per plant. While,

in the second season, golden coast recorded the highest seed yield per plant 27.97 and 27.65 g/ plant under sprayed and unsprayed conditions, respectively, followed insignificantly by balady green under sprayed. While the lowest seed yield per plant was recorded in balady red with 24.80 and 24.33 g/ plant under sprayed and unsprayed conditions, respectively, followed insignificantly by white velvet under both sprayed and unsprayed conditions, and by balady green under sprayed conditions. From t-test, it is evident that the differences between sprayed and unsprayed plots were insignificant for all cultivars for seed weight per plant in both seasons, except for balady green in 2018 season, which recorded 10.59% and 8.80% in the two seasons, respectively.

In both sprayed and unsprayed treatments, the highest seed yield per fed.was recorded in balady green with 573.90 and 573.78 kg/ fed. under sprayed and with 531.65 and 535.02 kg/ fed under unsprayed in 2017 and 2018 seasons, respectively, followed insignificantly by golden coast, except the first season of unsprayed. On the other hand, balady red recorded the lowest mean numbers of 538.19 and 538.27 kg/ fed. under sprayed and 501.67 and 511.07 kg/ fed. under unsprayed in 2017 and 2018 seasons, respectively, followed insignificantly by white velvet in both seasons. It is clear that white velvet recorded the lowest loss in seed yield (kg/fed.) with 5.54% and 4.62% in the two seasons, respectively, however, the highest one was recorded in Golden Coast with 7.3 to 8.47%.

Table (5): Seed yield and quality characteristics of four okra cultivars under sprayed and unsprayed conditions in Sohag Governorate during 2017 and 2018 seasons.

Plant characteristic	Treatment	Season	Okra cultivars				F. value	L.S.D.
			White Velvet	Balady Red	Golden Coast	Balady Green		
100 seeds weight	Unsprayed	2017	3.77	5.04	5.56	5.60	33.00*	0.51
		2018	3.68	5.05	5.48	5.59	584.27 *	0.13
	Sprayed	2017	4.49	6.00	5.82	6.43	28.71*	0.54
		2018	4.48	5.99	5.59	6.25	157.61 *	0.21
No. seeds per dry fruit	Unsprayed	2017	85.33	52.83	70.13	60.40	6.04*	19.75
		2018	86.22	56.72	70.27	60.87	322.5*	2.52
	Sprayed	2017	88.73	71.67	72.00	84.07	34.4*	5.09
		2018	87.17	68.40	71.67	84.87	116.79 *	3.00
Seed weight per plant (g)	Unsprayed	2017	24.27	22.31	25.79	24.28	1.83	N.S.
		2018	25.30	24.33	27.65	24.59	7.19*	1.95
	Sprayed	2017	26.13	26.03	27.72	27.15	0.72	N.S.
		2018	25.53	24.80	27.97	26.97	9.88*	1.57
Seed yield (kg/fed.)	Unsprayed	2017	511.29	501.67 c	522.66	531.65	6.62*	17.61
		2018	514.98	511.07 c	526.02	535.02	54.5*	5.09
	Sprayed	2017	541.28	538.19 b	571.05	573.90	61.72*	8.35
		2018	539.90	538.27 b	567.47	573.78	57.57*	8.39

(*): The F value is significant at P ≤ 0.05

Table (6): Reduction percentages on some seed yield components of four okra cultivars caused by certain piercing sucking pests in Sohag Governorate during 2017 and 2018 seasons.

Plant characters	Reduction%							
	2017 season				2018 season			
	White Velvet	Balady Red	Golden Coast	Balady Green	White Velvet	Balady Red	Golden Coast	Balady Green
100 seeds weight	15.90*	16.06*	4.41	12.90*	17.78*	15.65*	1.91	10.51*
No. seeds per dry fruit	3.83	26.28*	2.59	28.15*	1.09	17.08*	1.95	28.28*
Seed weight per plant (g)	7.14	14.30	6.96	10.59	0.91	1.88	1.13	8.80*
Seed yield (kg/fed.)	5.54*	6.79*	8.47*	7.36*	4.62*	5.05*	7.3*	6.76*

(*): The difference between sprayed and unsprayed is significant at P ≤ 0.05

These results are in the same line with Samaila and Oaya (2014) who reported that the dry fruit yields in the sprayed plants were significantly greater as compared to the unsprayed plants for both tall and short cultivars during the two seasons. Furthermore,Wagan *et al.* (2014) revealed that the huge application of pesticides did not improved yield of okra crop. Also, Poudel *et al.* (2018) studied the effect of different management practices on reduction in yield of five okra varieties. They stated that Julie variety can be the promising variety that showed the relatively lower yield reduction compared to other variety.

It was concluded that among the four tested cultivars none of them was found completely resistant against piercing sucking pests. Golden coast gave the highest yield fresh fruit per feddan followed by white velvet. Both cultivars received moderately levels of most tested pests. On the other hand, white velvet recorded the lowest reduction followed by golden coast. So, It can be suggest that the two previous cultivars could be successfully cultivated as a part of integrated pest management system. Furthermore, these results could be helpful for varieties screening programs.

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Microwave energy as an alternative control method for stored grain pests

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Abstract:

Chemicals are widely used to kill pests in stored grain even though these affect the environment and the consumers, which make it necessary to seek safer methods of pest management. As an alternative for traditional chemical methods, microwave radiations are used to kill insects and mites infest stored grains. Samples of wheat grain were infested with adult insects and larvae of *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) (Coleoptera:Curculionidae) and *Rhyzopertha dominica* (Fabricius) (Coleoptera : Bostrichidae). Crushed wheat was used with adult mites, *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae) , *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus echinopus* (Fumouze and Robin) (Acari: Acaridae). Wheat kernels were subjected to microwave treatment at three powers; low, medium-low at 6 exposure times and medium at 5 times. The mortality of pests increased with either power as well as exposure time or both. *R. dominica* and *R. echinopus* were more sensitive to microwave radiation where LT_{50} values were 3.07, 2.067, 1.17 and 1.96 minutes at low and medium-low powers, respectively. Complete reduction in F_1-progeny of all insects was obtained at medium power for 4 min. of exposure. *D. farinae* was the most tolerant mite to microwave radiation with LT_{50} values 2.57, 2.47 and 2.22 min. for the three powers, respectively. Biochemical analyses of wheat grains at the maximum time at which high mortality was obtained showed no detectable changes in the quality of protein, hardness, moisture and color. Germination of microwave treated wheat decreased, whereas exposure time, power or both increased. Managing stored grains requires the use of various techniques to ensure that the quality of the grain entering the storage facility does not deteriorate over time. Using microwaves opens up a new field to get rid of insects and mites in stored grains without any chemical pollution.

Introduction

Wheat is a major source of food for both humans and animals. It occupies a central position in the agriculture sector and the national economy. Pest infestation may start from the field and continues through storage until the grain is processed for

consumption. Stored grain pests cause high economic losses by feeding on stored grains and harm public health by contamination of food.

A loss due to insect infestations is a great problem as they feed, bore and ruin grains and speed up the process of decay, so they may account for 20-30% production loss and, in severe cases, they cause a total loss. Mites are important pests of stored grains, seeds, and other stored foods. Contamination with it causes both the qualitative and quantitative losses. The mites consumed up to 3% by weight of the grain. Mites feed on germ and destroy it completely but consume very little of the remaining material. The infested seeds look healthy but not capable of germination,which results in less plant population and the low yield (Al-Akhdar *et al.,* 2015; Islam *et al.,* 2004; Singh and Kaur, 2018 and Mahmood *et al.,* 2011).

Historically, the managementof stored-product pests has depended on the application of chemical pesticides, but increasing attentiveness of the risks these chemicals pose to environmental quality and human health has made it necessary to seek safer methods. The evolution of insecticide resistance has aggravated the problem and increased the need to develop pest management programs that are less chemical-dependent.

Hence, an alternative technique of killi ng insects in stored grain is searched. As anal ternative technique of killing pests in stored c ereals, particularly wheat, microwave disinfestations appear to have excellent poten tial. In the agriculture and food industry, the utilization of microwave energy and drying already has a position (Hamid *et al.,* 1968; Hamid and Boulanger, 1969; Kirkpatrick and Roberts, 1970; Nelson and Stetson, 1974; Watters, 1976; Tilton and Brower, 1987; Shayesteh and Barthakur, 1996 and Vadivambal *et al.,* 2007 and 2010).

The focus of recent research has been on

1.The effect of microwave use on the populat ion of six of the most abundant grain pests st ored, three insects; lesser grain borer *Rhyzopertha dominica* (Fabricius) (Coleoptera:Bostrichidae), wheat weevil *Sitophilus granarius* (L.) and rice weevil *Sitophilus oryzae* (L.) (Coleoptera:Curculionidae) and three mites:house dust mites *Dermatophagoides farinae* Hughes (Acari: Pyroglyphidae), mould mite *Tyrophagus putrescentiae* (Schrank) and *Rhizoglyphus echinopus* (Fumouze and Robin) (Acari: Acaridae), on wheat at various microwave power levels and exposure times.

2.The quality of the treated grains:

2.1. Sodium dodecyl sulphate (SDS) -Protein electrophoresis.

2.2. Physical properties (moisture, hardness and color).

2.3.Germination potential of microwave treated wheat.

Materials and methods

1. Microwaves:

The microwave frequency used in the study is a standard 2450 MHz. A microwave oven, EM-280 M, Electra, Japan, capacity 28 L and cavity dimensions 21.9 × 35 × 35 was used.The oven operated at three energy levels (low, medium-low and medium) with 17%, 44% and 66% of power output (output: 800W).

2. Insects:

Adults of S. *granarius*, *S. oryzae* and *R. dominica* reared on wheat seeds in glass jars (each of approximately 200 ml) and each jar was covered with muslin cloths and fixed with rubber bands. To have an initial population of *insect* adults homogenous in age, about 200 adults were introduced into jars containing seeds for egg-laying and then kept in an incubator at 28±2°c and 65±5 % R.H. After three days, all insects were removed from the media and the jars were kept again at controlled conditions. Adults of (1-2 weeks old) and larvae of (10-15 days old) were used for the experiment.Low and medium-low powers were tested after 1, 2, 4,

6, 8 and 10 mintsof exposureand 1, 2, 3, 4 and 5 mintsfor medium power, on adults and larvae of three insects. Mortality percentages were recorded after 24 hrs.of treatment for adults and were left till F_1-progeny appeared,while treated larvae stage left to estimate percentages of decrease.Reduction percentages in the progeny offspring were calculated by the following equation (El-Lakwah *et al.,* 1996).

$$\% \text{ Reduction} = \frac{\text{No. of progeny of control} - \text{No. of progeny of treat}}{\text{No. of progeny of control}} \times 100$$

3. Mites:

Strains of *T. putrescentiae*, *D. farinae* and *R. echinopus* were collected from infested grain samples. To obtain a pure culture, adults were placed in rearing plastic rings containing crushed wheat at 30±2°c and 70±5 % RH. Likewise, the three microwave powers were tested for 1,2,3,4 and 5 minutes, adult stages only were evaluated.The tested pests were identified and reared in the laboratories of Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt.

4. Quality of the treated grains:

4.1. Sodium dodecyl sulfate (SDS) -Protein electrophoresis:

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used by an analytical method in biochemistry to study banding patterns of treatments to separate protein mixtures by their molecular mass. Separation of charged molecules in an electric field under study were evaluated according to the method of Laemmli (1970) and Studier (1973).

4.2. Physical eroperties of treated wheat:

All test quality was carried out in the Agricultural Engineering Research Institute

4.2.1. Hardness: hardness testing was carried out by the Penetrometer system (Digital Force Gauge Model FGN-20G).Nidec-Shimpo Corporation, Japan.

4.2.2. Moisture: humidity of seed testing were carried out by grain moisture meter (Draminski electronics in agriculture SN: 24435).

4.2.3. Color: color testing was carried out by Color Analyzer (model RGB-1002). The resulted data were changed to images by website:https://htmlcolors.com/color-picker.

5. Determination of germination:

Germination of microwave-subjected wheat seeds was evaluated by placing 25 seeds on Whatman No. 3 filter paper in a 9-cm diameter Petri dish saturated with 5.5 mL of distilled water (Wallace and Sinha, 1962). To prevent filter paper from being desiccated, the plates were placed in a plastic bag and kept at 20°C for 7 days. The germinated seeds were counted on the seventh day and the percentage of germination was calculated.

6.Statistical analysis:

Statistical analysis of data was carried out according to Duncan's multiple range test (Duncan, 1955).Lethal effects of microwave energy were evaluated as percentages of cumulative mortality due to powers; corrected for mortality in the control variant according to Abbott's formula (Abbott, 1925). Virulence of the isolates was estimated by median lethal time (LT_{50}) which wes calculated by the probit analysis (Finney, 1971) for the variants treated with different microwave powers. Confidence intervals of varying LT_{50} values were calculated at p-level < 0.05.

Toxicity index (Ti) was calculated using Sun (1950) equation as follow:

$$Ti = \frac{LT_{50} \text{ of the most effective power}}{LT_{50} \text{ of less effective power}} X 100$$

Results and discussion:

1. Mortality of insects:

Mortality of *S.oryzae*, S. *granarius* and *R. dominica* adult stages at different temperatures and exposure times is shown in Table (1). For the three insects, the control mortality was zero. LT_{50} values at the low power were 3.07, 3.17 and 4.31 mints for *R. dominica*, *S. oryzae* and *S. granarius*, respectively. *R. dominica* was more sensitive to medium-low power with Lt_{90} 3.95 min.

followed by *S. oryzae*, while *S. granarius* was more tolerant to heat to 10.42 min. *S. granaries* appeared relatively heat tolerant compared with the other two species.

Table (1): Mortality of adult insects after exposure to microwave radiations in min.

Power	Insect	LT90	Slope	RR	Index	LT50	Lower limit	Upper limit
Low	*Rhyzopertha dominica*	8.49	2.9	1	100	3.07	2.66	3.5
	Sitophilus oryzae	11.97	2.22	1.03	96.99	3.17	1.53	5
	Sitophilus granarius	17.58	2.04	1.36	73.74	4.31	2.3	7.91
Medium-low	*Rhyzopertha dominica*	3.95	1.82	1	100	1.17	0.79	1.52
	Sitophilus oryzae	5.91	2.65	1.11	90.1	1.3	1.02	1.56
	Sitophilus granarius	10.42	1.52	1.28	78.22	1.5	0.42	2.06
Medium	*Sitophilus oryzae*	1.05	0.79	1	100	0.03	-	-
	Rhyzopertha dominica	2.41	1.78	15.69	6.37	0.41	0.07	0.72
	Sitophilus granarius	4.31	2.1	40.69	2.46	1.06	0.703	1.35

Complete reduction in F_1-progeny was found to *S. oryzae* after 8 min. of exposure to low power and to the three insects after 4 min. of medium power. On the other hand, there was100% reduction in F_1-progeny to larvae of *S. granarius* which was exposed to low and medium-low power after 4 min. (Table , 2).

Table (2): Reduction % in F_1-progony of infected wheat with adults and larvae of insects after treatment with microwave radiation.

F1-progeny	Time	Low			Medium-low			Time	Medium		
Adults		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***	***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***
	1	7.933	0	50	29.7	62.8	65.38	1	92.5	79.6	85.88
	2	21.66	16.66	51.27	67.82	81.3	78.19	2	96.66	98.33	96.15
	4	24.66	61.75	69.23	72.52	89.1	93.58	3	98.33	100	100
	6	41.15	75.94	78.19	87.98	93	100	4	100	100	100
	8	89.27	100	97.42	96.66	100	100	5	100	100	100
	10	100	100	100	100	100	100	-	-	-	-
Larvae		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***	***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***		***Rhyzopertha dominica***	***Sitophilus oryzae***	***Sitophilus granarius***
	1	0	31.1	93.81	73.33	78.2	89.44	1	98.3	65.5	95.24
	2	20	80.5	94.3	93.33	83.3	97.08	2	100	96.11	96
	4	40	84.4	100	95	85	100	3	100	98.33	97.7
	6	55	86.6	100	100	93.3	100	4	100	100	100
	8	75	91.6	100	100	100	100	5	100	100	100
	10	96.67	100	100	100	100	100	-	-	-	-

These results are in agreement with those obtained by Vadivambal *et al.* (2007) who reported that the mortality of *S. granarius* at 250, 300, 400 and 500W was 41%, 64%, 84%, and 100%, respectively, for 28 Sec. exposure time,while at an exposure time of 56 Sec., 100% mortality was obtained at 300W. as compared to 400W. for *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), respectively. Bedi and Singh (1992) also studied the effects of microwaves on three stored-grain insect species: larvae of *Corcyra cephalonica* (Stainton) (Lepidoptera Pyralidae), adults of *Callosobruchus chinenesis* (L.) (Coleoptera : Chrysomelidae) and *R. dominica*. The experiments were conducted at varying frequencies between 12 and 18 GHz and with exposure times of 2, 5, and 10 min. Their results suggested that mortality of insects increased significantly with an increase in both the frequency and the exposure times. At the same trend, Microwave radiation was used to control *Oryzaephilus surinamensis* (L.) (Coleoptera:

Silvanidae) adult beetles on dried stored figs. At 900 W. and 50 sec., complete mortality was accomplished (Reza Sadeghi *et al*., 2019).

2. Mortality of mites:

The mortality data for *T. putrescentiae, D. farinae* and *R. echinopus* at various thermal levels and exposure times are shown in Table (3). There were not any mortality observed in the controls. At a low power and exposure time of 2.75, 3.59 and 7.51 min. there was 50% mortality of *R. echinopus, T. putrescentiae* and *D. farinae*, respectively. As the temperature was increased to medium power, the mortality of *T. putrescentiae, R. echinopus* and *D. farinae* after a 1.44, 1.66 and 2.22-m exposure increased to 50%, respectively. Similar results for *D. pteronyssinu s*and *D. farinae* were reported by Ernieenor and Ho (2010) who found that the mortalities of adult mites exposed to 2,450 MHz microwave radiation produced by 3 ovens at various exposure times and power settings were 99.4, 84.1 and 44.8, respectively at 3 power settings. At high and medium powers, there was 100% mortality in both species when exposed for 300 seconds. The mean mortality rates at low power were 10.8 ± 0.7% for *D. pteronyssinus* and 9.7 ± 2.6% for *D. farinae*. Due to the heat produced by high-frequency oscillation of dielectric molecules, such as water and body fluid of mites, direct absorption of microwaves is extremely efficient in killing mites.

Table (3): Mortality of adult mites after exposure to microwave radiations in min.

Power	Mite	LT_{90}	Slope	RR	Index	LT_{50}	Lower limit	Upper limit
Low	***Rhizoglyphus echinopus***	9.39	1.99	1	100	2.067	2.75	1.52
	Tyrophagus putrescentiae	8.1	2.95	1.4	71.34	2.078	3.59	2.49
	Dermatophagoides farinae	16.32	2.38	2.22	44.86	2.57	7.51	3.74
Medium low	***Rhizoglyphus echinopus***	7.76	2.23	1	100	2.06	1.52	2.57
	Tyrophagus putrescentiae	7.73	2.24	1.01	99.47	2.07	1.8	2.34
	Dermatophagoides farinae	5.76	3.48	1.2	83.62	2.47	2.06	2.87
Medium	***Tyrophagus putrescentiae***	4.48	2.58	1	100	1.44	0.99	1.79
	Rhizoglyphus echinopus	3.6	3.79	1.15	86.55	1.66	1.35	1.95
	Dermatophagoides farinae	5.19	3.48	1.55	64.64	2.22	1.85	2.28

3. Quality of the treated grains:

To maintain the quality of the treated wheat that was exposed to microwave power, the lowest time that causes 100% mortality to one of the three insects at each power was tested for the following evaluations.

3.1. Protein electrophoresis:

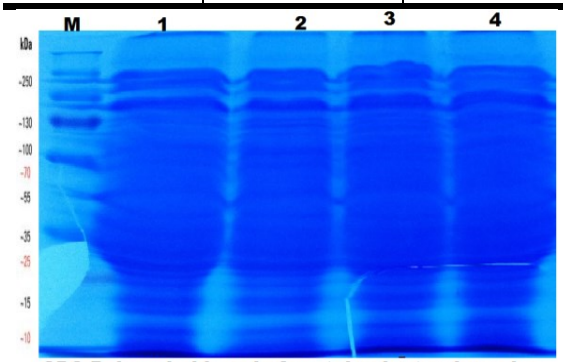
Data represented in Table (4) and illustrated in Figure (1) showed that due to the exposure of wheat grains to different powers of the microwave, some bands of electrophoresis of protein contents were generated and some disappeared. In low treatment, the band with mw 79 pb generated and the band with mw was 94 pb disappeared, but in low medium treatment two bands with mw 145 and 85 pbwere generated while the band with mw 35 disappeared. Also for medium treatment the band with mw 85 pbwas generated and the band with mw 35 pb disappeared compared tocontrol treatment. On the other hand, total the polymorphic bands for microwaves treatments were 2, 3, 2 with polymorphism ratios of 11.76 %, 17.65 % and 11.76 % for low, medium-low and medium power, respectively, as shown in Table (5). Kasarada *et al.* (1998) and Jaramillo *et al.* (1999) reported that SDS-PAGE was widely used to separate proteins related to genetic background and can be used to certify the genetic make-up of wild, cultivars, or newly derived cereal plants. Campana *et al*. (1993) cleared the physical and chemical properties of wheat dried with microwave energy and reported that the protein content was not affected but the functionality of gluten was altered gradually with increasing the time of exposure.

Table (4): Effect of microwaves on protein electrophoresis of wheat grains.

	Control	Low	Low medium	Medium
Total bands	17	17	18	17
Generated bands	-	1	2	1
Disappeared bands	-	1	1	1
Polymorphic bands	-	2	3	2
Polymorphisms %	-	11.76	17.65	11.76

Table (5): Effect of microwaves on generated bands and polymorphisms of wheat grains.

Bands No.	M.W.(k.da)	Control	Low	Low medium	Medium
1	145	-	-	+	-
2	133	+	+	+	+
3	121	+	+	+	+
4	105	+	+	+	+
5	94	+	-	+	+
6	88	+	+	+	+
7	85	-	-	+	+
8	79	-	+	-	-
9	71	+	+	+	+
10	50	+	+	+	+
11	41	+	+	+	+
12	35	+	+	-	-
13	30	+	+	+	+
14	26	+	+	+	+
15	25	+	+	+	+
16	23	+	+	+	+
17	21	+	+	+	+
18	19	+	+	+	+
19	16	+	+	+	+
20	14	+	+	+	+



SDS-Polycrylmide gel of protein electrophorasis of wheat treated with microwave

Figure (1): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS – PAGE) protein banding patterns of tested wheat seeds as affected by microwave treatment. Where: M = Standard marker 1= control 2= low 3= medium low 4=medium.

3.2. Physical properties of treated wheat:

3.2.1. Moisture loss:

The initial moisture content (IMC) of untreated wheat kernels was found to be 7.23%. Table (6) indicated that with an increase in power level, there was a non-significant decrease in moisture content from 7.12% to 7.06 %, and 6.93% at low, mid-low and medium, respectively.

3.2.2.Hardness:

The values of hardness attributes of wheat grains were tested after exposure to microwave radiation in periods that 100% mortality was achieved. However, hardness of wheat did not get affected by treatment (Table, 6).

Table (6): Effect of microwaves on hardness and moisture of wheat grains.

	Low	Mid-low	medium	control	LSD 0.05	F-value	*p*-value
Moisture	7.12[a]	7.06[a]	6.93[a]	7.23[a]	0.326	1.2015	.323ns
Hardness	48.64[a]	49.06[a]	54.95[a]	48.61[a]	9.98	0.792	.506ns

3.2.3.Color:

As demonstrated in Figure (2), the color of treatedgrainswith mid-low and medium powers showed more significant changes than control. For all colors, there were no changes between low power treated grains and control. Red, blue and green colorsshowed 237, 192 and 99 degrees in control and low power increase to 238, 196 and 1.6 with mid-low and 239, 199 and 114 with medium powers, respectively.

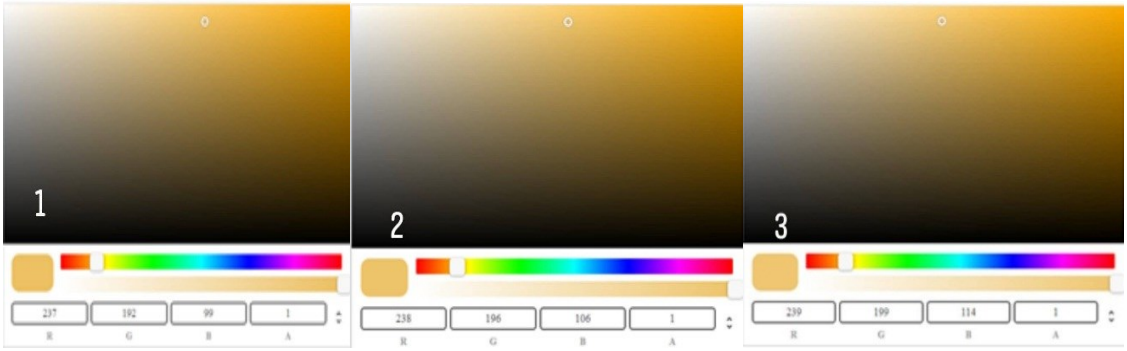


Figure (2): Effect of microwave on wheat colors. 1.Control and low power 2. Medium-low power 3-Medium power.

As for moisture content of wheat kernels that were subjected to microwave exposure, there were no significant differences between power levels, while it was significant in both time and periods and interaction between power and time levels (Abd El-Raheem and Saadiya , 2016). Manickavasagan *et al.* (2013) examined the date fruits which were not get affected by microwave treatment. But the gumminess of the dates treated at 300, 600 and 800 W. was significantly lower than the untreated and treated at 180 W. dates.

4. Determination of germination:

The control germination for wheat seeds was tested. Exposure times were 10, 6 and 4 for the three powers low, mid-low and medium, respectively. The germination decreased at high exposure time or power was due to the increase in temperature of the sample confirming that power ($F=37.65$; $df=12, 60$; $P<0.0001$) whereas moisture content had no significant effect ($F= 1.2$; $df= 36$; $P=.3231$ ns) on germination (Table, 7).

Table (7): Effect of microwave exposure on wheat germination.

	Low	Mid-low	Medium	control	LSD 0.05	F-value	*p*-value
Germination	14.75[b]	8.25[c]	3[d]	22.25[a]	4.178	37.65	.0000
Percentage	59%[b]	33%[c]	12%[d]	89%[a]	16.71	37.65	.000

Our results recommended that the microwave is a good application to get rid of insects and mites from stored grains and products that will be used in feeding. But it is not recommendeded to utilize with seeds because of microwave radiations and high temperature which were supposed to affect the germination capacity of the seeds. These results are at the same line with Vadivambal *et al.* (2007) who reported that germination of wheat kernels was lower after treatment with microwave energy of 500W. for an exposure time 56 sec. Campana *et al.* (1993) studied the chemical, physical and baking properties of wheat heated and dried with microwave energy. They founded that germination capacity was affected by exposure to microwave energy. The final temperature and the initial moisture content of the grains are responsible for the decrease in germination capacity. Bhaskara *et al.* (1998) studied the effect of microwave treatment on the quality of wheat seeds infected with *Fusarium graminearum* (Schwabe). Their results showed that seed viability and seedling vigor decreased accordingly. Similar results were obtained by Aladjadjiyan (2002) who studied the influence of microwave radiation on germination of ornamental perennial crops. Their results proved that at 850 W. the seed germination decreased.

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Impact of adding chitosan on bioefficacy of insecticides against cotton spiny bollworm *Earias insulana* (Lepidoptera: Noctuidae) infesting cotton bolls and yield measurable under field conditions

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Abstract:

Cotton spiny bollworm (SBW) *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) is important pests of cotton. Its larvae bore into the growing shoots, flower buds, flowers and bolls of cotton resulting in considerable losses in quality and quantity. The aim of this research work is to study, adding impact of chitosan as newcomer material on bioefficacy of some insecticides against *E. insulana* infesting cotton bolls and on cotton yield measurable. The results of statistical analysis showed there are highly significant differences in case 1st and 2nd spray with initial and residual effect. In case initial effect , Tracer + chitosan come in the first group recorded 75.33 reduction % followed by indoxacarb (52.00), chitosan (47.09), runner (34.67), indoxacarb + chitosan (43.48), runner + chitosan (24.33) and tracer alone (16.29) reduction % , respectively. On the other hand , adding chitosan to indoxacarb increase residual effect to indoxacarb recorded 74.53 reduction %, compared with other treatments , while chitosan alone recorded less reduction % 37.36 against larvae of *E. insulana* . The 2nd spray, chitosan spray on cotton bolls recorded highest reduction % 75.51 % in case initial effect. But , indoxacarb + chitosan come in the first category 81.13 % reduction in residual effect. In addition to , runner + chitosan and tracer recorded the highly number of healthy bolls 8.8 and 8.2 bolls / plant and in case of boll weight, indoxacarb + chitosan record 3.40 gm / bool followed by indoxacarb 3.18, runner 2.26, control 2.89 , runner + chitosan 1.97, tracer 1.96, chitosan 1.93 and tracr + chitosan 1.44 gm / boll .

Introduction

Cotton spiny bollworm is the larva of *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae). It is one of the most important pests infesting cotton allover the world (Mirmoayedi, 2009). There are varying amount of insecticides which are using regularly for controlling cotton spiny bollworm (SBW) (Gupta *et al.*, 2005). Pesticides are comparatively better option to avoid economic damage to this high value crop (Shanower *et al.*, 1994). However, chemicals pesticides cause healthy hazards,

environmental pollution, resistance development in insects, resurgence of new insect pests and toxicity to natural biological agents (Croft, 1990; Bhati *et al*., 1993; Horowitz *et al.*, 1993 and Gill and Garg, 2014). In China, when spinosad was used for control of pests in vegetables such as eggplant, Chinese cabbages and cotton the quantities found in sprayed plants didn't surpassed, the norm of that country (Gao *et al*., 2007). Methoxyfenozide is new chemistry insecticide, the latest and most persuasive member of the moult-accelerating compounds (MACs) against Lepidoptera (Smagghe *et al.*,2003). MACs directly binding to the same natural hormone receptors stimulates, the molting hormone receptor and cause an anticipated lethal moult (Dhadialla and Carlson, 1998). Several new chemistries with unique modes of action spinosad and methoxyfenozide are useful for pests control and can use as an important pest control option for integrated pest management (IPM) because of their low eco-toxicological effects and short time persistence in the environment (Osorio *et al*., 2008 and Arif *et al*., 2009). The objective of this stidy is adding impact of chitosan as newcomer material on bioefficacy of some insecticides against *E. insulana* infesting cotton bolls and on cotton yield measurable.

Materials and Methods

1. Experimental design and insecticides :

A field trial was conducted at Plant Protection Research Station at Qaha, Qalubiya Governorate , on cotton seedling, cultivated on 15th March, 2018. Cotton plants was left for bolls production beginning from June 2018 till cutting at 23rd September, 2018. Used 7th treatments (Table, 1) was distributed in a randomized complete block design (RCBD) in field trial during 2018 , number of treatments were eight each treatment include three replicates and a plot area was 1/200 of faddan (6x7 m^2), experimental area was 1008 m^2. Cotton plants were examined after bolls production about 20th day from constitute. When observed attack bolls with young larvae of *E. insulana* in the field, the cotton plants was subjected to insecticide spray. For determination of quantity of water, calibration was done by spraying water in the nontreated plots.

Table (1): Information on used insecticides, trade name , common name , formulation and rate of application .

Compounds		Formulation types	Rate of application/ faddan
Trade Name	**Common Name**		
Runner	Methoxyfenozide	28%SC	105 ml
Tracer	Spinosad	48%SC	42 ml
Avento	Indoxacarb	15%Sc	42 ml
Chitosan	Chitosan	81.2WP	200 gm

Spraying was done using a knapsack sprayer. Before the application of each insecticide, the knapsack spray was cleaned before and after used with clean water to avoid pollution . Insecticides were sprayed alone and in binary mixtures with chitosan twice , 10th days between twice sprays. The bolls number were 10th bolls / plot (medium size) were collected randomly before directly application and after application 1,3,5,7 and 10 days, the samples were put in cloth bags, then transporting to the laboratory in Plant Protection Research Institute , Sharkia branch , to examine and data record. Reduction percentage was calculated by using **Henderson and Tilton formula (1955).**

2. Yield measurable:

Mean number of healthy bolls, 1/3 bolls, 2/3 infestation with larvae , bolls branch number and length of plant was recorded from each treatment .

3. Data Analysis :

Data related to the impact of different insecticides tested on reduction

percentages of infestation bools with larvae in cotton plants were evaluated by analysis of variance using one-way ANOVA. Means of treatments were separated using Duncan's multiple range test at P = 0.05 .

Results and discussion

1. Adding impact of chitosan as newcomer material on bioefficacy of some insecticides against *Earias insulana* infesting cotton bolls after 1st spray:

The data in Tables (2 and 3) showed impact of adding chitosan on efficacy of various insecticides for the control of *E.insulana* in experimental cotton field during 2018 summer season, after twice spray. Statistical analysis illustrated that there are highly significant differences (***) and LSD_{05} values , recorded 3.914 and 2.98) in case 1st spray with initial and residual effect (Table, 2). In the same table, the treatments divided seven groups according to statistical analysis, tracer + chitosan come in the first group recorded 75.33 reduction % followed by indoxacarb (52.00), chitosan (47.09), runner (34.67), indoxacarb + chitosan (43.48), runner + chitosan (24.33) and tracer alone (16.29) reduction % , respectively. On the other hand , adding chitosan to indoxacarb increase residual effect to indoxacarb recorded 74.53 reduction %, compared with other treatments , while chitosan alone recorded less reduction % 37.36 in larvae of *E. insulana* after 1st spray.

2. Adding impact of chitosan as newcomer material on bioefficacy of some insecticides against *Earias insulana* infesting cotton bolls after 2nd spray :

Data tabulated in Table (3) , indicated that , adding effect chitosan on bioefficacy to insecticides against larvae of *E. insulana* infesting cotton bolls after 2nd spray . Chitosan spray on cotton bolls recorded highest reduction % 75.51 % , due to may be repellant effect or making cotton bolls are unpalatable , while the rest treatments are ordered a descending as follow , indoxacarb, runner, tracer + chitosan, indoxacarb + chitosan and tracer, respectively, while runner mixing with chitosan recorded less reduction %.

Table (2) : Impact of adding chitosan on bioefficacy to some insecticides after 1st spray during 2018 summer season.

Treatments	NO. of larvae before spray	No. of larvae and reduction % after 1st spray .						Residual effect
		Initial effect		3 Days	5 Days	7 Days	10 Days	
Indoxacarb	15	**NO**	**10**	**6**	**5**	**3**	**2**	**4**
		Red%	52.00b	69.34b	55.21b	69.00 b	71.99b	66.39 b
Runner	9	NO	8	6	4	1	3	2.25
		Red%	34.67d	50.66c	40.07d	81.99a	33.33e	51.51 d
Tracer	8	NO	9	1	2	3	3	2.5
		Red%	16.29f	89.62a	65.99a	42.41d	24.00 f	55.05 c
Chitosan	7	NO	5	5	4	3	2	3.25
		Red%	47.09c	45.75d	23.46e	36.37e	43.85d	37.36 e
Indoxacarb + chitosan	11	NO	10	5	3	1	1	4
		Red%	34.48d	66.56b	64.63a	85.69a	81.14a	74.53 a
Runner + chitosan	5	NO	5	1	2	3	3	2.25
		Red%	24.33e	86.00 a	47.67c	10.00 f	19.33g	40.75 e
Tracer+ chitosan	9	NO	3	4	4	3	2	2.5
		Red%	75.33a	67.45b	41.08 d	50.00c	56.32c	53.71 d
Control	12	NO	16	16	9	8	6	3.25
F-test		***		***	***	***	***	***
$LSD_{0.05}$		3.914		4.562	2.908	4.357	3.195	2.98

***Means of reduction % followed by similar letters and in the same column are not significantly different by LSD at P < 0.05 LSD = Least Significant Difference.**

In the same Table (3), data showed effect of mixing chitosan on residual effect to insecticides , noticed the same trend in Table (2) , Wherever, indoxacarb + chitosan come in the first category 81.13 % reduction while runner + chitosan recorded lowest reduction % (52.66) .

Table (3) : Impact of adding chitosan on bioefficacy to some insecticides after 2nd spray during 2018 summer season.

Treatments	NO. of larvae Before spray	No. of larvae and reduction % after 2nd spray .						Residual effect
		Initial effect		3 Days	5 Days	7 Days	10 Days	
Indoxacarb	**15**	**NO**	**3**	**1**	**2**	**2**	**2**	**1.75**
		Red%	66.04b	53e	73.33b	68cd	79.33d	**68.41c**
Runner	**9**	NO	2	2	2	1	1	1.5
		Red%	65.23 b	46.99f	55.89c	74.33b	83.34c	65.46d
Tracer	**8**	NO	3	1	1	1	1	1
		Red%	36.04d	70c	75.66b	69.33c	81.58c d	73.8 b
Chitosan	**7**	NO	1	1	2	1	2	1.5
		Red%	75.51a	66.37d	42.85d	66.04d	57.14e	58.43 e
Indoxacarb+Chitosan	**11**	NO	3	1	1	1	1	1
		Red%	53.24c	77.51a	82.81a	77.84a	86.36b	81.13 a
Runner + Chitosan	**5**	NO	2	2	2	1	-	1.25
		Red%	32.42e	40g	19e	52.66e	99a	52.66 f
Tracer+ Chitosan	**9**	NO	2	1	2	1	-	1
		Red%	63.9b	73.33b	55.56c	73.33b	97.66a	74.63 b
Control	**12**	NO	7	5	6	5	8	6
F-test		***		***	***	***	***	***
$LSD_{0.05}$		2.396		2.767	2.997	2.796	2.679	2.32

3.Adding impact of chitosan as a newcomer material on cotton yield measurable .

From data in Table (4), illustrateed adding impact of chitosan as a newcomer material on cotton yield measurableie (Mean Number of fruit branches / plant, total bolls / plant , healthy bolls / plant, infested bolls / plants and length of plants weight of plants . The results showed not significant differences between treatments in case of mean no. fruit branches / plant, no. total bolls / plant and no. infested bolls / plant, but there are significant differences between in case healthy bolls / plant, length of plant and weight of bolls /plant , wherever LSD values were 2.49, 3.96 and 1.07 , respectively. In the same table noticed that runner + chitosan and tracer recorded the highly healthy bolls 8.8 and 8.2 / plant, followed by other treatments, while control recorded 3.2 healthy bolls/ plant. Especially length of plant , control length of plant was 100.2 cm but tracer + chitosan recorded 79.8 cm , other treatments lie between it. On the other hand, indoxacarb + chitosan record 3.40 gm / bool followed by indoxacarb 3.18, runner 2.26, control 2.89 , runner + chitosan 1.97, tracer 1.96, chitosan 1.93 and tracer + chitosan 1.44 gm / boll .

Table (4): Impact of adding chitosan to insecticides on cotton yield .

Treatments	Mean No. fruit branches / plant	No. bolls of plant	Healthy bolls of plant	No. infested bolls of plant	Length of plant cm	Weight of bolls /plant gm	Mean weight / bool gm
Indoxacarb	6.00 a	6.80 a	4.60 bc	2.20 b	94.80 b	21.65 a	3.18
Runner	7.40 a	8.20 a	4.40 bc	3.80 ab	94.80 b	18.60 cd	2.26
Tracer	7.40 a	10.20 a	8.20 a	2.00 b	87.00 cd	20.05 b	1.96
Chitosan	7.00 a	9.80 a	4.80 bc	5.00 a	94.80 b	18.90 c	1.93
Indoxacarb +chitosan	6.20 a	9.60 a	6.20 ab	3.40 ab	89.80 c	21.51 a	3.40
Runner + chitosan	6.60 a	10.20 a	8.80 a	1.40 b	85.20 d	20.11 b	1.97
Tracer+ chitosan	6.00 a	10.80 a	6.40 ab	2.40 b	79.80 e	15.54 d	1.44
Control	5.20 a	6.60 a	3.20 c	3.40 ab	100.20 a	19.06 bc	2.89
F-test	NS	NS	***	NS	***	***	
$LSD_{0.05}$	2.0773	4.4230	2.4905	2.3269	3.9681	1.0778	

***Means followed by similar letters and in the same column are not significantly different by LSD at P < 0.05**
LSD = Least Significant Difference

Cultural, biological and chemical are being implemented globally for the management of lepidoptera on various crops. But the success of any control measure is judged by the outcome and the most acceptable control strategy is the one that gives appropriate control against the target organism, and saves the crop from economically important injury. Among various approaches of control, chemicals are considered as fast acting control measures. To overcome the lepidoptera insecticides are considered the only source of quick control measures that save the crop and prevent yield losses and is an important practice of IPM (Gogi *et al*., 2013). Similar results have been achieved by Stanley *et al.* (2009) who reported that the *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae are highly susceptible to spinosad insecticides. Methoxyfenozide (ecdyson receptor agonist) significantly reduced the SBW population and bolls infestation. The possible reason might be due to their effect on insect blood cells like other reported insecticides to affect the blood cells in different insects (Iqbal *et al*., 2002).

The highest cotton yield was recorded in tracer and methoxyfenozide treated plots followed by chitosan (Iqbal *et al.*, 2014) who studied that methoxyfenozide gave good yield.These insecticides not only gave best control of this notorious pest but also increase in seed yield of berseem was recorded. Similar results were found by Meena *et al.* (2013). It is concluded that the results here provided information for making better management decisions and improving cotton production.

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Survey and faunistic studies on moths and butterflies in New Valley Governorate, Egypt. 1. Butterflies

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Abstract:

A survey of moths and butterflies together with faunistic studies were carried out in Dakhla, Kharga and Baris Oases in New Valley Governorate using light traps and sweeping nets during a period covered two years, 2017 - 2018. As for the butterflies, the survey revealed the presence of 24 species in 20 genera belonging to 5 families (Danaidae, Hesperiidae, Lycaenidae, Nymphalidae and Pieridae).The largest number of species was belonged to family Lycaenidae (eleven species), followed by family Pieridae (six species). Kharga Oasis was found to be the richest in species (21 species). Three species were found most common in the area of study, these are: *Danaus chrysippus* (L.) (Lepidoptera:Nymphalidae), *Colotis chrysonome* (Klug) and *Pieris rapae* (L.) (Lepidoptera: Pieridae), eight species were found in fair numbers and thirteen species are to be rare. Taxonomic notes with common names, synonyms and notes on the distribution, habitats and host plants for each species are given. Occurrence and distribution in the zoogeographical zones of Egypt are also pointed.

Introduction

Butterflies are popular everywhere and there are over 19000 butterfly species are known in the world. The name "butterfly"is believed to have originated from a member of the family Pieridae, the brimstone, *Gonepteryx rhamni* (L.) (Lepidoptera: Pieridae), which was called the "butter-coloured fly" by early British naturalists. Butterflies are often polymorphic and many species make use of camouflage, mimicry and aposematism to evade their predators. Some migrate over long distances. Some species are pests, others are pollinating agents of some plants, while others live as mutualists in association with ants (Larsen, 1994).

Knowledge about Egypt's butterflies has progressed slowly and there have been only three major reviews (Andres and Seitz, 1923; Wiltshire, 1948 and Larsen, 1990) to- date and little else has been published over a half century ago. According to Gilbert and Zalat (2007), there are 63 species of butterflies

recorded from Egypt, two of them are endemic to Egypt. Benyamini (1984) surveyed the butterflies of Sinai Peninsula. El Moursy (1996) in the work "Biological diversity of Egypt" listed the butterflies of Egypt together with their distribution. Mabrouk (2003) presented a comprehensive list of Egyptian Lepidoptera including the butterflies. Mahbob and Mahmoud (2013) listed the insect fauna of Kharga Oasis, New Valley, Egypt. Salem (2017) presented a comprehensive checklist of insects recorded in Egypt including the butterflies.

As for the New Valley, most of the faunistic work on butterflies was no more than mere, fragment and scattered work such as Ibrahim, 1934; Wiltshire, 1948 and Al-Gamal *et al*., 2001. On account of the scarcity of knowledge and to overcome the lack of information regarding the fauna of the butterflies in the New Valley, the present work is to be presented. It is hopped to be of assistance for more detailed and prospective studies.

Materials and methods

The present work was carried out at the New Valley Governorate using light traps and sweeping net during two years (2017-2018) and covered the following areas in the New Valley Governorate: Dakhla, Kharga and Baris Oases. The surveyed areas were cultivated with variable field crops, vegetables and fruit trees. Captured insects were sorted out into species, identified and recorded then listed in alphabetical order according to families, genera and species. Data are presented here showing the recent scientific names and position of the species together with their collecting area, state of abundancy (Common, fair and rare) and their distribution in the geographical regions in Egypt.

Common names, synonyms, taxonomic notes and notes on the distribution, habitats and host plants for each species are given. Occurrence and distribution in the zoogeographical zones of Egypt are also pointed. Identification of species with updates of nomenclature and species status were carried out in the Insect Identification and Classification Department (IICD) in the Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Egypt.

Results and discussion

The present survey resulting 24 species belonging to 20 genera of five families of butterflies (Danaidae, Hesperidae, Lycaenidae, Nymphalidae and Pieridae). Table (1) below indicate that, family Lycaenidae is represented by eleven species, Pieridae six species, Hesperidae and Nymphalidae each is represented by three species and Danaidae by only one species. The largest number of species was collected from Kharga Oasis (21 species), followed by Dakhla Oasis (16 species) and then Baris Oasis (13 species). It was also found that, six species were collected from all the surveyed localities, these are: *Tarucus balkanicus*, *T. rosaceus* (Lycaenidae); *Cynthia cardui*, *Vanessa atalanta* (Nymphalidae), *Colotis chrysonome* and *Pontia glauconome* (Pieridae). Four species, each was collected from three localities, seven species, each was collected from two localities and five species, each was collected from only one locality.

Table (1): List of butterflies in the New Valley Oases, together with their abundancy and distribution in Egypt.

Taxa	Surveyed zones in the New Valley Oasis			Abundancy	Distribution in ecological zones of Egypt						
	Dakhla	Kharga	Baris		N. coast	W. desert	Lower Egypt	Upper Egypt	E. desert	Gebel Elba	Sinai
Fam. Danaidae											
Danaus chrysippus (Linn.)	*	*		+++	*	*	*	*	*	*	*
Fam. Hesperiidae											
Borbo barbonica Lederer		*		+		x	*	*	*		*
Gegenes nostrodomus Fab.	*	*		+		x	*	*			*
Gomalia elma(Trimen)		*		+	*	*	*	*	*	*	*
Fam. Lycaenidae											
Deudorix livia (Klug)		*		++		x	*	*		*	
Freyeria trochylus Freyer	*	*		+	*	x	*	*	*		*
Iolana alfierii Wiltshier	*			+		x	*		*	*	*
Lampides boeticus (Linn.)	*	*	*	++	*	*	*	*			*
Leptotes pirithous (Linnaeus)	*	*		+	*	x	*	*			*
Lycaena phlaeas Linnaeus		*	*	+		x	*	*			*
Lycaena thersamon omphali Klug	*	*	*	++	*	x	*	*		*	*
Polyommatus Icarus zelleri Verity	*			+		x	*	*			*
Tarucus balkanicus Freyer	*	*	*	++	*	*	*	*			*
Tarucus rosaceus Austant	*	*	*	+	*	x	*	*			*
Zizeeria karsandra (Moore)		*	*	++		*	*	*			*
Fam. Nymphalidae											
Cynthia cardui(Linnaeus)	*	*	*	++	*	*	*	*	*	*	*
Melitaea deserticola Oberthür		*		+		*					
Vanessa atalanta (Linnaeus)	*	*	*	+	*	*	*	*			*
Fam. Pieridae											
Colias croceus (Geoffroy)	*	*		++	*	*	*	*	*	*	*
Colotis chrysonome Klug	*	*	*	+++		x	*				
Colotis protomedia Klug			*	+		x	*				*
Pieris rapae (Linnaeus)	*	*	*	+++	*	*	*	*	*		*
Pontia daplidice Linnaeus		*	*	+	*	x	*	*			*
Pontia glauconome Klug	*	*	*	++	*	x	*	*	*		*
Total 5 fam., 24 sp., 20 gen.	16	21	13		14	24	23	20	9	7	21

+++ Common ++ Fair + Rare x Present record E. Eastern N. North W. Western

The table also indicated that, three species were found to be most common in the area of study, these are: *Danaus chrysippus* (Nymphalidae),*Colotis chrysonome* and *Pieris rapae* (Pieridae). Eight species were found in considerable numbers (more than 3 individuals) and thirteen species are to be rare (less than 3 individuals). On the other hand and according to the records of distribution for the mentioned species in the geographical regions in Egypt, it is noticed that, fourteen species were recorded from the coastal region, ten species previously recorded, in addition to fourteen species recorded in the present work from the Western Desert, twenty three species from lower Egypt, twenty species from Upper Egypt, nine species from Eastern Desert, seven species from Gebel Elba and twenty

one species from Sinai peninsula. It is found also that, four species are found inhabiting all the geographical regions in Egypt, these are: *Danaus chrysippus* (Danaidae), *Gomalia elma* (Hesperiidae), *Cynthia cardui* (Nymphalidae) and *Colias croceus* (Pieridae). Four species are recorded from six regions, eight species from five regions, five species from four regions, only one species from three regions and other one species from two regions and only one species, *Melitaea deserticola* (Nymphalidae) from only one region that is previously recorded from the Western Desert.

Families, genera and species of butterflies in New Valley Governorate , Egypt

1.Family: Danaidae (Milkweed butterflies):

A small family of large tropical butterflies, some of 300 species of Danaidae exist worldwide. Most of them are found in tropical Asia and Africa and are diverse in the Neotropics. Some are restricted to Australia and the Oriental region. The monarch butterfly is by far the most famous, being one of the most recognizable butterflies. Milkweed butterflies are now classified as the subfamily Danainae within the family Nymphalidae; however, the previous family name Danaidae is still used. They lay their eggs on various milkweeds on which their larvae (caterpillars) feed. Danaidae is represented in Egypt by two species within one genus. It is recorded here by one species.

1.1. *Danaus chrysippus* (Linnaeus)

Common name: Tiger milkweed butterfy, plain tiger and African queen

Local distribution: Fayoum, Al Arish (North Sinai), Kharga Oasis, Dakhla Oasis, Baris Oasis, El-Baharia Oasis (W. Desert), Alexandria, Nubaryia (Behaira), Fayed (Ismailia), Belbis, Al Kanater (Qalubiya), Giza, Seds (Kafr El-Skeikh) and Matrouh.

Geographic distribution: Wide spread in Asia, Australia and Africa

Recorded hosts: Host plants are from several families, most importantly Asclepiadoideae (Apocynaceae). Adults obtain nectar from various flowering plants.

Habitats: It prefers arid, open areas, and is most often found in a variety of habitats including deserts, mountains, deciduous forests, and human-tended gardens in cities and parks. It is comfortable at altitudes ranging from sea level to around 1,500 m (5,000 ft.)

Remarks: A 3500-year-old Egyptian fresco in Luxor features the oldest known illustration of this species. Strong fliers from dawn to dusk. Flight period from June to September.

2. Family: Hesperiidae (Skippers):

Butterflies of family Hesperiidae, were previously placed in a separate superfamily, Hesperioidea; however, the most recent taxonomy places the family in the superfamily Papilionoidea. They are named for their quick, darting flight habits. More than 3500 species of skippers are recognized and they occur worldwide, but with the greatest diversity in the Neotropical regions of Central and South America. They are now classified in the following subfamilies: Coeliadinae, Euschemoninae, Eudaminae, Pyrginae, Heteropterinae, Hesperiinae, Megathyminae and Trapezitinae.

The family is represented in Egypt by 13 species within 7 genera. It is recorded here by three species.

2.1. *Borbo borbonica* (Boisduval)

Common name: The borbo skipper and Zeller's skipper or olive haired swift.

Local distribution: Fayoum, Al Arish, Rafah (N. Sinai), Saint Kathrine (South Sinai), Nobaryia (Behaira), Fayed (Ismailia) and El-Baharia Oasis (Western Desert).

Geographic distribution: It is found along the southern coasts of the Mediterranean Sea, but mainly in Syria, Arabia, North Africa, Middle East, Sub-Saharan Africa, including Mauritania, Senegal, Gambia, Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Northern Nigeria, Zambia, Mozambique, Zimbabwe, Northern Botswana, Northern Namibia, South Africa, Swaziland, Madagascar, Reunion, Rodrigues and Mauritius.

Recorded hosts:*Leersia oryzoides*, *Sorghum halepense*, *Panicum*, *Ehrharta erecta*, *Oryza,Pennisetum* and *Zea mays*.

Habitats: Associated with the banks of slow moving rivers and damp areas in lowlands.

Remarks: Adults are on wing from September to October.

2.2. *Gegenes nostrodomus* Fabricius

Common name: The dingy swift, light pygmy skipper and Mediterranean skipper

Local distribution: Al Kanater, Belbais (Qalubiya), Fayoum and El tur (S. Sinai), Kharga Oasis, Dakhla Oasis, Bahariya Oasis (W. Desert), Giza and Aswan.

Geographic distribution: It is found from the Mediterranean Sea (South Europe, North Africa) through Anatolia to Turkestan (Western Asia**)** and India.

Recorded hosts: The larvae feed on various grasses, including Gramineae, *Aeluropus* (in the Sinai Desert) and *Aerulopus* and *Panicum* species.It is pest on Maize.

Habitats: It is found in dry places.

Remarks: Adults are on wing from May to October in multiple generations.

2.3.*Gomalia elma* (Trimen)

Common name: The marbled skipper, African marbled skipper, the African Mallow skipper and Green-marbled skipper or sandman.

Local distribution: Allover Egypt's geographical regions

Geographic distribution: It is found in Africa in the Cape region, Orange Free State, Botswana, Zimbabwe, Mozambique, Benin, Burkina Faso, Gambia, Ghana, Guinea, Ivory Coast, Kenya, Namibia, Nigeria, Senegal, Sierra Leone, South Africa, Togo, Zambia, and parts of Asiaranges from Saudi Arabia, Oman; Yemen; Sri Lanka and India.

Recorded hosts:*Malvaceae*: *Abutilon indicum*, *Abutilon mauritianum,Abutilon intermedium, Abutilon sonneratianum*, *Croton gratissimus, Sida cordifolia* and *Wissadula rostrata*

Habitats: Savannah

Remarks: Flight period: All year in warmer areas and from August to April in colder parts. Males establish territories in clearings and along paths in the bush. They often use several perching spots within the territory that they are defending.

3.Family: Lycaenidae (gossamer-winged butterflies):

This family is known as blues, coppers and hairstreaks. Lycaenidae is the second-largest family of butterflies (behind Nymphalidae, brush-footed butterflies), with over 6,000 species worldwide, whose members are also called gossamer-winged butterflies. They constitute about 30% of the known butterfly species.

The family is traditionally divided into the subfamilies of the blues (Polyommatinae), the coppers (Lycaeninae), the hairstreaks (Theclinae) and the harvesters (Miletinae).

Lycaenids are diverse in their food habits and apart from phytophagy, some of them are entomophagous feeding on aphids, scale insects, and ant larvae. Some lycaenids even exploit their association with ants by inducing ants to feed them by regurgitation, a process called trophallaxis. Not all lycaenid butterflies need ants, but about 75% of

species associate with ants, a relationship called myrmecophily. These associations can be mutualistic, parasitic or predatory depending on the species.

The family is represented in Egypt by 36 species within 20 genera. It is recorded here by eleven species.

3.1. *Deudorix livia* (Klug)

Common name: The pomegranate butterfly or the pomegranate playboy

Local distribution: Al Kanater (Qalubiya), Fayoum, Al Arish (N. Sinai), St. Kathrine (S. Sinai), Kharga Oasis, Dakhla Oasis, Baharyia Oasis (W. Desert), Burg Al Arab (Alexandria), W. El Natroun (Behaira), Fayed (Ismailia) and Aswan.

Geographic distribution: It is found in Senegal, Gambia, Burkina Faso, Cameroon, Chad, Sudan, Uganda, Kenya, Tanzania, Somalia, Djibouti, Yemen, Saudi Arabia, United Arab Emirates, Oman, Algeria, Egypt and the eastern Mediterranean, including Greece.

Recorded hosts: The larvae feed on *Punica granatum, Eriobotrya japonica, Acacia, Phoenix, Allium, Psidium, Gardenia* and *Lycopersicum* species.

Habitats: The habitat consists of savanna, including arid savanna.

Remarks: It is a somewhat migratory species. Dangerous pest.

3.2. *Freyeria trochylus* (Freyer)

Common name: The grass jewel.

Local distribution: Fayoum, Kharga Oasis, Dakhla Oasis (W. Desert), Rafah, Zaranik (N. Sinai), Tur Sinai (S. Sinai) and Fayed (Ismailia).

Geographic distribution: Found in Africa, Arabia (United Arab Emirates, Oman, Saudi Arabia), southern Europe, India and southern Asia. In Europe, only from a few areas of Greece and several of the Greek islands.

Recorded hosts:*Heliotropium strigosum* and *Goniogyna hirta.*

Habitats: Hot dry rocky grassy ground with sparse grasses. Disturbed ground and edges of cultivation. Attracted to flowers which in summer at least are commoner nearer water sources, ditches or roadside drains.

Remarks: From March to October in a number of broods.

3.3. *Iolana alfierii* Wiltshire

Common name: The burning bush blue

Local distribution: Al Arish (N. Sinai),Wadi-el-Rabaa (S. Sinai), Al Kanater, Belbais (Qalubiya), Nobariya, W. El Natroun (Behaira), Seds (Kafr El Skeikh), Gabal Elba (Red Sea) and Bahariya Oasis (W. Desert).

Geographic distribution: Egypt and Israel.

Recorded hosts: *Colutea istria* (Leguminosae).

Habitats: Ravines, bounded by relatively steep banks and high steeps.

Remarks: Resident in Egypt. Powerful fast flying along cliff faces and places near the host plants, flying during February-April, and September.

3.4.*Lampides boeticus* (Linnaeus)

Common name: The legumes butterfly and the pea blue or long-tailed blue**.**

Local distribution: Al Kanater (Qalubiya), Fayoum, Al Arish, Rafah (N. Sinai), Kharga Oasis, Dakhla Oasis (W. Desert), Burg Al Arab (Alexandria) and Helwan (Cairo).

Geographic distribution: Cosmopolitan. This species can be found in Europe, Africa, South and Southeast Asia and Australia.

Recorded hosts: Clover, cow pea, peas, cassia and sesban (*Medicago*, *Crotalaria*, *Polygala*, *Sutherlandia*, *Dolichos*, *Cytisus*, *Spartium* and *Lathyrus* species. It has also been recorded on *Crotolaria pallida*.

Habitats: This species inhabits the edge of forests, mountain meadows and hot flowery places at an elevation up to 2,700 metres above sea level.

Remarks: Strong migrants. The larvae feed on flowers, seeds and pods of many Fabaceae species. It is minor pest.

3.5. *Leptotes pirithous* (Linnaeus)

Common name: The Lang's short-tailed blue or common zebra blue

Local distribution: Al Kanater (Qalubiya), St. Kathrine (S. Sinai), Rafah (N. Sinai), Alexandria, W. El Natroun (Behaira), Seds (Kafr El Skeikh), Aswan, Baris Oasis and Dakhla Oasis (W. Desert).

Geographic distribution: Southern Europe (Spain, France and Italy), along the Mediterranean coast, in Asia Minor up to the Himalayas, and in most of Africa and Madagascar.

Recorded hosts: Found on sespan, millet and clover,feed on the flowers and fruits of Fabaceae, Rosaceae and Plumbaginaceae species, including *Plumbago capensis, Indigofera, Rynchosia, Vigna, Burkea, Mundulea, Melilotus, Crataegus, Quercus suber, Medicago sativa, Trifolium alexandrium, Arachis hypogaea, Lythrum, Calluna, Genista, Dorycnium, Lythrum salicaria, Calluna vulgaris, Onobrychis viciifolia, Ulex* and *Melilotus alba.*

Habitats: This species prefers varied wasteland, cultivated areas and gardens.

Remarks: These butterflies fly from February to November depending on the location. They are regular migrants.

3.6. *Lycaena phlaeas* Linnaeus

Common name: The small copper and American copper or common copper

Local distribution: Al Kanater (Qalubiya), Fayoum, Rafah (N. Sinai), Kharga Oasis, Baris Oasis (W. Desert), Nobariya,Wadi El Natroun (Behaira), Giza and Assiut.

Geographic distribution: The small copper is a very widespread and common across Europe, almost all of Europe including sub-arctic areas of Scandinavia, and across temperate Asia, as far east as Japan, North America, occurring in Canada, the eastern United States, the Canary Isles and also found from the Atlas - mountains and north African grasslands, in North Africa south through to Ethiopia, Kenya and Malawi.

Recorded hosts: Buckwheat (Polygonaceae) family including sheep sorrel (*Rumex acetosella*), curled dock (*Rumex crispus*) and *Oxyria digyna*.

Habitats: The small copper occurs in many different habitats including heaths, chalk and limestone grasslands, sand dunes, cliff tops, woodland rides and clearings, hay meadows, pastures and almost anywhere else where the larval food plants grow.

Remarks: They usually breed in sheltered hollows, or at the bottom of sunny slopes, where vegetation is sparse, and areas of bare ground are available for basking. The species overwinters as a caterpillar. Both sexes are subject to variation regarding the size of the black spots on the forewings. Flight period from April to September.

3.7. *Lycaena thersamon* Klug

Common name: The lesser fiery copper

Local distribution: Al Kanater, Belbais (Qalubiya), Fayoum, Al Arish (N. Sinai), Kharga Oasis, Dakhla Oasis, Baris Oasis (W. Desert), Alexandria, Burg Al Arab, Nobariya, Wadi El Natroun (Behaira), Seds (Kafr El Skeikh) and Gabal Elba (Red Sea).

Geographic distribution: It is found from Eastern Europe, Italy and South-East Europe to Mongolia and North-Western China.

Recorded hosts:*Eryngium creticum* (Umbelliferae), *Rumex cyprius*, *Polygonum* spp. and *Sarothamnus* (Polygonaceae).

Habitats: Dry grasslands

Remarks: The butterfly flies from March to October depending on the location, form in April and May and again as omphale from July onward, flying on dry sunny hillsides, not being rare at their flight-places.

3.8. *Polyommatus icarus zelleri* Verity

Common name: The common blue butterfly

Local distribution: Al Kanater (Qalubiya), Fayoum, Tur Sinai (S. Sinai), Bahariya Oasis and Dakhla Oasis (W. Desert).

Geographic distribution: The common blue butterfly is found in Europe, North Africa, the Canary Islands, and temperate Asia to Northern China. Recently it was discovered in Quebec and Canada. It is widespread in the British Isles.

Recorded hosts: Family Leguminosae (bean family). Recorded food plants are *Lathyrus* species, *Vicia* species, *Vicia cracca*, *Oxytropis campestris*, bird's foot trefoil (*Lotus corniculatus*), *Oxytropis pyrenaica*, *Astragalus aristatus, Astragalus onobrychis, Astragalus pinetorum*, black medick (*Medicago lupulina*), *Medicago romanica, Medicago falcata*, common restharrow (*Ononis repens*), wild thyme (*Thymus serpyllum*), lesser trefoil (*Trifolium dubium*), *Trifolium pratense* and white clover (*Trifolium repens*).

Habitats: Meadows, coastal dunes, woodland clearings, heathlands, sand dunes, and under cliffs and also many man-made habitats, anywhere their food plants are found. These butterflies inhabit flowery or grassy places, warm and cool, open or wooded areas and at all altitudes up to high alpine meadows at an elevation of 0–2,700 metres above sea level. It mostly resides on chalk or limestone grassland.

Remarks: Resident in Egypt, flight period from April to July, rare species.

3.9. *Tarucus balkanicus* Freyer

Common name: the Balkan Pierrot or little tiger blue and Little Tiger Pierrot.

Local distribution: Al Kanater, Belbais (Qalubiya), Fayoum, Al Arish (N. Sinai), Kharga Oasis, Dakhla Oasis, El-Baharia Oasis (W. Desert), Burg Al Arab (Alexandria), Giza and Assiut.

Geographic distribution: Mauritania, Niger, Sudan, Uganda, Saudi Arabia, the United Arab Emirates, Oman, North Africa, the Balkans, western Asia, parts of central Asia and India.

Recorded hosts: *Ziziphus spina - christi* (Rhmanaceae).

Habitats: Very arid savanna and desert areas near host plant.

Remarks: Vagrant, Flying period from March to October.

3.10. *Tarucus rosacea* (Austaut)

Common name: The Mediterranean Pierrot or Mediterranean tiger blue

Local distribution: Fayoum,Al Arish (N. Sinai), Kharga Oasis, Dakhla Oasis, Baris Oasis, El-Baharia Oasis (W. Desert), St. Kathrine (S. Sinai), Matrouh, Nobaryia and Seds (Kafr El Skeikh).

Geographic distribution: From Sahel to North West India.

Recorded hosts: *Ziziphus spina -christ* (Rhmanaceae).

Habitats: Occurs wherever its food plants occur.

Remarks: Flight period from February to October.

3.11. *Zizeeria karsandra* (Moore)

Common name: The dark grass blue**.**

Local distribution: Al Kanater (Qalubiya), Fayoum, Al Arish (N. Sinai), Baris Oasis, El-Baharia Oasis (W. Desert) and Nobariya (Behaira).

Geographic distribution: From the Southern Mediterranean, in a broad band to India, Sri Lanka, Burma, Thailand, Malaysia, Yunnan, Indonesia, the Philippines, United Arab Emirates, Saudi Arabia and Oman, New Guinea and Northern and Eastern Australia.

Recorded hosts: Leguminous plants, alfalfa and Tribulus.

Habitats: Cultivated and Oasis habitats.

Remarks: Flight period from March up to November, Resident in Egypt.

4.Family: Nymphalidae (brush-footed butterflies):

The Nymphalidae are the largest family of butterflies with more than 6,000 species distributed throughout most of the world, belonging to the

superfamily Papilionoidea. These are usually medium-sized to large butterflies. Most species have a reduced pair of forelegs and many hold their colourful wings flat when resting. They are also called four-footed butterflies, because they are known to stand on only four legs while the other two are curled up**.** Many species are brightly coloured and include popular species such as the emperors, monarch butterfly, admirals, tortoiseshells, and fritillaries.However**,** the under wings are, in contrast, often dull and in some species look remarkably like dead leaves, or are much paler, producing a cryptic effect that helps the butterflies blend into their surroundings. It is represented in Egypt by 9 species within 5 genera. It is recorded here by three species.

4.1. ***Cynthia cardui*** **(Linnaeus)**

Common name: The painted lady**.**

Local distribution: Al Kanater (Qalubiya), Fayoum, Alexandria, W. El Natroun (Behaira), Fayed (Ismailia), Giza, Seds (Kafr El Skeikh), Al Arish, Rafah (N. Sinai), Kharga Oasis, Dakhla Oasis, Baharyia Oasis and Baris Oasis (W. Desert).

Geographic distribution: Widespread and Holarctic**.**

Recorded hosts: Artichoke, *Helianthus* and *Mulva*. It has a wide range of host plants.

Habitats: Occurs in any areas with flowers.

Remarks: Migrant species, not resident in Egypt. flight period from February until November. Pests on Malvaceae.

4.2. ***Melitaea deserticola*** **Oberthür**

Common name: The desert fritillary**.**

Local distribution: Northern Egypt, Sinai.

Geographic distribution: It is found in North Africa (Morocco, Algeria, Libya and Egypt), Lebanon, Israel, Jordan, Saudi Arabia and Yemen.

Recorded hosts: The larvae feed on *Linaria aegyptiaca*, *Plantago media*, *Anarrhinum fruticosum* and *Anarrhinum* species and other Scrophulariaceae.

Habitats: Desert Wadis.

Remarks: Flight period from May to Sebtember. Resident in Egypt.

4.3. ***Vanessa atalanta*****(Linnaeus)**

Common name: The red admiral.

Local distribution: Al Kanater (Qalubiya), Fayoum, Al Arish, Zaranick (N. Sinai), El Tur (S. Sinai), Kharga Oasis, Dakhla Oasis, Baharyia Oasis, Baris (W. Desert), W. El Natroun and Nubaria (Behaira).

Geographic distribution: The red admiral is widely distributed across temperate regions of North Africa, the Americas, Europe, Asia and the Caribbean.

Recorded hosts: The red admiral's main host plant, stinging nettle (*Urtica dioica*), false nettle (*Boehmeria cylindrica*), often feeding on the flowers of ivy on sunny days. The adult butterfly drinks from flowering plants like Buddleia and overripe fruit.

Habitats: Typically found in moist woodlands.

Remarks: Red admirals are territorial. It is known as an unusually people-friendly butterfly, often landing on and using humans as perches. The butterfly flies on sunny winter days, especially in southern Europe. It resides in warmer areas, but migrates north in spring and sometimes again in autumn.

5. **Family: Pieridae (Cabbage butterflies):**

The Pieridae are a large family of butterflies with about 76 genera containing about 1,100 species, mostly from tropical Africa and tropical Asia with some varieties in the more northern regions of North America. Most pierid butterflies are white, yellow, or orange incoloration, often with black spots. The sexes usually differ, often in the pattern or number of the black markings. They are notorious agricultural pests.

Males of many species exhibit gregarious mud-puddling behavior when they may imbibe salts from moist soils. The Pieridae are generally divided into these four subfamilies: Dismorphiinae (mostly Neotropical; this group includes several mimetic species. The host plants are in the family Fabaceae), Pierinae (whites, yellows, and orange-tips; many of these species are strongly migratory. Host plants are in the families Capparidaceae, Brassicaceae, Santalaceae, and Loranthaceae), Coliadinae (sulphurs or yellows; many of these species are sexually dimorphic. Some, such as Colias, have wing patterns that are visible only under ultraviolet) and Seudopontiinae (includes only the genus *Pseudopontia,* with the sole species in this subfamily, *Pseudopontia paradoxa*, which is endemic to West Africa).

It is represented in Egypt by 27 species within 10 genera. It is recorded here by six species.

5.1. *Colias croceus* (Geoffroy)

Common name: Clouded yellow butterfly**.**

Local distribution: Allover Egypt.

Geographic distribution: One of the most-widespread species in Europe. The common clouded yellow's breeding range is North Africa and southern Europe and eastwards through Turkey into the Middle East but it occurs throughout much of Europe as a summer migrant. In Asia, its range extends into central Siberia in the north and barely into India in the south; it is not found in Central Asia.

Recorded hosts: Mainly alfalfa with *Tephrosia purpurea* and *Astragalus* spp.

Habitats: Live in any open cultivated areas in the countryside, including downland, coastal cliffs and fields containing the caterpillar's host plants, at an elevation up to 1,600 metres above sea level.

Remarks: A truly migratory European butterfly, this species is famous for occasional mass migrations and subsequent breeding. Resident in Egypt. Flight period from April till November.

5.2. *Colotis chrysonome* Klug

Common name: The golden Arab tip

Local distribution: Gebel Elba (Red Sea), Kharga Oasis, Dakhla Oasis, Baharyia Oasis, Baris Oasis (W. Desert),Giza, W. El Natroun (Behaira), Belbais (Qalubiya) and Seds (Kafr El Skeikh).

Geographic distribution: Mauritania, northern Senegal, Mali, Burkina Faso, Nigeria, Niger, the central and eastern part of the Sahara, Sudan, Ethiopia, Somalia, southern Arabia, northern Uganda, Kenya, northern Tanzania, Israel and Jordan.

Recorded hosts:*Maerua crassifolia* (Capparaceae).

Habitats: The habitat consists of arid savanna.

Remarks: Resident in Egypt, but very rare species. Flight period April and June.

5.3. *Colotis protomedia* (Klug)

Common name: The yellow splendour tip

Local distribution: Al Arish (N. Sinai), W. El Natroun (Behaira) and Baharyia Oasis (W. Desert).

Geographic distribution: It is found in north-eastern Nigeria, northern Cameroon, Chad, southern Sudan, northern Uganda, Ethiopia, Somalia, south-western Saudi Arabia, Yemen, Kenya, Tanzania and the Democratic Republic of the Congo.

Recorded hosts:*Maerua* species (Capparaceae).

Habitats: The habitat consists of dry savannah.

Remarks: Adults have a fast flight. They are attracted to flowers, especially those of *Maerua* species.

5.4. *Pieris rapae* (L.)

Common name: Cabbage butterfly, the small white,the small cabbage white, white butterfly

Local distribution: All over Egypt

Geographic distribution: Cosmopolitan.It is widespread and is believed to have originated in Europe or Asia. It is also found in North Africa and has been accidentally introduced to North America, Bermuda, Australia and New Zealand.

Recorded hosts: herb Cruciferae – *Arabis glabra*, *Armoracia lapthifolia*, *Armoracia aquatica*, *Barbarea vulgaris*, *Barbarea orthoceras*, *Barbarea verna*, *Brassica oleracea*, *Brassica rapa*, *Brassica caulorapa*, *Brassica napus*, *Brassica juncea*, *Brassica hirta*, *Brassica nigra*, *Brassica tula*, *Cardaria draba*, *Capsella bursa-pastoris*, *Dentaria diphylla*, *Descurainia Sophia*, *Eruca sativa*, *Erysimum perenne*, *Lobularia maritima*, *Lunaria annua*, *Matthiola incana*, *Nasturtium officinale*, *Raphanus sativus*, *Raphanus raphanistrum*, *Rorippa curvisiliqua*, *Rorippa islandica*, *Sisymbrium irio*, *Sisymbrium altissimum*, *Sisymbrium officinale*, *Streptanthus tortuosus*, *Thlaspi arvense*; Capparidaceae: *Cleome serrulata*, *CapBaris sandwichiana* and Tropaeolaceae: *Tropaeolum majus*; Resedaceae: *Reseda odorata.*

Habitats: The species can be found in any open area with diverse plant association. It can be seen usually in towns, but also in natural habitats, mostly in valley bottoms.

Remarks: The caterpillar of this species is seen as a pest for commercial agriculture. Often referred to as the "imported cabbageworm" they are a serious pest to cabbage and other mustard family crops.

5.5. *Pontia daplidice* L.

Common name: The bath white

Local distribution: Aswan, Kharga Oasis, Baris Oasis (W. Desert), Al Kanater (Qalubiya) and Fayoum.

Geographic distribution: Palearctic region. It is common in central and Southern Europe, Asia Minor, Persia and Afghanistan. In Central Asia, the Bath white ranges from Baluchistan, Peshawar, Chitral, Kashmir and along the Himalayas right across the Central Himalayas up to Darjeeling. The butterfly appears to be extending its range westwards along the Himalayas.

Recorded hosts: family Brassicaceae and vary according to locality. They include tower mustard (*Arabis glabra*) and sea rocket (*Cakile maritima*).

Habitats: It is usually found on dry slopes and rough ground with little vegetation. The butterfly lives in the Mediterranean coastal dunes, on rocky, hot slopes.

Remarks: migrating northwards in the summer. In Central Asia.

5.6. *Pontia glauconome* Klug

Common name: the desert white or desert bath white**.**

Local distribution: Fayoum, Al Arish (N. Sinai), Kharga Oasis, Dakhla Oasis, Bairs Oasis, El-Baharia Oasis (W. Desert), El Tur (S. Sinai), Alexandria, Nubaryia (Behaira), Fayed (Ismailia), Belbis (Qalubiya), Giza, Seds (Kafr El Skeikh) and Assuit.

Geographic distribution: Mauritania, Senegal, Gambia, Niger, Chad, Sudan, Ethiopia, Somalia, Kenya, Arabia, Egypt, the Middle East, Pakistan, Afghanistan, the southern part of the former Soviet Union, Uzbekistan, Tajikistan and Turkmenistan.

Recorded hosts: *Epicastrum arabicum, Zilla spinosa, Caylusia, Dipterygium, Erucastrum, Moracandia, Diplotaxis, Cleome arabica* and *Ochradenus baccatus* and *Reseda.*

Habitats: The habitat consists of sub-deserts.

Remarks: Adults are on wing from March to October or from April to

November in three to four generations per year.The pupae have a facultative diapause of at least four years. Resident in Egypt.

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Effect of insect infestation by *Macrosiphoniella sanborni* (Hemiptera: Aphididae) and *Frankliniella tritci* (Thysanoptera: Thripidae) on morphological characteristics of chrysanthemum flowers under glasshouse

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Abstract:

The aim of this resreach work is to study the comparison between effects of insect infestation by chrysanthemum aphid *Macrosiphoniella sanborni* (Gillete) (Hemiptera: Aphididae) and ornamental plant thrips *Frankliniella tritci* (Fitch) (Thysanoptera: Thripidae) on some morphological characteristics of chrysanthemum flowers under glasshouse conditions at two locations in Cairo and Giza Governorates during 2018 seasons. Morphological characteristics which studied were, color of the flower, number of the petals per flower, the flower diameter, weight of the flower, the stem length, vase life period (flowers life after picking) and annual production. Results showed that the infestation by *M. sanborni* (Aphid) and *F. tritci* (Thrips) affected on all morphological characteristics except the color of the flowers. The effect of infestation by aphid was higher than the effect of infestation by thrips, this is compared to control (chrysanthemum flowers did not infested by any insects). Also, present study showed clearly that the effect of aphid and thrips infestation concentration on the vascular pundles of the flowers petals and with the increase in the infestation by the two insects and the damage in the vascular bundles increases.

Introduction

Chrysanthemums flowers are very important cut flowers crop with an economical value in international floral industry. Chrysanthemum has long post harvest life and it continues to look attractive even when semi dry. It has wide range of colors, shapes and sizes. Chrysanthemum is ranked as the second most economic important cut flower in the world after rose (Kafi and Ghahsareh, 2009).

Chrysanthemum flowers infested different species of insects causes much damages to the flowers both to its quantity and quality. Chrysanthemum aphid *Macrosiphoniella sanborni* (Gillette) (Hemiptera: Aphididae) is one of the most dangerous insect pests infesting chrysanthemum flowers. Chrysanthemum aphid *M. sanborni*) had caused great damage to chrysanthemum production and affected seriously on the flowers in

quantity and quality (Sumei *et al*., 2014). Chrysanthemum aphid *M. sanborni* represent the most destructive of chrysanthemum pests to cultivation and caused many damage to the flowers and production (Yanming *et al.*, 2010 and Wang *et al.*, 2015).

Also, ornamental plant thrips *Frankliniella tritci* (Fitch) (Thysanoptera: Thripidae) is considered as one of the most dangerous insects infested chrysanthemum flowers both in open field and under glasshouse conditions. Candica *et al.* (2012) reported that thrips insects cause damage to chrysanthemum flowers growth under glasshouse conditions. Carl and Diann (2015) studied the injury to various plant tissues by ovipositing thrips and feeding injuries by thrips species to pollen, flowers, fruit and leaves are characterized for different economic plants. Murugan and Jagadish (2014) in India reported that chilli thrips *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) has become a serious pest on chrysanthemum flowers in recent times. It feeds all stages of chrysanthemum flowers by remaining concealed between the petals. They reported four stages of chrysanthemum flowers, unopened buds, opened young buds, harvestable flowers and fully opened flowers.

This research work was carried out to study the comparison between effect of insect infestation by chrysanthemum aphid *M. sanborni* and ornamental plant thrips *F. tritci* on some morphological characteristics of chrysanthemum flowers under glasshouse conditions at two locations in Cairo and Giza Governorates during 2018 season.

Materials and methods

1. Experimental design:

This study was conducted on chrysanthemum plants grown in two locations, International Garden (Cairo Governorate) and El-Orman Garden, (Giza Governorate) under glasshouse conditions during successive seasons 2018. The glasshouse in each garden with an area of 27x45 m. Each glasshouse was divided into three parts, first part left as control, second part had artificially infestation by chrysanthemum aphid *M.sanborni* and the third part had artificially infestation by ornamental plant thrips *F.tritci*. Each part contains 5 plots (3x5 m^2) for each, and each part isolated completely from others. Chrsanthemum seedlings were planted in glasshouse conditions at the same time on September (the planting time of chrysanthemum plants). All agricultural operations of irrigation and fertilization and others are completely identical in the two glasshouses were done without application of any insecticide.

At both of the two glasshouses all postharvest treatments were identical but conducted separately until the arrival of the flowers for the final stage, then took these flowers to the laboratory. At the end of the first growing season, 50 flowers were collected from each part at the two locations and all morphological parameters carried out of them at the laboratory.

2. Laboratorial design:

This experiment was carried out also to study the effect of both the two studied insects on the morphological characteristics of chrysanthemum flowers. Morphological characteristics studied were, color of the flower, number of the petals per flower, the flower diameter, weight of the flower, the stem length, vase life period (flowers life after picking) and annual production. Also effect of the two insects on the interior tissues of chrysanthemum betals especially the vascular bandles which consider the food factory of the flowers and all the plant.

3. Statistical analysis:

Effect of the insect infestation by both chrysanthemum aphid *M. sanborni* and ornamental plant thrips *F. tritci* on the morphological characteristics of certain chrysanthemum varieties were subjected to analysis of variance (ANOVA) and the means were compared by LSD test at 0.05 level, using SAS program **(SAS institute,1988)**.

Results and discussion

1. Effect of infestation by *Macrosiphoniella sanborni* and *Frankliniella tritci* on the morphological characteristics of chrysanthemum flowers:

Data tabulated in Table (1) showed the effect of insect infestation by aphid *M. sanborni* and thrips *F. tritci* on some morphological characteristics of chrysanthemum flowers for different varieties (colors) compared to control which non infested by these insects. The results showed that red flowers, the color not changed after infestation by the two insects. Number of petals per flower decreased from 50-55 petals/flower in control to 37-39 and 41-43 petals/ flower; the flower diameter changed from 8-10 cm in control to 4-5 and 5-6 cm ; weight of the flower also decreased from 15-17 gram in control to 10-12 and 12-14 gram ; the stem length decreased from 25-30 cm in control to 17-19 and 20-22 cm ; the vase life period decreased from 8-11 days in control to 4-5 and 5-6 days and the annual production reached to 125-130 flower/m^2/year in control but decreased to 85-90 and 90-95 flower/m^2/year after infestation by *M. sanborni* and *F tritci* , respectively. For (Yellow flowers), the color not changed after infestation by the two insects. Number of petals per flower decreased from 45-50 petals/flower in control to 31-33 and 36-39 petals/flower ; the flower diameter changed from 9-11 cm in control to 5-6 and 6-7 cm ; weight of the flower also decreased from 17-19 gram in control to 11-13 and 14-15 gram ; the stem length decreased from 27-31cm in control to 19-21 and 22-24 cm ;the vase life period decreased from 9-11 days in control to 5-7 and 6-8 days and the annual production reached to 120-125 flower/m^2/year in control, but decreased to 85-90 and 90-95 flower/m^2/year after infestation by *M. sanborni* and *F tritci* , respectively.

For (blue flowers), the color not changed after infestation by the two insects. Number of petals per flower decreased from 47-50 petals/flower in control to 35-37 and 38-40 petals/flower ; the flower diameter changed from 10-12 cm in control to 6-8 and 7-9 cm ; weight of the flower also decreased from 16-18 gram in control to 10-12 and 12-14 gram ; the stem length decreased from 28-32cm in control to 20-22 and 23-24 cm ; the vase life period decreased from 10-12 days in control to 5-6 and 7-8 days and the annual production reached to 115-120 flower/m^2/year in control, but decreased to 80-85 and 85-90 flower/m^2/year after infestation by *M. sanborni* and *F. tritci* , respectively. For (White flowers), the color not changed after infestation by the two insects. Number of petals per flower decreased from 48-52 petals/flower in control to 33-35 and 38-41 petals/flower ; the flower diameter changed from 9-11 cm in control to 5-7 and 6-8 cm ; weight of the flower also decreased from 17-19 gram in control to 10-12 and 13-15 gram ; the stem length decreased from 26-30 cm in control to 18-20 and 22-23 cm ;the vase life period decreased from 9-11 days in control to 5-6 and 7-9 days and the annual production reached to 120-125 flower/m^2/year in control, but decreased to 85-90 and 90-95 flower/m^2/year after infestation by *M. sanborni* and *F. tritci* , respectively.

Table (1) : Effect of infestation by *Macrosiphoniella sanborni* and *Frankliniella tritci* on the morphological characteristics of chrysanthemum flowers.

Adjective	Red			SA	Yellow			SA	Blue			SA	White			SA
	Control	Aphid	Thrips		Control	Aphid	Thrips		Control	Aphid	Thrips		Control	Aphid	Thrips	
Colour	Red	Red	Red	ns	yellow	yellow	yellow	ns	Blue	blue	blue	ns	White	White	white	ns
No. of Petals/flower	50-55	37-39	41-43	F =2.52* LSD=6.17	45-50	31-33	36-39	F=9.27** LSD=6.25	47-50	35-37	38-40	F=7.08* LSD=7.12	48-52	33-35	38-41	F=15.31** LSD=6.23
Flower diameter/cm	8-10	4-5	5-6	F=12.01** LSD=1.29	9-11	5-6	6-7	F=14.00** LSD=1.26	10-12	6-8	7-9	F=15.01** LSD=2.03	9-11	5-7	6-8	F=13.01*** LSD=1.43
Weight/g	15-17	10-12	12-14	F=14.01** LSD=1.98	17-19	11-13	14-15	F =8.03* LSD =1.99	16-18	10-12	12-14	F=13.01** LSD=1.97	17-19	10-12	13-15	F=14.01** LSD=1.88
Stem length/cm	25-30	17-19	20-22	F=22.14*** LSD=7.56	27-31	19-21	22-24	F=7.41** LSD =7.82	28-32	20-22	23-24	F=12.00** LSD =8.78	26-30	18-20	22-23	F =12.0** LSD=8.79
Vase life/day	8-11	4-5	5-6	F =14.36* LSD =2.36	9-11	5-7	6-8	F=13.01** LSD =2.36	10-12	5-6	7-8	F =8.03** LSD=1.58	9-11	5-6	7-9	F =9.4* LSD=1.57
Annual production flower/m^2 /year	125-130	85-90	90-95	F=53.03*** LSD=8.59	120-125	85-90	90-95	F=32.74** LSD=12.31	115-120	80-85	85-90	F=32.5*** LSD=13.15	120-125	85-90	90-95	F=14.01** LSD=15.35

SA = Statistical analysis ns - non significant * - significant ** - significant *** - high significant

The obtained results are agreement with those obtained by Jaskiewicz (2016) in Poland who studied the effect of feeding of chrysanthemum aphid, *M. sanborni* on the flowering of chrysanthemum and reported that the *M. Sanborni* when found in greater numbers caused deformation of the leaf blades, the shorting of shoots and flowers petioles, as well as deformation of the flowers. Also Pham *et al*. (2008) in Netherlands who studied the effect of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) on chrysanthemum plants and stated that flower damage caused by *F.occidentalis* depends on the season and number of thrips on the flower and conducted a study to determine the relationship among silver damage on the leaves and flower damage and Sauer (2012) in Germany reported that petal damage could not always be attributed to thrips infestation (number of thrips) only but also attributed to the time of the infestation, total infestation percentages depending on the average colonization /week. On the other side, Gary *et al.* (2015) found that the aphid has not only direct effect on chrysanthemum flowers but also has indirect effect on these flowers through transmitted virus diseases of chrysanthemum seedlings. Also, Mokenny (2016) agreement with this opinion that the effect of aphid insects not only the direct effect on flowers but also the most effect on chrysanthemum flowers through as a vector of virus "breaking" which transmitted it to chrysanthemum flowers.

2. Effect of infestation by aphid and thrips on the vascular bundles of chrysanthemum flowers:

The obtained results showed that the effect of chrysanthemum aphid *M. sanborni* and ornamental plant thrips, *F. tritci* concentrated on the vascular bundles in the tissue of the petals of chrysanthemum flowers which consider the factory of the food in the plant. The damage in theses vascular bundles increase with the high infestation by the two insects and decrease with the low infestation by them. This is show clearly from Figure (1) which showcross - sections for petals of chrysanthemum flowers and show the effect of infestation by aphid and thrips concentration on the vascular bundles (bundle sheeth, xylem vessels and phloem), which make an important role in transporting water and nutrient from soil to plants and from leaves to all parts of chrysanthemum plant. As shown as in this figure by increase infestation with the two insects the damage in vascular bundles increase and so more bad, also morphological and physiological effects will occur due to deficient in water and important dissolved salts.

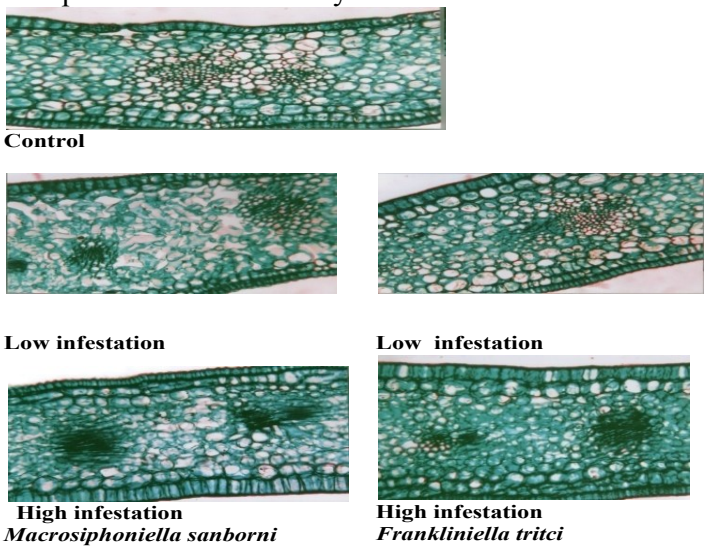

Figure (1):The damage symptoms of chrysanthemum flowers tissues (vascular bundles) after infestation by *Macrosiphoniella sanborni* and *Frankliniella tritci*.

The obtained results are agreement with those obtained by Pollard (2013) who studied the feeding penetration of *Myzus persicae* (Sulz.) (Hemiptera: Aphididae) nymphs into tulip leaf epidermis, as shown by a study of stylets and tracks, may be intercellular, or stomatal with the former predominating, and reported also that the contact between the stylet sheath or track and cell cytoplasm is at a maximum during intracellular entry but occurs to a limited extend during intercellular penetration due to partial rupture of the epidermal end walls, in the mesophyll the stylet path is intercellular but a few cells were penetrated by tracks.

Also, the obtained results are agreement with those obtained by Peng and Miles (2015) in Australia, detected that *M. sanborni* feeding on the chrysanthemum flowers that occurs and concentration in the parenchymal and vascular tissues of the chrysanthemum tissues. The aphids will feed on tissues and on aqueous diets containing low concn. Davidson (2014) reported that the plant exhibits many pathological features as a result of aphid attack, the food of aphids is the cell sap of plants derived from various cells of the plant tissues, especially the vascular bundles. Also, Zuniga *et al.* (2016) studied the effect of gramine on the feeding behavior of the aphids *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) and found that gramine found only in the vascular bundles, and it is suggested that gramine content and location may affect the feeding behavior of aphids in these plants. Kindt *et al.* (2003) studied characterization of the feeding behavior of Western flower thrips (Ornamental plant thirps) *F. occidentalis* and found that insect causes damage to plants when it is feeding, also this thrips species transmits *Tomato spotted* Wilt virus (TSWV) during styles penetration and also investigated that the penetration behavior (probing) of thirps on leaves causes more damage on vascular bundles.

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New records of Encyrtidae, Eulophidae, Eurytomidae, Mymaridae, Pteromalidae and Torymidae (Hymenoptera: Chalcidoidea) from Iran

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Abstract:

In this faunistic paper, totally 18 species of Chalcidoidea (Hymenoptera) within 6 families, Encyrtidae (4 species, 4 genera), Eulophidae (3 species, 3 genera), Eurytomidae (one species), Mymaridae (2 species, 2 genera), Pteromalidae (6 species, 6 genera), and Torymidae (2 species, 2 genera) are given as new records for the fauna of Iran.

Introduction

Chalcidoid wasps (Hymenoptera: Chalcidoidea) are a fascinating group of insects, which show exquisite life histories and diverse types of host relationships (Narendran *et al*., 2007 and Heraty, 2009). Chalcidoid wasps are extremely diverse with over than 23,000 valid species (Noyes, 2019) and an estimated diversity of up to 500,000 distinct species (Heraty and Gates, 2003 and Munro *et al*., 2011). Most species of this superfamily are powerfull parasitoids of agricultural pests and have efficient role in biological control programs (Godfray, 1994; Bellows and Fisher, 1999 and Noyes, 2000).

The fauna of Iranian Chalcidoidea was studied rather well and several contributions have been published recently which most of the families were catalogued: Aphelinidae:146 species in 12 genera, Azotidae: 11 species in one genus, Eriaporidae: 2 species in 2 genera (Abd-Rabou *et al*., 2019), Chalcididae: 68 species in 18 genera (Falahatpisheh *et al*., 2018), Encyrtidae (Fallahzadeh and Japoshvili, 2017: 159 species within 48 genera and Guerrieri and Ghahari, 2018: 180 species), Eulophidae: 176 species in 44 genera (Hesami *et al*., 2018), Eucharitidae:5 species in 2 genera and Ormyridae:13 species in one

genus (Ghahari and Gençer, 2017), Eurytomidae:89 species in 8 genera (Saghaei *et al*., 2018), Leucospidae:6 species in one genusand Tetracampidae:4 species in 4 genera (Ghahari, 2019), Pteromalidae:227 species in 114 genera(Ghahari *et al*., 2015), Signiphoridae:11 species in 3 genera (Ghahari *et al*., 2014) and Torymidae:80 species in 18 genera (Ghahari and Doğanlar, 2017). The aim of this paper is a faunistic study on chalcidoid wasps of Iran.

Materials and methods

Chalcidoid wasps of Iranwere collected from some regions of Iran by Malaise traps and sweeping net, additionally some specimens deposited in insect collections of Islamic Azad University and private collections of colleagues were studied. Here we follow Noyes (2019) for classification, nomenclature and distribution.

Results and discussion

List of collected species

1.Family Encyrtidae Walker

1.1. *Anagyrus vladimiri* Triapitsyn

Material examined: Khorasan-e Shomali province, Farooj, 37°22′N 58°28′E, 2♀, May 2017.

General distribution: Israel, Italy, Russia, Spain, Tunisia, Turkmenistan, United States of America **(Noyes, 2019)** and Iran (this study).

3.2. ***Arrhenophagus chionaspidis* Aurivillius**

Material examined: Sistan and Baluchestan province, Qasr-e Qand, 26°24′N 60°73′E, 2♀, October 1998.

General distribution: Afrotropical, Argentina, Australia, Azerbaijan, Barbados, Bermuda, Brazil, British Virgin Islands, Canary Islands, Cape Verde Islands, China, Czech Republic, France, Georgia, Guyana, Hungary, India, Jamaica, Japan, Korea, South, Madeira, Mauritius, Mexico, New Zealand, Peru, Poland, Puerto Rico, Réunion, Russia, Senegal, Slovakia, Spain, Sri Lanka, Sweden, Switzerland, Taiwan, Tanzania, Uganda, United Kingdom, United States of America, former USSR **(Noyes, 2019)** and Iran (this study).

1.3.*Choreia inepta* (Dalman)

Material examined: Gilan province, Lahijan, 37°13′N 50°01′E, 2♀, April 2012. Zanjan province, Khorram-Darreh (Eslam-Abad), 1♀, July 2014.

General distribution: Armenia, Austria, Azerbaijan, Bosnia Hercegovina, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Hungary, Italy, Moldova, Mongolia, Montenegro, Netherlands, Poland, Romania, Russia, Slovakia, Spain, Sweden, Ukraine, United Kingdom, former Yugoslavia (Noyes, 2019) and Iran (this study).

1.4. *Diversinervus elegans* Silvestri

Material examined: Ardebil province, Aslandooz, 39°45′N 47°41′E, 2♀, 1♂, 26 July 2010.

General distribution: Afrotropical, Angola, Argentina, Australia, Brazil, China, Colombia, Cuba, Egypt, Eritrea, Ethiopia, France, Greece, Hawaii, India, Israel, Italy, Kenya, Mexico, Morocco, New Caledonia, Peru, South Africa, Spain, United States of America+, former Yugoslavia (Noyes, 2019) and Iran (this study).

2. Family Eulophidae Westwood

2.1.*Aprostocetus elongatus* (Foerster)

Material examined: Luristan province, Nur-Abad, 34°06′N 47°95′E, 1♀, 1♂, 8 June 2013.

General distribution: Austria, Bulgaria, Croatia, Czech Republic, Denmark, France, Georgia, Germany, Hungary, Italy, Montenegro, Netherlands, Poland, Slovakia, Spain, Sweden, Switzerland, United Kingdom (Noyes, 2019) and Iran (this study).

2.2.*Baryscapus daira* (Walker)

Material examined: Azarbaijan-e Sharghi province, Horand, 38°84′N 47°35′E, 1♀, August 2013.

General distribution: Argentina, Austria, Bulgaria, Canada, Chile, Czech Republic, France, Germany, Greece, Hungary, Italy, Moldova, Netherlands, Russia, Slovakia,

Spain, Sweden, Switzerland, Turkey, United Kingdom, United States of America, former USSR (Noyes, 2019) and Iran (this study).

2.3. *Pediobius foliorum* (Geoffroy)

Material examined: Kuhgiloyeh and Boyerahmad province, Kakan, 30.65′N 51.82'E, 2♀, 1♂, September 2012.

General distribution: Austria, Canada, Czech Republic, Finland, France, Germany, Hungary, Israel, Italy, Japan, Moldova, Netherlands, Romania, Russia, Serbia, Slovakia, Slovenia, Sweden, United Kingdom, United States of America (Noyes, 2019) and Iran (this study).

3.Family Eurytomidae Walker

3.1. *Eurytoma aethiops* Boheman

Material examined: Qazvin province, Rostam-Abad, 35°66′N 49°85′E, 1♀, 1♂, August 2012. Mazandaran province, Behshahr,36°44′N 53°46′E, 2♀, April 2016.

General distribution: Bulgaria, Caucasus, Hungary, Italy, Sweden, Transcaucasus, United Kingdom (Noyes, 2019) and Iran (this study).

4. Family Mymaridae Haliday

4.1.*Polynema ovulorum* (Linnaeus)

Material examined: Kerman province, Jiroft, 28°51′N 57°33′E, 1♀, 1♂, October 2014.

General distribution: Austria, Belgium, Bulgaria, France, Germany, Greece, Romania, Sweden, Turkey (Noyes, 2019) and Iran (this study).

4.2.*Ooctonus insignis* Haliday

Material examined: Azarbaijan-e Gharbi province, Mahabad, 36°47′N 45°43′E, 2♀, 9-11.viii.2014.

General distribution: Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Ireland, Italy, Netherlands, Norway, Romania, Russia, Slovakia, Sweden, Switzerland, United Kingdom, United States of America (Noyes, 2019) and Iran (this study).

5. Family Pteromalidae Dalman

5.1. *Anogmus hohenheimensis* (Ratzeburg)

Material examined: Zanjan province, Zanjan,36°33′N 48°12′E, 1♀, 1♂, July 2014.

General distribution: Austria, Belgium, Czech Republic, Finland, Germany, Hungary, Luxembourg, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Ukraine, former USSR (Noyes, 2019) and Iran (this study).

5.2.*Eunotus areolatus* (Ratzeburg)

Material examined: Chaharmahal & Bakhtiary province, Borujen,31°54′N 51°12′E, 3♀, May 2015.

General distribution: China, Czech Republic, Denmark, France, Germany, Hungary, Italy, Kazakhstan, Netherlands, Romania, Spain, Sweden, Turkey (Noyes, 2019) and Iran (this study).

5.3.*Homoporus luniger* (Nees, 1834)

Material examined: Fars province, Abadeh, 31°16′N 52°32′E, 1♀, 2♂, April 2010.

General distribution: Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Kazakhstan, Moldova, Netherlands, Romania, Russia, Slovakia, Spain, Sweden, Transcaucasus, Ukraine, United Kingdom (Noyes, 2019) and Iran (this study).

5.4. *Psilonotus achaeus* Walker

Material examined: Khuzestan province, Dezful, 32°31′N 48°42′E, 3♀, 2♂, October 2013.

General distribution: Belgium, Bulgaria, Canada, Czech Republic, Germany, Hungary, Kazakhstan, Netherlands, Sweden, Turkey, Ukraine, United Kingdom, United States of America (Noyes, 2019) and Iran (this study).

5.5.*Stenomalina favorinus* (Walker)

Material examined: Mazandaran province, Tonekabon, Jangal-e 3000, 36°37′N 50°48′E, 2♀, September 2009.

General distribution: Belgium, Bulgaria, Croatia, Czech Republic, Germany, Ireland (north and south), Netherlands, Romania, Sweden, Turkey, United Kingdom (Noyes, 2019) and Iran (this study).

5.6.*Tritneptis affinis* (Nees)

Material examined: Tehran province, Shahreyar (Zarrindeh), 1♂, September 2015.

General distribution: Austria, Belgium, Canada, China, Czech Republic, Germany,

Kazakhstan, Lithuania, Montenegro, Netherlands, Sweden, United States of America, former USSR (Noyes, 2019) and Iran (this study).

6. Family Torymidae Walker

6.1.*Eridontomerus isosomatis* (Riley)

Material examined: Zanjan province, Abhar,36°16′N 49°03′E, 2♀, June 2014.

General distribution: Bulgaria, Czech Republic, Hungary, Kazakhstan, Mongolia, Slovakia, Tadzhikistan, Ukraine, United States of America, former USSR (Noyes, 2019) and Iran (this study).

6.2.*Torymus caudatus* Boheman

Material examined: Qazvin province, Taleghan, 35.46°N 50.57°E, 1♀, 2♂, August 2012.

General distribution: Croatia, Czech Republic, Finland, France, Georgia, Germany, Hungary, Japan, Latvia, Lithuania, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Sweden, Switzerland, Ukraine, United Kingdom, United States of America, former Yugoslavia (Noyes, 2019) and Iran (this study).

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Honey bee worker as a bio-indicator for measuring environmental pollution in certain Upper Egypt Governorates.

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Abstract:

Pollution is damage caused to water, air, etc. by harmful substance, it is considered one of the major environmental problem, which is causing many diseases. This study aimed to use honeybee's workers body as bio-indicator to monitor pollutants. Samples were collected from some Upper Egypt Governorates. Mass analyze coupled to Inductively Coupled Plasma (ICP) systems include double focusing magnetic-electrostatic sector systems with both single and multiple collectorwas used, as well as time of flight systems (both axial and orthogonal accelerators have been used). The highest pollinate Governorate was (0.575 ppm) in Beni-Suef as the lowest one was (0.256 ppm) in Asyut and Aswan. The highest pollinate elements was 2.294 ppm in manganese (less than the Egyptian's stander 0.02 ppm), but the lowest elements were - 0.356 ppm in boron (Just at the Egyptian's stander). Honey bee *Apis mellifera* L. (Hymenoptera: Apidae) could be used as bio-indicator in ecosystem to ecotoxicological

Introduction:

Condition contamination by synthetic compounds and overwhelming metals quickened significantly during the most recent couple of decades because of mining, purifying, producing, utilization of horticultural composts, pesticides, metropolitan squanders, traffic outflows, modern effluents and mechanical synthetic concoctions and so forth. Wide event of metal contamination exists overall presently, including Egypt (Moussa and Abdelkhalek, 2007). The degree of ecological contamination and coming about human introduction to risky lethal substantial metals in the earth is hard to survey. Compound investigation of the earth network is the most immediate way to deal with uncover the overwhelming metal status in nature, while it can't manage the cost of the incredible proof in the incorporated impact and conceivable danger of such contamination on life forms and biological system.

One of the conceivable elective ways to deal with this issue is the utilization of organic pointers to exhibit ecological contamination. This methodology has all the earmarks of being especially appropriate for showing presentation to possibly lethal

follow components. Natural observing inside a quality control program includes the orderly utilization of living creatures for acquiring quantitative data on changes in the earth, frequently because of anthropogenic exercises (Bargagli, 1998). Creepy crawlies give the foundation of natural checking in oceanic frameworks, where there are well-created systems for utilizing them to survey organic respectability. The utilization of creepy crawlies as bio-markers in earthly biological system, interestingly, has been far less eagerly grasped.

The bumble bee, *Apis mellifera* L. (Hymenoptera: Apidae) has been explored as bio-pointer to screen toxins (Leita *et al.*, 1996). Its engaging quality as a biological finder relies upon a few highlights, for example, high conceptive rate, huge flying reach since they regularly rummage to 2–3 km away from the apiary, the body is secured with hairs that gather different particles and increment by this mean, close contact with the encompassing condition, touchy to poisonous substances, and the conceivable utilization of honey bee items as markers for ecological contamination (Porrini *et al.*, 2003 and Stark, 2003). This last point is significant in light of the fact that honey bees and honey bee items can be utilized as collective and receptive markers (Billalov *et al.*, 1992). Because of preparation by exercises of people, including mining, purifying, producing, utilization of farming manures, pesticides, city squanders, traffic outflows, mechanical effluents and modern synthetic substances, contamination of soils by change metals, for example, cadmium (Cd), nickel (Ni), zinc (Zn), lead (Pb), copper (Cu), has expanded significantly during the most recent couple of decades (Chibuike and Obiora, 2014). Defilement of the earth, including Egypt, by metals is presently far reaching (Al-Nagger *et al.* 2013) bringing about harmfulness (Nordberg *et al.*, 2011).

The objective of the present investigation is to check the adequacy of a bio-marker based technique including the examination of honey bee *A. mellifera* for the assurance of the natural contamination with overwhelming metals by looking at information acquired by various testing locales in various Governorates in Egypt.

Materials and methods

Study was carried out in laboratory of healthy ministry in 2019. Honey bee samples were collected from different Governorate`s apiaries Upper, Egypt. with 40 km intervallic distances between each apiary.

1. Samples:

Samples were collected from Giza, Beni-Suef, Asyut, Aswan, and New Valley.

2.Apparatuses:

Balance**,** microwave, digestive and micro plasma [Inductively Coupled Plasma (ICP)]

3.Procedure:

Samples were collected into plastic packages using gloves, were stored at –18°C before analyses and were dried in microwave for 10 minute at 120 °C. Dried bees (50 g) were placed into vessels with 8 ml of nitric acid (HNO3) and 2 ml of hydrogen peroxide (H_2O_2) put in digestive per one hour then leaved it cold for 24 hours, samples were filtrated through 1–2.5 μm filter paper and brought to a final volume of 25 ml with distilled water. Then analysis to determine the following heavy metal (Boron-Zinc-Ferric-Nickel-Lead-Molybdenum-Cadmium-Copper-Menganese- Chromium) in micro plasma [Inductively Coupled Plasma (ICP)], is highly sensitive and capable of multi-element trace analysis and ultra-trace analysis, often at the parts-per-trillion level. Testing for trace elements can be performed on a range of materials from super alloys to high purity materials.

Mode of action that coupling to mass spectrometry, the ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration. The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards inductively coupled

plasma mass spectrometry (ICP-MS). Also lends itself to quantitative determinations through isotope dilution, a single point method based on an isotopically enriched standard.

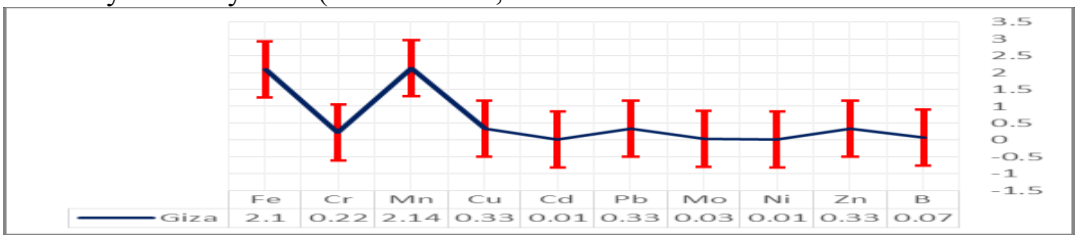
Other mass analysers coupled to ICP systems include double focusing magnetic-electrostatic sector systems with both single and multiple collector, as well as time of flight systems
(both axial and orthogonal accelerators have been used). The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards. ICP-MS also lends itself to quantitative determinations through isotope dilution, a single point method based on an isotopically enriched standard. Ceased to be discharged, the excess nitric acid was removed by increasing the temperature to 100°C. The solution was carefully evaporated until the perchloric acid began to evaporate. The solution was then cooled, 10 ml of distilled water was added, and the mixture was filtrated through a 1–2.5 µm filter paper and brought to a final volume of 25 ml with distilled water.

Microelements in the samples were quantified by atom-absorption spectrometry with an Analyst-400 system (Perkin-Elmer, Waltham, MA, USA) with flame atomization. The following heavy metals were analyzed: Boron (B), zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe). All samples were analyzed in duplicate.

Results and discussion

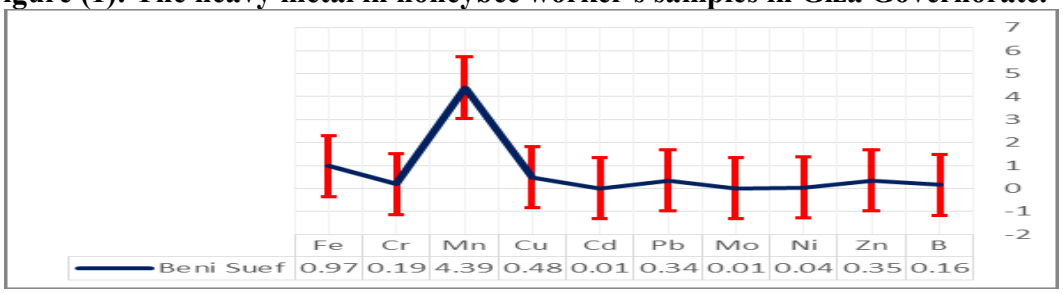
Data in Figure (1) showed the heavy metal (ppm) in honeybee workers samples in Giza Governorate. It's recorded the high value of heavy metal at manganese **(**2.14) while the nickel and cadmium recorded the low value (0.01).

Data in Figure (2) showed the heavy metal (ppm) in honeybee workers samples in Beni-Suef Governorate. It's recorded the high value of heavy metal at manganese (4.39) while the low value was recorded at the cadmium and molybdenum (0.1). Data in Figure (3) showed the heavy metal (ppm) in honey bee workers samples in Asyut Governorate. It's recorded the high value of heavy metal at ferric **(**1.45) while the nickel and cadmium recorded the low value (0).



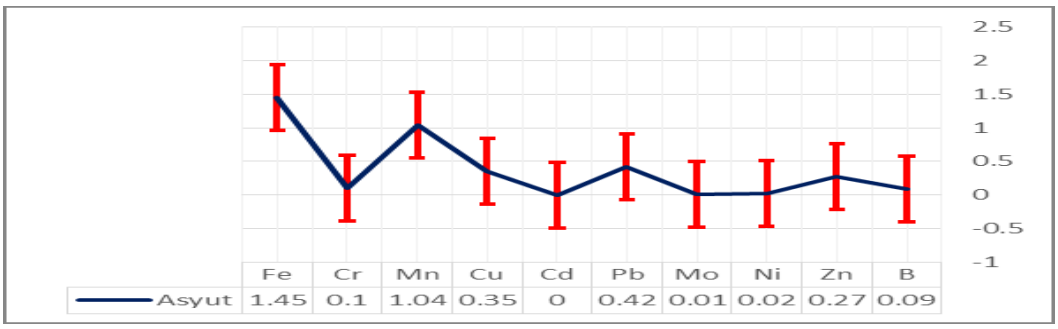
Boron (B), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

Figure (1): The heavy metal in honeybee worker's samples in Giza Governorate.



Boron (b), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

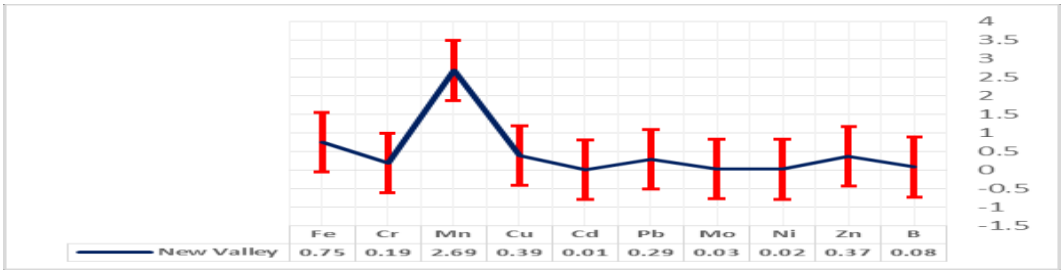
Figure (2): The heavy metal in honeybee worker's samples in Beni-Suef Governorate.



Boron (b), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

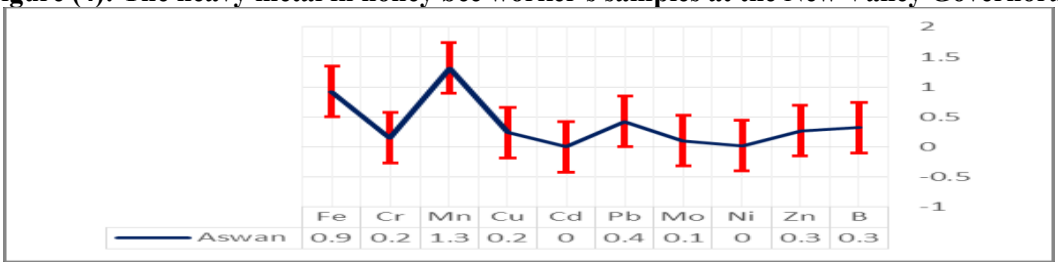
Figure (3): The heavy metal in honeybee worker's samples in Asyut Governorate.

Data in Figure (4) showed the heavy metal in honeybee workers samples in New Valley Governorate. It's recorded the high value of heavy metal at manganese (2.69) while the low value was recorded at the cadmium (0.01). Data in Figure (5) showed the heavy metal (ppm) in honeybee workers samples in Aswan Governorate. It's recorded the high value of heavy metal at manganese (1.3) while the cadmium and nickel recorded the low value (0).



Boron (b), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

Figure (4): The heavy metal in honey bee worker's samples at the New Valley Governorate.



Boron (b), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

Figure (5): The heavy metal in honey bee worker's samples in Aswan Governorate.

From the obtained results showed in Table (1) it could be summarized that, the high boron concentration was (-0.18 ppm) founded at Aswan. As the low was (-0.43 ppm) founded at Giza. While the high concentration of zinc was (0.36 ppm) founded at New Valley. Whereas the low concentration was (0.26 ppm) founded at Asyut and Aswan. But The high concentration of nickel was (0.02 ppm) founded at Beni-Suef . As the low one was (-0.01 ppm) founded at Giza. The high molybdenum concentration was (0.03) founded at Aswan. As the low concentration was (-0.06 ppm) founded at Beni-Suef and Asyut. The high lead concentration was (0.41 ppm) founded at Asyut. As the low one was (0.28 ppm) founded at New Valley. The cadmium high concentration was (0.009 ppm) recorded the at Giza, Beni-Suef and New Valley. As the low was (-0.001 ppm) founded at Asyut and Aswan's workers body. The high concentration of copper was (0.47 ppm) founded at Beni Suef's . As the low was

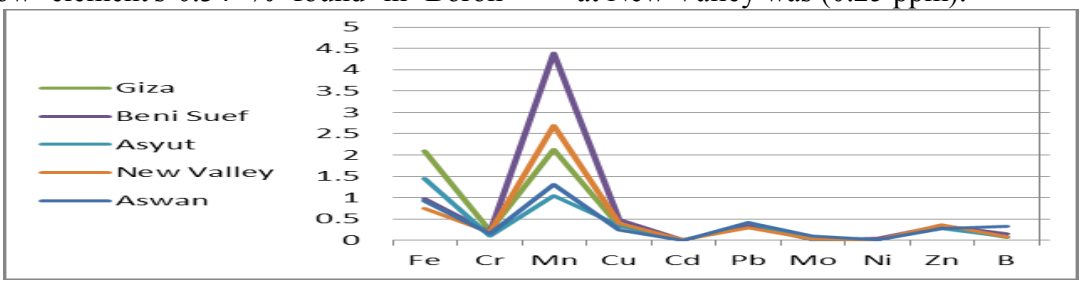
(0.23 ppm) founded at Aswan's workers body. The manganese high concentration was (4.37 ppm) founded at Beni-Suef. As the low concentration was (1.02 ppm) founded at Asyut. The chromium recorded high concentration at Giza, it was (0.17 ppm). As the low concentration was (0.05 ppm) founded at Asyut. The high concentration of Ferric founded at Giza was (1.6 ppm). As the low founded at New Valley was (0.25 ppm).

Table (1): Heavy metal percentage in honey bee worker's samples from some Upper Egyptian Governorates.

Governorates Differences	B	Zn	Ni	Mo	Pb	Cd	Cu	Mn	Cr	Fe	mean
Standards	0.5	0.01	0.02	0.07	0.01	0.001	0.01	0.02	0.05	0.5	0.119
Giza	-0.43	0.32	-0.01	-0.04	0.32	0.009	0.32	2.12	0.17	1.6	0.438
Beni –Suef	-0.34	0.34	0.02	-0.06	0.33	0.009	0.47	4.37	0.14	0.47	0.575
Asyut	-0.41	0.26	0	-0.06	0.41	-0.001	0.34	1.02	0.05	0.95	0.256
New Valley	-0.42	0.36	0	-0.04	0.28	0.009	0.38	2.67	0.14	0.25	0.363
Aswan	-0.18	0.26	0	0.03	0.41	-0.001	0.23	1.29	0.1	0.42	0.256
Mean	-0.356	0.308	0.002	-0.034	0.35	0.005	0.348	2.294	0.12	0.738	

Boron (b), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

From the obtained results showed in Figure (6), it could be summarized that,the Giza Governorate has manganese was the high concentration (2.12 ppm). But the low element's was (-0.43 ppm) found in boron (less than the Egyptian's stander). The manganese recorded high concentration at Beni-Suef Governorate was (4.37 ppm) . As the low element's-0.34 % found in Boron (less than the Egyptian's stander). However, the Asyut Governorate record that the high concentration was (1.02 ppm) founded in manganese (more than the Egyptian's stander). As the low one was (-0.41 ppm)(less than the Egyptian's stander). The high concentration of manganese founded at Aswan was (1.29 ppm). As the low founded at New Valley was (0.25 ppm).



Boron (b), Zink (zn), nickel (Ni), molybdenum (Mo), lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), chromium (Cr) and iron (Fe)

Figure (6): Heavy metal in honey bee worker's samples from several Upper Egyptian Governorates.

From the obtained data the following conclusion can be recommended; the most suitable Upper Egypt treatments Governorates for breeding honeybee was Asyut and Aswan. On the other hand, the Beni-Suef Governorate not recommended for breeding honey bee. The most pollution element was manganese and the less pollution was molybdenum.

Meany authors discussed this question and found that, lead accumulation happens slowly in young bees which feed mainly on pollen and is equally efficient when the contamination begins at foraging bees (Zhelyazkova *et al.,* 2004). The high content of cadmium on the surface of bees may occur as a result of very occasional atmospheric contamination, especially when the samples are not collected in the vicinity of industrial

areas (Perugini *et al*., 2011 and Lambert *et al*., 2012).

Porrini *et al.* (2003) indicated similar trends for distribution of lead and chromium, which are in higher concentrations on the surface than inside the body. It results from the fact that large part of chromium present in the environment is in the atmosphere (Seigneur and Constantinou, 1995).

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The relationship between formulation of insecticides and droplet distribution of cetain ground spraying equipment and controlling *Heteracris annulosa* (Orthoptera: Acrididae) infesting alfalfa plants

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Abstract:

Field experiments were carried out in an area of about 1.1 feddans planted with alfalfa *Medicago sativa* during season of 2019 in 5th September at El-Baharia Oasis, Western Desert, Giza Governorate. The selected area was split into 7 plots including control plot. Chlorpyrifos EC and U.L.V., deltamthrin E.C. and U.L.V., spinosad and chlorpyrifos +lufenuron were sprayed with recommended rate and one treatment left without spraying as un treated check by using motorized knapsack sprayer (solo) (52.5 L./fed.) and economy micron ULVA + sprayer (3.15 L/fed.). Data indicated that, all tested compounds induce a significant negative influence on *Heteracris annulosa* (Walker) (Orthoptera: Acrididae) nymphs survival. The most effective compounds is deltamthrin E.C. and chlorpyrifos E.C. followed by other compounds. It could be recommended to use those compounds with LV spraying equipment with not less than (3.15L/fed.).The data showed that motorized knapsack sprayer (solo) (52.5 L./fed.) was the most efficient equipment to control the 4th, 5th instar nymphs of *H. annulosa* on alfalfa ha plants.The rate of performance of motorized knapsack sprayer (solo) was 15.2 fed./day. But the lowest rate of performance was recorded with economy micron ULVA + sprayer since it could spray only 9.1 fed. /day.

Introduction

Locusts and grasshoppers (Orthoptera: Acrididae) were considered as a serious agricultural pests that cause considerable damage to different crops in Africa especialy during outbreaks (Showler, 1993). The major insect pests in Western Desert of Egypt were the species of family Acrididae. In the region of El-Baharia Oasis, it was found that the berseem grasshopper ***Heteracris annulosa*** **(**Walker**)** (Orthoptera: Acrididae) was the most dominant insect pest in this area. Much attention has been focused in compounds which disrupt the normal process of insect development. They are known as Insect Growth Regulators (IGR's). The use of bio agents to control pests has been known and practiced for a long time. In Egypt, majority of interest was directed to the type, dosage of insecticides used, while a lesser attention was given to the application methods. A comparative studies on the efficiency of certain ground sprayers was carried out by

(Hindy, 1992) , who recorded significant variation in the spray deposit due to arrangement of the nozzles, spray technique and rate of application. The world attention was directed to minimization of spraying volumes and the costs of control pests which may be achieved by using a cheap and effective insecticide or using developmental ground spraying technique with low cost of application per feddan (Magdoline *et al*. , 1992 and Matthews, 1992). The present work aimed to determine the most effective insecticide, formulation and equipment controlling *H. annulosa* on alfalfa plants.

Materials and methods

1. Tested compounds:

1.1. Chlorpyrifos - (Renocam®48% E.C.) 1 Liter/fed., Acetylcholinesterase inhibitor.
1.2. Chlorpyrifos – (Locban 45% U.L.V.) 400 cm^3\fed., Acetylcholinesterase inhibitor.
1.3. Deltamthrin (Deltafan®1.25% U.L.V.)400 cm^3\fed. (Axonic excitotoxins).
1.4. Deltamthrin (Kafrothrin ®2.5% E.C.)350 cm^3\fed. (Axonic excitotoxins).
1.5. Spinosad (Tracer ®24% S.C.) 50 cm /fed., Neuronal excitation.
1.6. Chlorpyrifos 25%+Lufenuron 5% (Tembo AX®30%E.C.)500cm^3\fed., Acetylcholinesterase inhibitor+Chitin synthesis inhibitor.

2. Spraying equipment tested on alfalfa plants:-

Two ground application machines were selected to perform the scope of this work as follows:
2.1. Economy micron ULVA + sprayer (3.15L./fed.).
2.2. Motorized knapsack sprayer (solo) (52.2 L./fed.) .
As commonly used equipment in applying pesticides on alfalfa plants, the tested equipment could be represented according to the technical categorization mentioned in Table (1). Calculations of productivity and rate of performance were recorded as described by **Hindy (1992).**

3. Execution of field experiments:

3.1. Arrangements of the experiments:

Field experiments were carried out during season 2019 on 5thSeptember in private alfalfa (*Medicago sativa*) field , located at El-Baharia Oasis, Western Desert, Giza Governorate. The experiments were done under local meteorological conditions of 29±2ºC average temperature, 35% average RH.% and 4-6 m/sec. average wind velocity , at 7 am. The selected area of 1.1 feddans was split into 7 plots including control plot , each plot about (35 x 15) = 525 m^2. The plots were isolated by a wide belt of 10 X 15 = 150 m^2 as barrier zones to avoid pesticide drift. Plots laying up wind of treatments were used as a control. The untreated cheek plot were not sprayed, suitable infested sites with the grasshoppers were selected at valley El-Baharia Oasis. These sites were characterized by high population density of grasshoppers (more than 15 insect/ m^2). The studied nymphs were 4th, 5th instars. Numbers of grasshoppers were collected, counted and placed in each cage.

Spraying operations have not been done with any insecticides before execution the field experiment. The experimental fields were sprayed with recommendeded rate of chloropyrifos E.C. and U.L.V., deltamthrin E.C. and U.L.V., spinosad and chloropyrifos + lufenuron, respectively. The spraying was done between 7 and 10 am in the morning.

3.2. Bioassay procedure:

To define *H. annulosa* nymphs numbers, each treatment was represented by three replicate cages 0.5m X 0.5m The insects were collected randomly from the same treatment after application directly by using sweep-net and placed 30 insects in each cage. The cages were kept and fed with treated plants (alfalfa) to the insects treated. Mortality counts were after different period of treatment, i.e., 1, 6, 12, 24 and 48 hrs. post treatment to insecticide treatments. Assessments: in cages daily, routine work includes removing the previous uneaten food, faces and dead nymphs and counting the

living insects before introducing the fresh food.

3.3. Phytotoxic effect:

It was determined by recording any color change, leaf curling or flaming up to 8 days after spraying, according to Badr *et al*. (1995).

3.4. Calculation and data analysis:

3.4.1. The percentage of reduction in the field experiment was calculated according to Henderson and Tilton (1955).

3.4.2. Statistical analysis of results was done according to SAS (1996) for biological studies: Duncan's for biological evaluation of insecticides in field.

4. Calibration and performance adjustment of the tested equipment:

4.1. Collection of spray deposit:

Before spraying each **alfalfa** field treatments, water sensitive paper size 26x76 mm. developed by (Novartis®) were hanged on **alfalfa** plants and on ground selected on parallel position to the ground wire collectors, Hindy (1992) at about one meter between two adjusted plants in order to estimate the spray lost on the ground between plants in diagonal line through the tested field. Measurements of size and number of spots were carried out by means of a scaled monocular (Strüben)® (X15) lens. All necessary corrections and calculations connected with such technique of measurements and determination of droplets were conducted according to Anonymous (1978). Sizing of droplets is a necessary and frequent routine procedure for the assessment of agricultural spray applications (Johnstone and Huntington, 1970). The spread factor of used sensitive paper was 2.2 (Ciba Geigy, 1990).

Table (1): Techno-Operational data of certain ground sprayers applied on alfalfa during season, 2019.

Items	Motorized knapsack (solo) sprayer	Hand-held spining disc ULVA^{+} sprayer
Type of atomization	Mechanical Pneumatic	Centrifugal (Rotary disc)
Nozzle type	Pneumatic-Flow rate 3	One restirector (Red)
Pump type	Centrifugal fan	-
Number of nozzles	1	1
Pressure (bar)	-	-
Spray tank (L.)	20	1+10
Rate of application (L/fed.)	52.5	3.15
Working speed (Km/h.)	2.4	2.4
Swath width (m.)	5	3
Flow rate (L/min.)	2.5	0.09
Spray height (m.)	0.5	0.5
Type of Spraying	Target in all sprayres	
Productivity * (fed./h.)	2.86	1.7
Rate of performance* (fed./day)	15.2	9.1

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

*** Calculations of productivity and rate of performance.**

***Number of workers =2.**

Results and discussion

1. Bioresidual activity of chlorpyrifos (U.L.V.) against *Heteracris annulosa* on alfalfa plants:

Efficiency of chlorpyrifos (U.L.V.) represented as mortality percentages after 1 and 2 days of treatments was indicated in Tables (2 and 4) .The general mean reduction% in population of *H. annulosa* 4 th ^{a}nd 5th instar nymphs was 77.3 using Economy Micron ULVA^{+} sprayer (3.15L./fed.). The droplet size was 143 µm and N/cm^{2} was 155 for recommended rate sprayed with economy micron ULVA^{+}sprayer (3.15L./fed.).

2. Bioresidual activity of chlorpyrifos (E.C.) against *Heteracris annulosa* on alfalfa plants:

Efficiency of chlorpyrifos (E.C.) represented as mortality percentages after 1 and 2 days of treatments was indicated in Tables (2 and 4). The general mean reduction% in population of *H. annulosa* 4 th and 5th instar nymphs was78 using motorized knapsack sprayer (solo)(52.2 L./fed.).The droplet size was 159 µm and N/cm^2 was 140 for recommendeded rate sprayed motorized knapsack sprayer (solo)(52.2 L./fed.) .

3. Bioresidual activity of spinosad against *Heteracris annulosa* on alfalfa plants:

Efficiency of spinosad (E.C.) represented as mortality percentages after 1 and 2 days of treatments was indicated in Tables (2 and 4) .The general mean reduction% in population of *H. annulosa* 4 th and 5th instar nymphs was 63.2 using motorized knapsack sprayer (solo)(52.2 L./fed.).The droplet size was 146 µm and N/cm^2 was 130 for recommendeded rate sprayed motorized knapsack sprayer (solo) (52.2 L./fed.)

4. Bioresidual activity of deltamethrin (U.L.V.) against *Heteracris annulosa* on alfalfa plants:

Efficiency of deltamethrin (U.L.V.) represented as mortality percentages after 1 and 2 days of treatments was indicated in Tables (2 and 4). The general mean reduction% in population of *H. annulosa* 4 th and 5th instar nymphs was 70 using economy micron ULVA$^+$ sprayer (3.15L./fed.).The droplet size was 150 µm and N/cm^2 was 160 for recommendeded rate sprayed with economy micron ULVA$^+$sprayer (3.15L./fed.).

5. Bioresidual activity of deltamethrin (E.C.) against *Heteracris annulosa* on alfalfa plants:

Efficiency of deltamethrin (E.C.) represented as mortality percentages after 1 and 2 days of treatments was indicated in Tables (2 and 4). The general mean reduction % in population of *H. annulosa* 4 th and 5th instar nymphs was 85 using motorized knapsack sprayer (solo) (52.2 L./fed.). The droplet size was 125 µm and N/cm^2 was 127 for recommendeded rate sprayed motorized knapsack sprayer (Solo)(52.2 L./fed.) .

6. Bioresidual activity of chlorpyrifos + iufenuron against *Heteracris annulosa* on alfalfa plants:

Efficiency of chlorpyrifos+ lufenuron (E.C.) represented as mortality percentages after 1 and 2 days of treatments was indicated in Tables (2 and 4) .The general mean reduction% in population of *H. annulosa* 4 th and 5th instar nymphs was 62 using motorized knapsack sprayer (solo) (52.2 L./fed.).The droplet size was 141 µm and N/cm^2 was 135 for recommendeded rate sprayed motorized knapsack sprayer (solo)(52.2 L./fed.) .

Table (2): The relation between droplet distributions obtained by the tested ground spraying equipment and the corresponding mortality of 4th and 5th instar nymphs of *Heteracris annulosa*, using the total recommended rate of insecticides on alfalfa plants during season 2019 in Giza Governorate.

Insecticide	Tested sprayer and formulation and dose rate\fed.	VMDµm	N / cm^2	% Mortality	
				Initial	Residual
Chlorpyrifos	Micron ULVA$^+$(U.L.V.) (400cm^3)	143	155	90	100
	Solo (sprayer)(E.C.)(1 L.)	159	140	96.6	100
Deltamethrin	Micron ULVA$^+$(U.L.V.) (400cm^3)	150	160	80	83.3
	Solo (sprayer)(E.C.) (350cm^3)	125	127	100	100
Spinosad	Solo (sprayer)(E.C.) (50cm^3/fed.)	146	130	76.6	83.3
Lufenuron+Chlorpyrifos	Solo (sprayer)(E.C.) (500cm^3)	141	135	80	83.3

VMD = Volume Mean Diameter. N / cm^2 = Number of droplets per square centimeter

Data in Table (3) showed that there were a negative correlation between lost spray on ground equipment and the bioresidual activity of insecticides used.

7. Economy micron ULVA^{+} sprayer (3.15 L/fed) :

Data in Table (3) showed that the lost spray percentages were 4.3 and 4.8 % from the total spray volume in the case of chlorpyrifos and deltamethrin.The general mean reduction% in population of *H. annulosa* 4 th and 5th instar nymphs were 77.3 and 70 % at total recommendeded rate, respectively.

8. **Motorized knapsak (solo) sprayer(52.5L/fed):**

Data in Table (3) showed that the lost spray percentages were 9 ,9.3 , 9.7 and 10 % from the total spray volume in the case of chlorpyrifos , deltamethrin, spinosad and chlorpyrifos+ lufenuron.The general mean reduction% in population of *H. annulosa* 4 th and 5th instar nymphs were 78 , 85 , 63.2 and 62 % at total recommended rate, respectively.

9. Relationship between the tested chemicals, formulation, techniques and the mortality percentages of *Heteracris annulosa* on alfalfa plants:

9.1. Bioassay evaluation:

Tables (2 ,3 and 4) showed that, the percentages of reduction of *H. annulosa* 4 th and 5th instar nymphs on alfalfa plants affected by certain insecticides sprayed with certain ground application techniques during the season of 2019 using total recommendeded rate. The productivity of motorized knapsack sprayer (solo) (52.2 L./ fed.) sprayer was 15.2 fed./day. Reviewing the obtained result it could be proved that solo achieved the superior equipment, but the lowest productivity was recorded with economy micron ULVA^{+} sprayer (3.15L./fed.) since it could spray only 9.1 fed./day.

Table (3): Lost spray on ground, as produced by low volume ground spraying equipment, by using certain insecticides at total recommendeded rate against 4th and 5th instar nymphs of *Heteracris annulosa* during season (2019).

Insecticide	Tested sprayer&Formulation and spray volume (L / fed.)	*N/ cm^2 of total spray droplets	N / cm^2 droplets (on ground)	% N/cm^2 (ground) ——— x 100 N/Cm^2 (Plants+ground)	% Mortality	
					Initial	Residual
Chlorpyrifos	Micron ULVA^{+}(U.L.V.)(3.15)	162	7	4.3	90	100
	Solo (sprayer)(E.C.)(52.5)	154	14	9	96.6	100
Deltamethrin	Micron ULVA^{+}(U.L.V.)(3.15)	168	8	4.8	80	83.3
	Solo (sprayer)(E.C.)(52.5)	140	13	9.3	100	100
Spinosad	Solo (sprayer)(E.C.)(52.5)	144	14	9.7	76.6	83.3
Lufenuron+Chlorpyrifos	Solo (sprayer)(E.C.)(52.5)	150	15	10	80	83.3

N / cm^2 = Number of droplets per square centimeter. * On alfalfa **plants and lost spray on ground**

Statistical analysis showed that, there was a significant differences between both the distribution percentages of droplet sizes (LSD= 9.405 for levels and 16.29 for compounds), for the droplets number/cm^2 (LSD= 5.8188 for levels and 10.078 for compounds) and for reduction percentages (LSD=2.5739 for compounds).

Table (4): Reduction percentages in *Heteracris annulosa* nymphs affected by insecticides sprayed with certain ground equipment during season 2019.

Treatments	Pretreat-ment	Post 1		Post 6 hours		Post 12		Post 24		Post 48		General	
		C	R %	C	R %	C	R %	C	R %	C	R %	C	R %
Chlorpyrifos EC (1L/fed.)	30	15	50	11	63.3	5	83.3	3	90	0	100	6.8	77.3
ChlorpyrifosULV (400cm3/fed.)	30	22	26.6	8	73.3	2	93.3	1	96.6	0	100	6.6	78
Spinosad (50cm3/fed.)	30	26	13.3	17	43.3	13	56.6	7	76.6	5	83.3	16.3	63.2
Deltamthrin E.C.(350 cm3/fed.)	30	10	66.6	5	83.3	3	90	0	100	_	_	4.5	85
Deltamthrin U.L.V.(400 cm3/fed.)	30	17	43.3	10	66.6	7	76.6	6	80	5	83.3	9	70
Chlorpyrifos+LufenuronE. C.30% (50cm3/fed.)	30	27	10	12	60	7	76.6	6	80	5	83.3	11.4	62
Untreated (control)	30	30	_	30	_	30	_	30	_	30	_	30	_

C = Count of life nymphs after treatment. R = % Reduction of nymphs

Field experiment was carried out on infested area with grasshopper *H. annulosa* nymph sat early season on alfalfa. For evaluation the field performance of low-volume spraying machines, motorized knapsack sprayer (solo) (52.5 L/fed.) and ultra low volume economy micron ULVA^{+} sprayer (3.15L./fed.); to spray chlorpyrifos E.C. and U.L.V.; deltamthrin E.C. and U.L.V.; spinosad and chlorpyrifos + lufenuron, with full recommended dose. A satisfactory coverage was obtained on alfalfa plants , the droplets spectrum was obtained in field experiment was agreed with the optimum droplet sizes which mentioned by Himel (1969).

The best obtained result was (52,5 L/fed.) as spray volume and droplet spectrum were 144μm and 141 droplets/cm^2 , these results agreed with (Himel and Moore, 1969) in the optimum droplet size to control insects in fields by ground equipment. Deltamthrin E.C. and chlorpyrifos E.C. revealed the best bioefficiacy results with motorized knapsack sprayer (solo) (52.5 L/fed.) followed by the other compounds and these results agreed with Hindy *et al.* (2004). Genidy *et al.* (2005) recommended KZ oil and pyriproxyfen followed by agerin by 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 motorized knapsack sprayer(20L/fed.) and Morsy *et al.*(2015) whom recommended using carbosolvan, acetamiprid and deltamethrin with low volume machines not less than (15 L/fed.). Also Dar (2016), recommended whenever using lufenuron followed by spinosad in controlling cotton leafworm on clover with low volume machines. Acetamiprid, thiaclopride, thiamethoxam, profenofos, flupyradifurone, revealed successeful results in controlling both *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) , *Empoasca decipiens* (Paoli) (Hemiptera: Cicadellidae) nymphs . Dar (2019) and Dar *et al*. (2019) whom achived best control results, spray volume per feddan , productivity and rate of performance with motorized knapsack sprayers.

Finnally, it could be recommended that using those compounds with LV spraying equipment with not less than (52.5 L./fed.). The data showed that motorized knapsack sprayer (solo) (52.5 L. fed.) was the best equipment to control *H. annulosa* on alfalfa. The rate of performance of motorized knapsack sprayer (solo) was 15.2 fed./day, it was the best equipment, the low spray volume and the low percentages of lost

spraying between plants about 9.5%, but the lowest rate of performance was economy micron ULVA^{+} sprayer (3.15L./fed.); since it could spraying only 9.1 fed./day and spray lost about 4.5% , these results were agreed with Hindy *et al.* (1997), they mentioned that, there was a positive correlation relationship between rate of application and lost spray on ground. There was a negative complete correlation between droplet sizes and the mean residual of mortality of *H. annulosa* nymphs and while there was a positive complete correlate between N/cm² and the mean residual of mortality of *H. annulosa* nymphs in all treatments.

It could be concluded that, using deltamthrin E.C. and chlorpyrifos E.C. followed by other compounds with low volume (LV) ground spraying equipment with not less than (3, 15 L. /fed.) by using recommended doses which revealed successful management against grasshoppers on alfalfa under our local conditions.

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Comparison between electrostatic spraying and traditional ground spraying techniques by using certain insecticides on *Thrips tabaci* (Thysanoptera: Thripidae) infesting onion in Qalubiya Governorate

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Abstract:

The present work was carried out to determine quality, spray deposited on the plant, lost spraying by drift and lost spraying on ground between plants. As well as the biological efficiency produced with possibility of using the least amount of pesticides to reach the highest efficiency against controlling *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) on onion crop. The used ground equipment were knapsack motor mist blower sprayer with shear unit (79 L/fed.), knapsack blower sprayer with electrostatic charging unit (42 L/fed.), rotary hand held sprayer (18 L/fed.), knapsack hydraulic hand held sprayer (56 L/fed.) and conventional ground motor sprayer with two spray guns (578 L/fed.). Marshal insecticide was used for controlling onion thrips (*T. tabaci*) infesting onion fields with recommended dose during season 2017. In the second season 2018 marshal and chinook insecticides were used with recommended doses and 3/4 recommended dose during season 2018. In the second season 2018 experimental results showed that, the highest mortality rate for *T. tabaci* infesting onion was revealed by knapsack motor mist blower with electrostatic unit spraying 95 % followed by knapsack mist blower motor sprayer with its shear unit, rotary hand held sprayer, knapsack hydraulic hand held sprayer and conventional ground motor sprayer with spray gun were 93, 91, 77 and 72 %, respectively. The lowest drift spray was done from electrostatic knapsack motor sprayer 42 L/fed. and the highest drift spray was done from rotary sprayer 18 L/fed. Conventional ground motor sprayer revealed the worst equipment in lost spraying in ground about 44% from spraying volume was lost on ground, but the best equipment saving lost spraying on ground was electrostatic Agrimondo 15.5% from spraying volume; also revealed 20% from droplets deposition on both sides of onion leaves, also pneumatic knapsack sprayer with electrostatic unit revealed a lowest drift spray but the highest equipment revealed drift spray was rotary spinning disk matabi.

Introduction

Onion had been one of the most important crops grown in Egypt for domestic use and exportation. The total area of onion were (113118 fed.) with a total production of (1504081) tons with an average of (13.3) tons per fed. Economic Affairs Sector Ministry of Agriculture (2010/2011). The volume of exports of onion crop was 669,358 thousand tons, which was equivalent to 19.4% of the total exports of the European Union as the average period (2009-2013) (Abdel- Hamid *et al*., 2016). Onion had been one of the important crops in the medical industry. Onion was important source of the rapetic agents containing active principles mainly in the form of cysteine derivatives. It contains enzymes acting possess and diabetic (Augusti, 1996). Onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) had been a key insect pest of onion and other *Allium* species in many parts of the world (Lewis ,1997). *T. tabaci* was destructive, polyphagus pest of agricultural and other economically important crop plants (Sathe and Mithari, 2015). Thrips had been among the most important agricultural pests globally because of the damage inflicted by their oviposition, feeding and ability to transmit plant viruses (Stuart *et al.,* 2011). High populations of onion thrips can damage the leaves of onions (*Allium cepa* L.) in the field resulting drastic reducing in crop yields (Edelson *et al*., 1989). The climate condations played major role in increasing or decreasing the number of onion thrips in the field (Domiciano *et al.*, 1993). Thrips population correlated negatively with relative humidity and positively with temperature. A population of 10 thrips/plant and temperature around 29ºC coupled with dry season could cause serious damages to the onion crop. Due to climate changes occurring in the world, the temperature in Egypt during the onion season exceeds 30 ºC as the average temperature during the season so we can say that thrips is permanent pest according to our climate condations. Farmers were using chemical control as an integrated pest control against onion thrips. The using chemical control without awareness caused pollution to the environment, humans and farm animals (Foqué *et al*., 2012). Many growers also erroneously believing that high spray volumes and pressures had been need to obtain good plant protection. Therefore, we should studied the traditional and modern spraying machines to determine the optimum droplet size of the appropriate spray volume and the ideal spray coverage on the onion crop. The reasons to be achieved to ensure proper chemical control with less spray volume, reduced the loss as of pesticides on the ground and drifting spray, through testing certain recent ground equipment with comparison of traditional ground sprayers.

Materials and methods

1. Qualitative spraying techniques:

1.1. Field experiment and sampling:

Field experiments were conducted over two sucssive seasons. Second season field experiments were carried out during season 2018 on 16th March in private onion field located at Qaha, Qalyubiya Governorate. The onion cultivated was (*Allium cepa* L.). The experiments were done under local meteorological conditions of 22ºC temperature, relative humidity 75% RH. and 2 m/sec.wind velocity. The selected areas of 2.0 feddans were split into 20.0 plots and control plot. The areas of each plot were about 400m², spraying operations have not been done with any insecticides before execution the field experiment. The experimental field was divided into ten plots were sprayed with recommended dose rate, ten plots were sprayed with ¾ recommended dose rate and one treatment left without spraying as a control with two insecticides (Marshal 25% WP) common name carbosulfan were sprayed at the recommended rate (150 gm. / 100 L.) and 3/4 recommended rate (112.5 gm. / 100 L) and (Chinook 35% SC) common name imidacloprid were sprayed at the

recommended rate (250 cm. / 100 L.) and 3/4 recommended rate (187.5 gm. / 100 L) . The spraying equipment were rotary hand held sprayer (Spinning-disc, MATABI)® (18 L/fed), knapsack motor mist blower sprayer with electrostatic (Spectrum electrostatic 3010 head)® (42 L/fed.), knapsack hydraulic hand held sprayer (MATABI)® with the nozzle types, 4 holes hollow cone nozzles (56 L/fed.), knapsack mist blower sprayer (AGRIMONDO)® with (shear nozzle) i.e normal unit (79 L/fed.) and conventional ground motor sprayer with spray gun (Wisconson)® ground motor (578 L/fed.), respectively.

1.2. Calibration and performance adjustment of the tested equipment:

The calibration of the equipment used in spraying application was done in the laboratory to fulfill the technical needs of the required field tests, the program of calibration tests for ground spraying equipment suggested by **Gabir (1995)** was applied as follows:

$$Q = \frac{T \times Rw \times Vo}{252}$$

Where:

Q=Flow rate (L/ min.).
T=Spraying Volume (L/ fed.).
Rw=Effective run width (m.).
Vo=Working speed (Km/h)
252=Constant value

and measure the swath width, using water sensitive papers (Novartis cards)® , with a minimum spot diameter of 100 micron (VMD) was calculated with each of the tested sprayers at 0.5 meter as spray height and average walking speed 2.4 km/h i.e 40.0 meter/min. Daily rate of performance fed. /day was counted as the following equation by Hindy (1992).

$$\text{productivity (fed/h.)} = \frac{60 \times \textit{swath width}(m) \times \text{working speed (m/min)}}{4200}$$

Where:

4200 (m²) = the area of one feddan.
60= Constant number to turn the values from minutes to hours.

Rate of performance (fed. /day.) =Productivity (fed. /h.)×*2/3×*6 hours.

Where:

*2/3= The time of actual spraying minus from it the time consumed in going and returning to the field during the spraying and the time consumed in feeding spray solution in spray tank
*6 hours = number of daily spraying working hour.

I.3. Quantity of insecticides:

Out of Techno- operational data of ground application techniques used in Table (1) determined the quantity of insecticides (Marshal 25% WP) and (Chinook 35%SC) with recommended doses and 3/4 recommended doses per feddan for each unit to applied spraying on onion field to control the (*T. tabaci*) data in Tables (2 and 3).

Table (1): Techno- operational data of some ground application techniques used against controlling *Thrips tabaci* on onion crop during season 2018.

Equipment	Tank capacity(L)	Spray volume (L/fed)	Flow rate (L/min)	Swath width (m)	Productivity (fed/h)*	Rate of performance (fed/day)*
Rotary sprayer	1	18	0.172	1.0	0.57	2.3
Knapsack motor sprayer with electrostatic unit	20	42	2.0	5.0	2.9	11.6
Hydraulic sprayer	20	56	0.807	1.5	0.86	3.4
Knapsack blower sprayer with normal unit	20	79	3.75	5.0	2.9	11.6
Conventioal ground motor sprayer	600	578	11	2.0	1.1	4.4
Type of Spraying	**Target spraying in all treatments**					

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

Table (2): Quantity of insecticide for used (Marshal 25% WP) with recommended doses and 3/4 recommended doses used against controlling *Thrips tabaci* on onion crop during season 2018.

Equipment	Quantity of insecticide (gm. / fed.) recommended doses	Quantity of insecticide (gm./ fed.) 3/4 recommended doses
Rotary sprayer	30	22.5
Knapsack motor sprayer with electrostatic unit	66	49.5
Hydraulic sprayer	84	63
Knapsack blower sprayer with normal unit	120	90
Conventional ground motor sprayer	**882**	**661.5**

Table (3): Quantity of insecticide used (Chinook 35% SC) with recommended doses and 3/4 recommended doses used against controlling *Thrips tabaci* on onion crop during season 2018.

Equipment	Quantity of insecticide (cm. / fed.) recommended doses	Quantity of insecticide (cm. / fed.) 3/4 recommended doses
Rotary sprayer	53	40
Knapsack motor sprayer with electrostatic unit	105	79
Hydraulic sprayer	136.5	102
Knapsack blower sprayer with normal unit	210	158
Conventional ground motor sprayer	1449	1087

I.4. Collection and measurement of lost spray on ground:

Before spraying each onion field treatment, a sampling line was consisted of five wire holders fixed in diagonal line with distance (2.0) meters between each holders inside each treatment to collect spraying chemicals between plants; each wire holder top has a water sensitive paper (Novarits cards)® on it. Also each five onion plants, was put at distances the water sensitive paper cards at the same height as the onion plant and on the ground to calculate the spray droplets slipping from the leaves of the onion plants. Receptors were fixed in the experiments were designed after Hindy (1989). Number and size of blue spots (deposited droplets) on water sensitive papers were measured with a special scaled monocular japanese lens (Struben)® with a magnification power of 15x with an accuracy of ±25 micrometers. The diameter data of the spots were corrected with the knowledge of the spread factor, and converted to actual volume mean diameter (VMD), and the number of droplets in one square centimeter according to Gabir (1995).

I.5.Collection and measurement of spray drift:

During spraying each onion field treatment, a sampling line was constructed of eight wire holders fixed in straight line with down wind trend outside each treatment to collect spraying drift; each wire holder top has two water sensitive paper (26 x 76 mm) (Novartis cards)® on it one horizontal position the other at vertical position. The holders were placed in straight line with a distance of 50 cm between the each others by a long distance of 4 meter. All cards were collected and transferred carefully to the laboratory for measuring and calculated the drift droplets in all treatments by a special scaled monocular Japanese lens (Struben)®

1.6.Bioassay procedure:

Filed experiment was conducted on onion field highly infested with insect *T. tabaci*. Insects were active on onion leaves and their number exceed the critical economic limit. In order to evaluate the tested compounds on onion thrips, pre-treatment count was recorded at onion field. Plants at

random for each treatment, each treatment was divided into four replicates, in which five plants were randomly examined to calculate the number of moving thrips individuals fallen from the plant on white paper and posttreatment count was recorded after 1,7 and 12 days after treatment onion thrips and control treatment. These calculations were done before and after the treatment.In the field experiment results were calculated according to **Henderson and Tilton (1955).**

Results and discussion

1. Spray coverage and mortality:

Data from Table (4) and Figures (1 and 2) were revealed that, relationship between spray coverage of certain ground equipment and reduction percentages of onion thrips sprayed by marshal recommended dose insecticide during season (2018). It was noticed that, no significant difference between initial spraying and mean of residual spraying mean at rotary matabi sprayer 18 L/fed., knapsack motor sprayer with charging unit 42 L/fed and knapsack motor sprayer with normal unit 79 L/fed. the initial and mean residual were 98, 96, 91, 94, 93 and 95%, respectively. But a drastic efficiency of marshal recommended dose the low value of reduction percentages were mortality with conventional ground motor sprayer (578 L/fed.) and hydraulic matabi sprayer 56 L/fed. The initial and mean residual were 63, 76, 67 and 68% respectively. Data on Table (4) and Figures (3 and 4) showed that, the relationship between spray coverage of certain ground equipment and reduction percentages of onion thrips sprayed by marshal ¾ recommended dose during season (2018). It was found that, no significant difference between initial spraying and mean of residual spraying at rotary matabi sprayer 18 L/fed., knapsak motor sprayer with charging unit 42 L/fed. and the same equipment with shear unit 79 L/fed. The initial and residual mean were 93, 94, 87, 89, 90 and 92%, respectively, with droplet spectrum ranged between 55-62µm (VMD) droplets sizes and (222-265) droplets/cm². But it was noticed that, the same initial and mean residual was reaveled in hydreulic matabi was 76% and 76%. But in case of conventional ground motor sprayer initial and mean residual were 63% and 76% respectively the droplet spectrum was differantly distribution than recent sprayers which ranged 116-385 µm (VMD) as droplet sizes and 52-68 droplets/cm² . these phenomena of asmmitrical droplet distrbution lead to the poor results in bio-residual activity of marshal ¾ recommended dose with hyduralic equipment.

Table (4): Relationship between spray coverage of certain ground equipment and reduction percentages of onion thrips *Thrips tabaci* sprayed by marshal insecticides recommended dose and 3/4 recommended dose during season 2018.

Days after spraying / Equipmet	Insecticides	Spray volume (L/fed)	Initial spraying after 24 hours	Residual spraying mean	VMD	N/cm²
Rotary sprayer	Marshal recommended dose	18	98 %	96 %	58	228
	Marshal ¾ recommended dose		93 %	94 %	60	222
Electrostatic sprayer	Marshal recommended dose	42	91 %	94 %	47	265
	Marshal ¾ recommended dose		87 %	89 %	55	241
Hydraulic sprayer	Marshal recommended dose	56	67 %	68 %	119	60
	Marshal ¾ recommended dose		76 %	76 %	116	68
Knapsack blower sprayer	Marshal recommended dose	79	93 %	95 %	53	280
	Marshal ¾ recommended dose		90 %	92 %	62	265
Conventional ground motor sprayer	Marshal recommended dose	578	63 %	76 %	370	50
	Marshal ¾ recommended dose		63 %	76 %	375	52

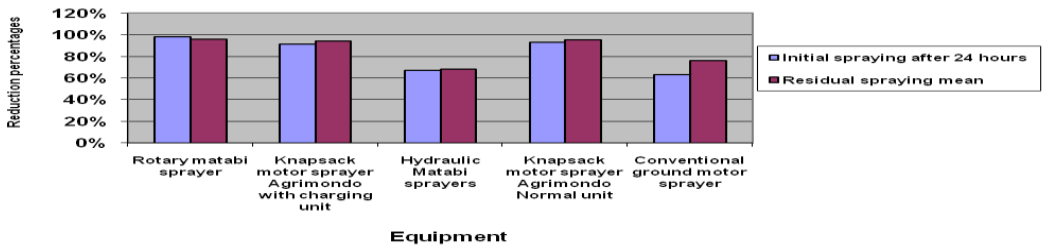
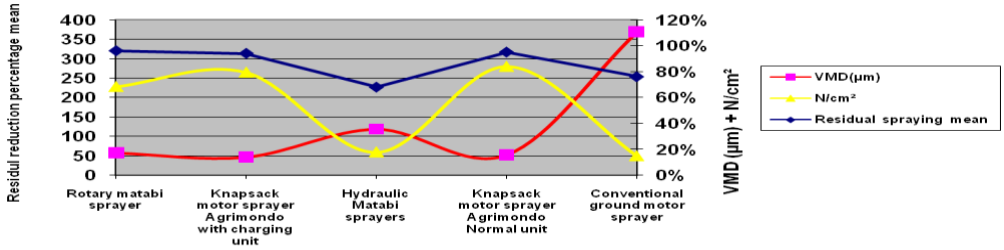


Figure (1): Relationship of reduction percentages and different spraying volume of certain ground equipment for insecticides marshal recommended dose during season 2018.



Figue (2): Relationship between percentage reduction means and distribution coverage of certain ground equipment for insecticides marshal recommended dose during season (2018).

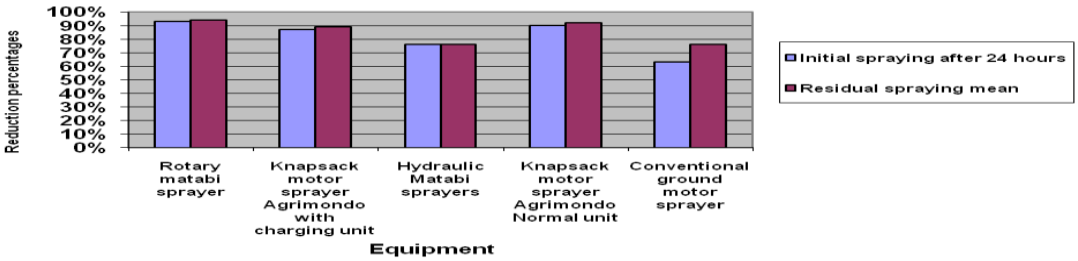


Figure (3): Relationship of reduction percentages and different spraying volume of certain ground equipment for insecticides marshal 3/4 recommended dose during season 2018.

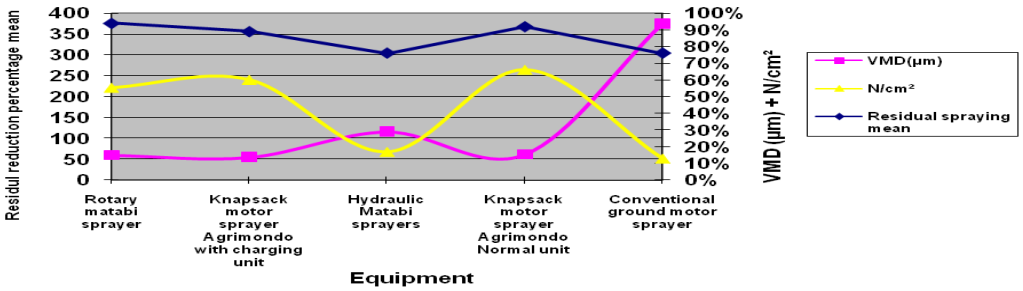


Figure (4): Rrelationship between percentage reduction means and distribution coverage of certain ground equipment for insecticides marshal 3/4 recommended dose during season 2018.

Data in Table (5) and Figures (5 and 6) illustrated that the relationship between spray coverage of certain ground equipment and reduction percentages of onion thrips sprayed by chinook recommended dose during season (2018). It was found that in recent sprayers like rotary matabi sprayer 18 L/fed., initial spraying and residual spraying mean were 99 and 96%, respectively. But knapsack motor with charging unit and normal spraying mean 92, 93, 95 and 95%, respectively. On other hand, hydraulic matabi 56 L/fed. and conventional ground motor sprayer reaveled initial spraying and residual spraying mean as 88, 77, 65 and 80% respectively. Data in Table (5) and Figures (7 and 8) illustrated the relationship between spray coverage of certain ground equipment and reduction percentages of onion thrips sprayed by chinook ¾ recommended dose during season (2018), in recent equipment rotary sprayer and knapsack motor sprayer with charging unit and normal unit there was no significant differences between initial spraying and residual spraying means the reduction percentages were 96, 93, 94, 90, 93 and 92%, respectively. But in hydraulic matabi and conventional ground motor sprayr the reduction percentages were 90, 75, 63 and 75%, respectively.

Data in Tables (4 and 5), illustrated that, there was no significant differences between recommended dose and ¾ recommended dose with resent equipment. It was mean that, it could be sawed 25% of the price of insecticides used in controlling *T. tabaci* and save the agriculture environment from pollution with using recent machine to control thrips pests. Data also showed that, there were no significant results between initial spraying after 24 hours and residual after 7 days and 12 days after spraying in recent sprayers. But a drastic differences between initial and residual spraying in both of conventional ground motor sprayer and hydraulic matabi sprayer these data agree with Smith and Goodhue, 1945), Potts and Garman (1950) and Gabir (1975 and 1995).

Table (5): relationship between spray coverage of certain ground equipment and reduction percentages of onion thrips sprayed by chinook insecticides recommended dose and 3/4 recommended dose during season 2018.

Days afterspraying Equipment	Insecticides	Spray volume (L/fed)	Initial spraying after 24 hours	Residual spraying mean	VMD	N/cm²
Rotary sprayer	Chinook recommended dose	18	99 %	96 %	55	230
	Chinook ¾ recommended dose		96 %	93 %	61	220
Electrostatic sprayer	Chinook recommended dose	42	92 %	93 %	50	258
	Chinook ¾ recommended dose		94%	90 %	52	260
Hydraulic sprayer	Chinook recommended dose	56	88 %	77 %	120	66
	Chinook ¾ recommended dose		90%	75 %	125	70
Knapsack blower sprayer	Chinook recommended dose	79	95 %	95 %	60	271
	Chinook ¾ recommended dose		93 %	92 %	65	264
Conventional ground motor sprayer	Chinook recommended dose	578	65 %	80 %	375	58
	Chinook ¾ recommended dose		63 %	75 %	380	60

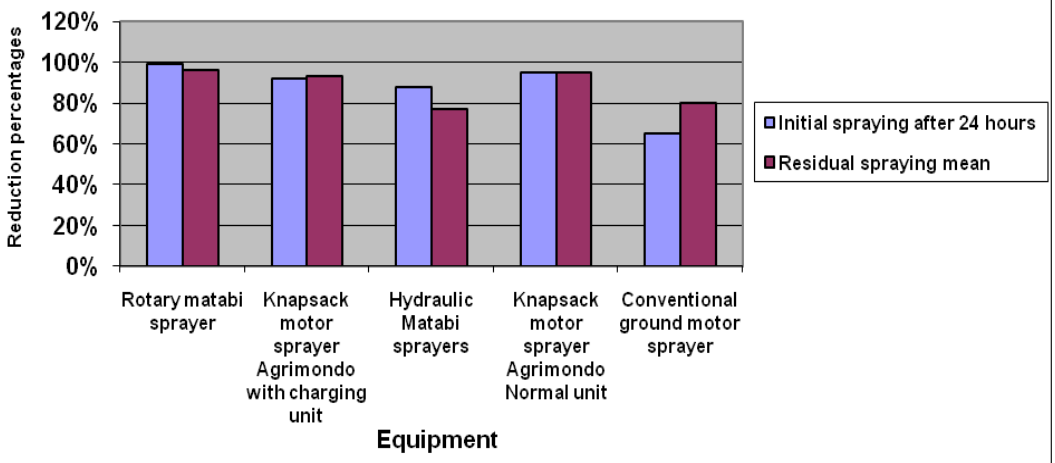


Figure (5): Relationship of reduction percentages and different spraying volume of certain ground equipment for insecticides chinook recommended dose during season 2018.

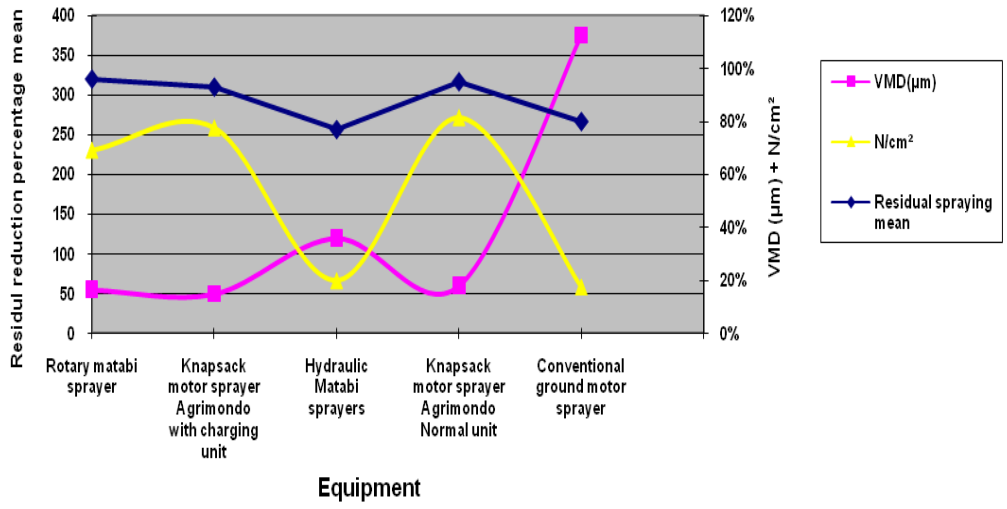


Figure (6): Relationship between percentage reduction means and distribution coverage of certain ground equipment for insecticides chinook recommended dose during season 2018.

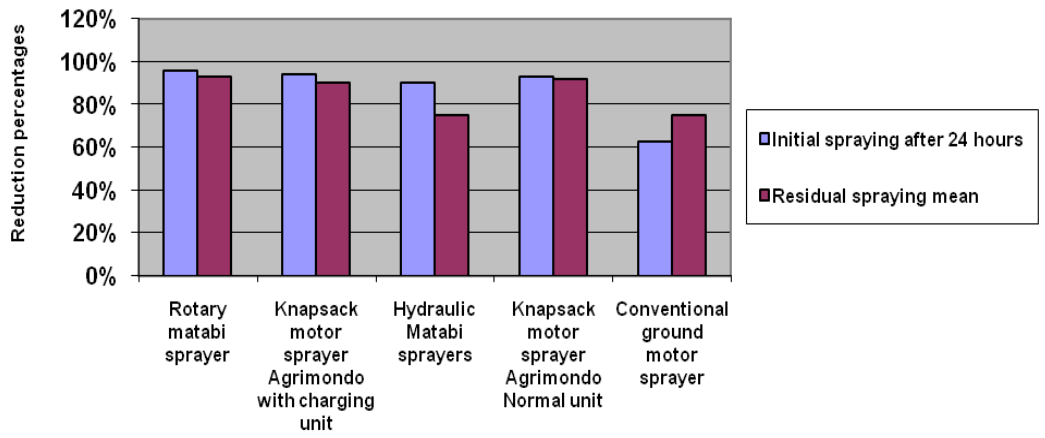


Figure (7): Relationship of reduction percentages and different spraying volume of certain ground equipment for insecticides chinook ¾ recommended dose during season 2018.

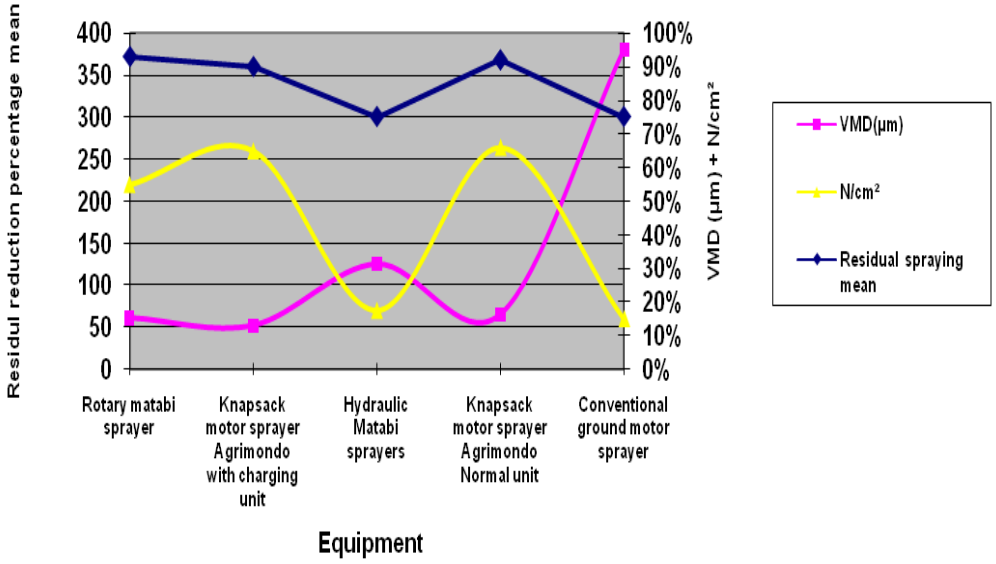


Figure (8): Relationship between percentage reduction means and distribution coverage of certain ground equipment for insecticides chinook 3/4 recommended dose during season 2018.

2. Drift:

Drift spray measurements for certain equipment under field conditions during season 2018 as shown in Table (6) and Figures (9-14), the front of at each treatment with down wind direction watery sensitive papers were put at 0.5m until 4.0 meter to collect the drift spray resultant from each tested ground sprayers, data showed that, the highly drifted sprayer was rotary matabi (spinning disk) sprayer 18 L/fed. drift distance was 3.5m down wind of the treatment. But the lowest drift spray distance was in case of knapsack motor sprayer with charging unit, the drift distance was 1.0m from spraying treatment, the short distance of drift due to the electrostatic foresees which controlled the movement of droplets sizes and captured its at the target spraying and the effect of wind was weak effect due to the charging power of droplets keep its on target spray directly. This results agreed with Mattwes (1989), Celen *et al.* (2009), Gitirana Neto *et al.* (2015) and Jaferi *et al.* (2018). Drift spray measurements were hydraulic matabi sprayer and knapsack motor sprayer Agrimondo with normal unit (Sheear nozzle). The drift distances were 3.0m down wind and the conventional ground motor sprayer was 1.0 m from treatment the short distance due to the of drift spray was big volumes droplets falls terminal velocity of vertically and the wind velocity could not easily to horizontal direction down wind. Therefore the big amount of lost spray of conventional motor sprayer was between the plants on ground and contaminated the local area treated with lost spray on ground. This data agreed with Gaber (1975) and Hindy *et al*. (1991).

Table (6): Spray drift of certain ground equipment during season 2018.

Equipment	Spray volume (L/fed)	Drift distance (m)	General mean	
			VMD (µm)	N/cm²
Rotary sprayer	18	3.5	46	35
Electrostatic sprayer	42	1	50	8
Hydraulic sprayer	56	3	109	56
Knapsack blower sprayer	79	3	50	73
Conventionalground motor sprayer	578	1	150	177

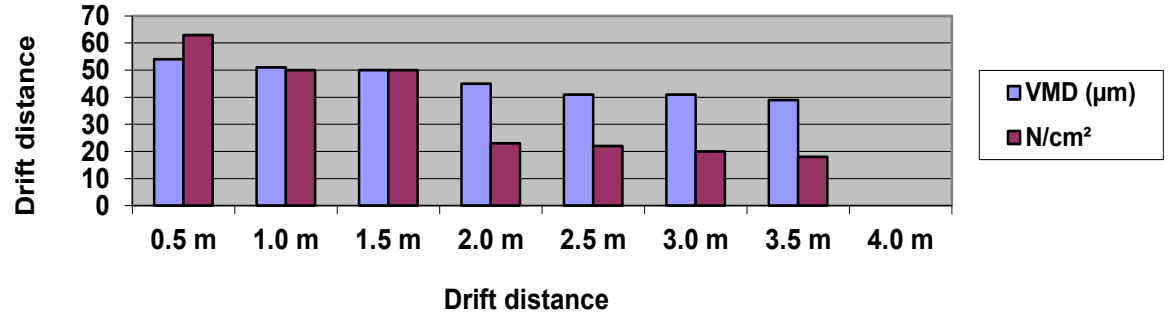


Figure (9): Drift rotary matabi sprayer.

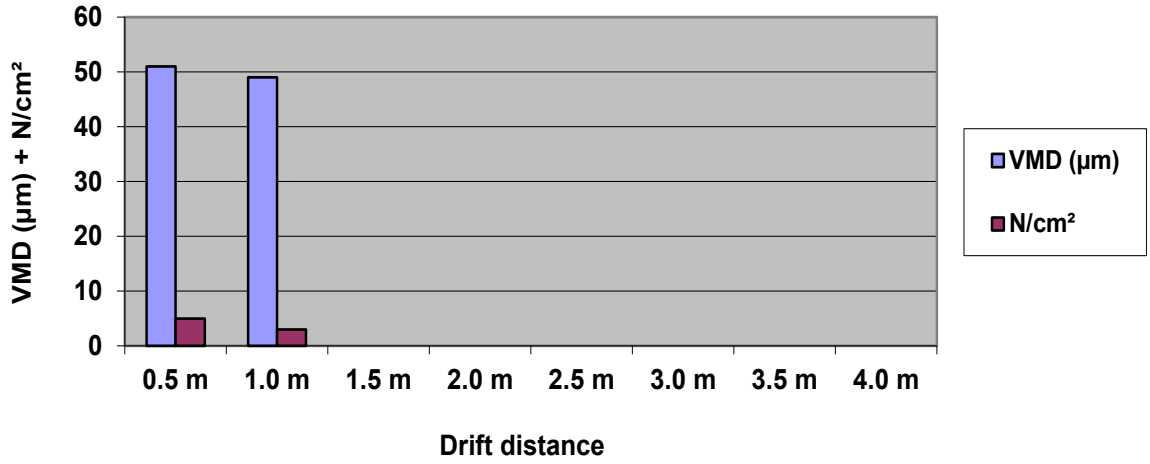


Figure (10): Drift knapsack motor sprayer Agrimondo with charging unit.

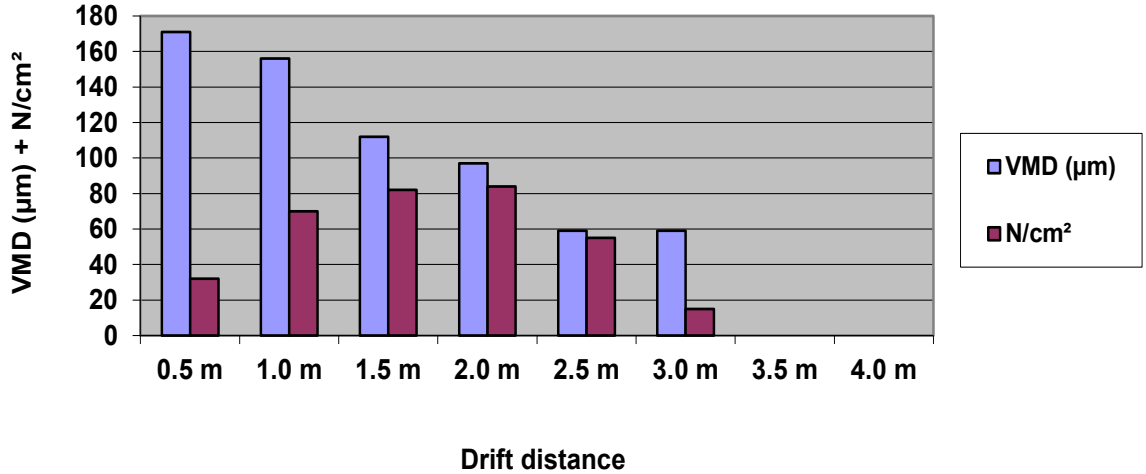


Figure (11): Drift hydraulic matabi sprayers.

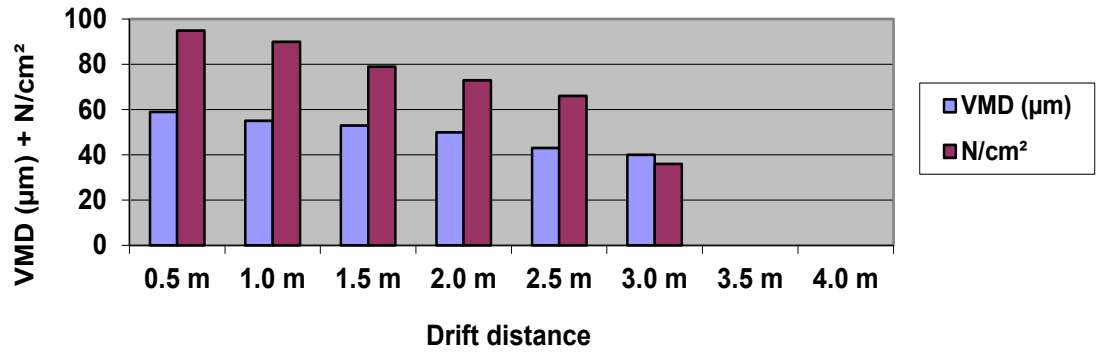


Figure (12): Drift knapsack motor sprayer Agrimondo normal unit.

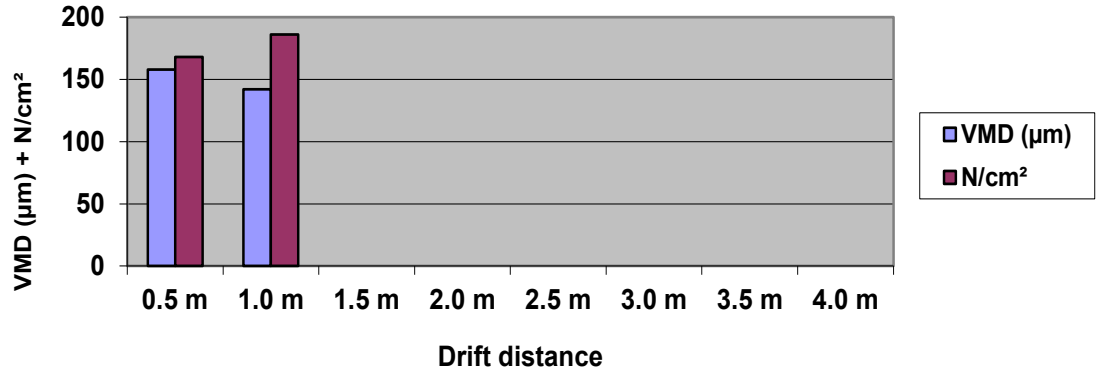


Figure (13): Drift conventional ground motor sprayer.

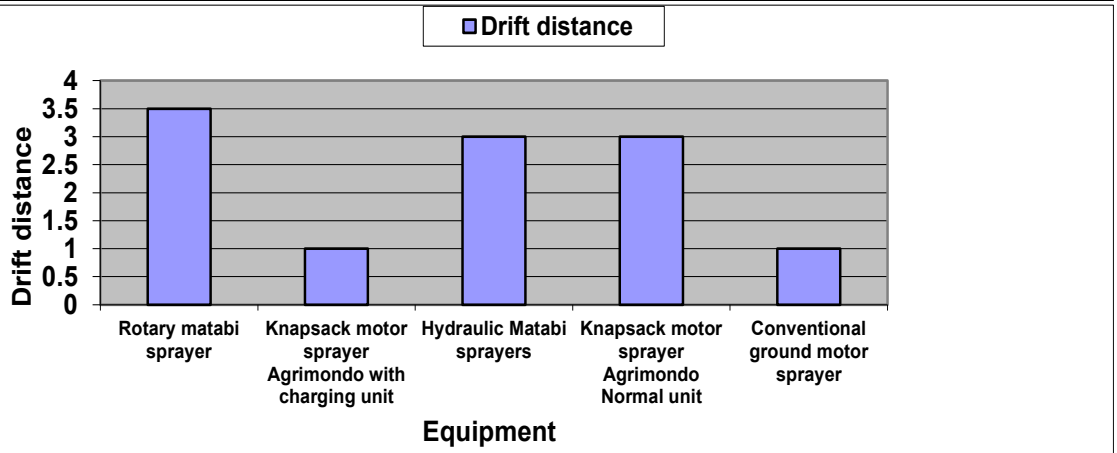


Figure (14): Relationship of drift distance between equipment.

3. Spray mass:

Data in Table (7) and Figures (15 a,b,c,d and e) illustrated that, percentages of spray mass on onion plant, lost spray on ground and the drift by using marshal and chinook recommended and ¾ recommended doses by certain ground equipment during season 2018. The range of spray coverage on the onion plant was ranged from 79% by Agrimondo® with charging unit (24 L/fed.) and 26% by conventional motor sprayer (587 L/fed.). But in case of rotary matabi® sprayer (18 L/fed.) the coverage on the onion plant was 43%. On other hand the Agrimondo® with normal unit the coverage spray on onion plant was 51% and the hydraulic matabi® sprayer (56 L/fed.) the coverage spray on onion was 31%.

Table (7):Relationship between deposit on plant, lost spray on ground and lost spray by drift for sprayer equipment during season 2018.

Spray mass / Equipment	% of the spray mass on the plant	% of the lost spray mass on ground	% of the lost drift mass
Rotary matabi sprayer	43 %	52 %	5 %
Knapsack motor sprayer Agrimondo with charging unit	79 %	19 %	2 %
Hydraulic Matabi sprayers	31 %	46 %	23%
Knapsack motor sprayer Agrimondo Normal unit	51 %	38 %	11 %
Conventional ground motor sprayer	26 %	42 %	32 %

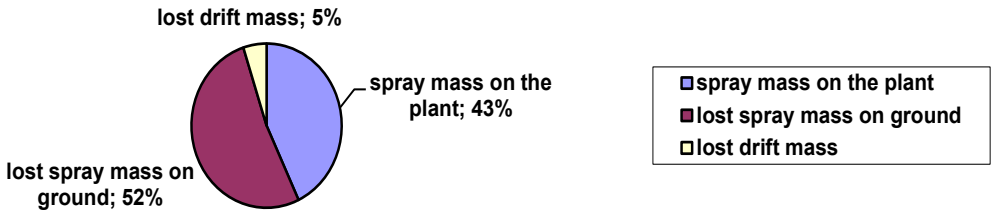


Figure (15a): Distribution of spray mass on the plant, lost on ground and the drift spray forrmatabi sprayer (18) L / fed.

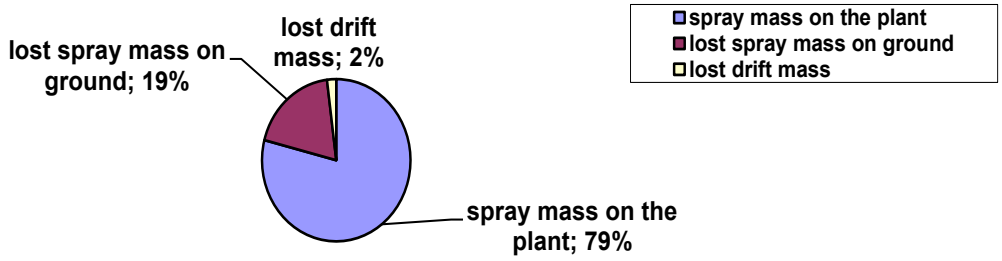


Figure (15b): Distribution of spray mass on the plant, lost on ground and the drift spray for knapsack motor sprayer Agrimondo with charging unit (42) L / fed.

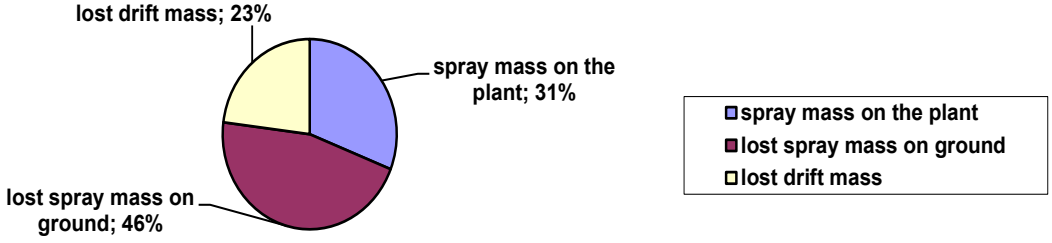


Figure (15c): Distribution of spray mass on the plant, lost on ground and the drift spray for hydraulic matabi sprayers (56) L / fed.

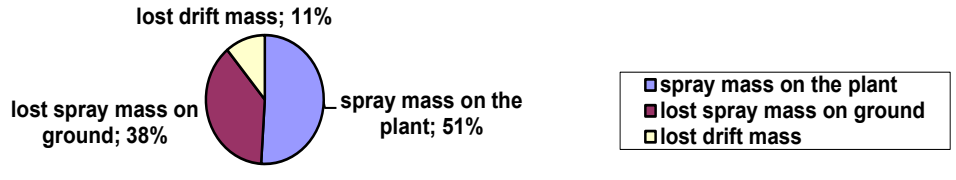


Figure (15d): Distribution of spray mass on the plant, lost on ground and the drift spray for knapsack motor sprayer Agrimondo Normal unit (79) L / fed.

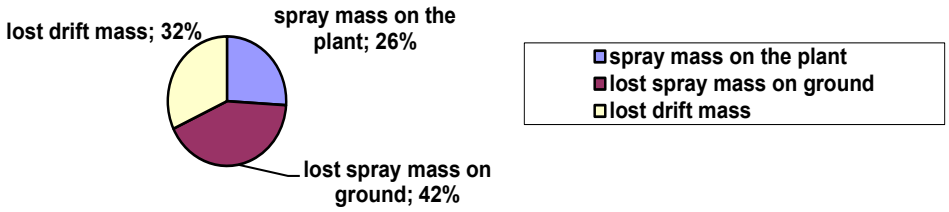


Figure (15e): Distribution of spray mass on the plant, lost spray on ground and the drift spray for conventional ground motor sprayer (578) L /fed.

Data showed that, there was great relationship between droplet (i.e. decreasing droplet sizes (VMD) and increasing number of s given a high efficiency of insecticides used against thrips on onion. It must be controlled with low volume spraying machines reneged from 18-42 L/fed. Through using electrostatic and pneumatic energy or both of them or by using centrifugal energy by spinning disc sprayer the worst bad quality spray and a poor efficiency of bio residual activity of insecticides sprayed had been revealed through using hydraulic energy through ground motor spray or hand held hydraulic spray. Data also showed that, there was no significant differences between recommended doses and ¾ recommended doses with recent equipment. It could saved 25% of the insecticides prices used in controlling thrips and saving agricultural environment from pollution on land and air.

From another side, data found that, there was no significant results between initial spraying after 24 hours and residual spraying after spraying 7 days and 12 days by resent sprayer and hydraulic matabi sprayer. Also, data showed a lowest drift spray was resulted from electrostatic sprayer, but the biggest drift spray was resulted from rotary matabi spinning disc. Similar result had been obtained from using two insecticides with recommended and ¾ recommended dose with using the same five tested spraying techniques. So, a sati's factory spray coverage, as well as lost spray on ground between plants and drift spray outside the treatment with down wind were determined. Data showed that the ideal spray quality on onion crop, and a highest reduction of lost spray on ground was about 15.6%. and a least spray drift out side the treatment was electrostatic Agrimondo sprayer, but the worst equipment was conventional ground motor sprayer was 44.6% as lost spray on ground due two a big droplets, amount of water and a high operational pressure used.

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Insecticidal activity of peels oil of *Citrus sinensis* and summer oil against two scale insects *Aulacaspis tubercularis* (Hemiptera: Diaspididae) and *Milviscutulus mangiferae* (Hemiptera: Coccidae)

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Abstract:

Essential oil extracted from peels of balady orange (*Citrus sinensis* L.) was tested for its insecticidal activity at three different concentrations (1000, 5000 and 10000 ppm) against nymphs, adults and gravid females of white mango scale *Aulacaspis tubercularis* (Newstead) (Hemiptera: Diaspididae) and mango soft scale *Milviscutulus mangiferae* (Green) (Hemiptera: Coccidae). Formulated oil of balady orange was bioassayed against the two scale insects and the results revealed that, the formulated oil of balady orange achieved high toxicity against nymphs, adults and gravid females of both *A. tubercularis* and *M. mangiferae* at all used concentrations. Balady orange oil concentrations (0.1, 0.5 and 1%) were more potent against the two scale insects than the reference product (summer oil 1.5%). The essential oil of balady orange was isolated by hydrodistillation and the analysis of gas chromatography–mass spectrometry (GC/MS) revealed the presence of 12 peaks, approximately all peaks were identified. The chemical composition showed that limonene was the main constituent in citrus oil (83.28%). The results of the present study suggested that, formulated balady orange oil used as safe, potential natural products for control of *A. tubercularis* and *M. mangiferae* on mango trees and may be used as alternatives to the reference products after application of these results in the semifield and field experiments.

Introduction

Mango trees are considered one of the most popular and economic fruit trees in Egypt. They occupy the third rank from the commercial point of view. Mango trees are liable to be infested with many serious pests during their growth stages including white mango scale *Aulacaspis tubercularis* (Newstead)(Hemiptera, Diaspididae) and *Milviscutulus mangiferae* (Green)(Hemiptera: Coccidae). The white mango scale insect *A. tubercularis* injures mangoes by feeding on the plant sap through leaves, branches and fruits, causing defoliation, drying up of young twigs, poor blossoming and so affecting the commercial value of fruits and their export potential especially to late cultivars where it causes conspicuous pink blemishes around the feeding sites of the

scales. *A. tubercularis* is a tropical species that may have originated in Asia. It has been recorded mainly from hosts belonging to four plant families: Palmaceae, Lauraceae, Rutaceae, Anacardiaceae, particularly on mangoes and cinnamon (Borchsenius, 1966).

The mango shield scale or mango soft scale *M. mangiferae* a serious pest of mango trees in various parts of the world, is reported on *Mangifera indica* in Egypt which represents the first record of this species in the country (Abd-Rabou and Evans, 2017). In general, continuous and heavy use of the synthetic pesticides has created serious problems such as environmental pollution, toxicity to non-target organisms (parasitoids and predators), pest resistance and pesticide residues (Mohan and Fields, 2002). Therefore, there is an urgent need to develop new, convenient and safer alternatives to synthetic pesticides. Essential oils and their major constituents, attracted research attention in recent years as potential alternatives to synthetic insecticides.

The genus *Citrus* includes several important fruits such as oranges, mandarins, lime, lemons and grapefruits. The essential oils of some citrus species have been reported to have insecticidal properties against insect pests (Elhag, 2000). The major active component of citrus oil is limonene and using 1% limonene mixture was safe for most plants and provided good control of mealybugs and scale insects (Hollingsworth, 2005).

The present study aimed to investigate the efficacy of Egyptian citrus peel essential oil of balady orange (*C. sinensis*), belonging to Family Rutaceae against nymphs, adults and gravid females of both scale insects *A. tubercularis* and *M. mangiferae* on mango trees compared with the summer mineral oil 1.5%. Also, extraction, determination and chemical analysis of essential oil were studied.

Materials and methods

1. Tested citrus species:

The experimental citrus species, balady orange was selected for this study. This citrus species was obtained from a private citrus orchard.

2. Insects source:

Infested leaves with both *A. tubercularis* and *M. mangiferae* for laboratory experiments were collected from the Agricultural Experimental Station at El-Kanater El-Khayria, Qalubiya Governorate. Samples were collected randomly from each of the four cardinal directions (East, West, North and South). Leaves were packed in plastic bags and transferred immediately to the laboratory. *A. tubercularis* and *M. mangiferae* were identified by Department of Mealybug and Scale insects, Plant Protection Researches Institute, Agriculture Researches Center. The identification was assured by the aid of Dr. Jean François German in the laboratory of Anses, Laboratoire de la santè des vègètaux, CBGP, Campus Internationalde Baillarguet, France (Attia *et al.,* 2018).

3. Extraction of citrus oil:

Citrus oil was extracted by Cavalcanti *et al*. (2004). The essential oil was extracted from the fresh peels (200g weight and 400 ml of distilled water) by hydrodistillation using a modified Clevenger type apparatus for 4 h. The distilled was extracted with diethyl ether after saturation with sodium chloride. The extracted oil was dried over anhydrous sodium sulfate, then packed in dark container and stored at 4°C until used for gas chromatography–mass spectrometry (GC-MS) analysis and bioassays.

4. Chemical analysis of essential oil:

4.1. Chemical analysis of citrus peel oil constituents:

The extracted citrus oil was subjected to GC/MS analysis using Shimadzu GC/MS–QP-5050A. Column: DB5, 30m, 0.53mm ID, 1.5µm film. Carrier gas: Helium (flow rate 1.2 ml/min.). Ionization mode: (70ev). The injection volume was 0.5µl (split ratio of 1:100), Temperature program: 50^{o}C (static for 2 min) with gradually increasing (a rate of 4^{o}C/ min.) up to 200^{o}C then (10^{o}C/ min.) to

280^0C. The detector temperature was 290^0C, while, the injector temperature was 250^0C.

4.2. Identification of the chemical constituents:

Qualitative identification of the essential oil was achieved by library searched data base Willey 229 LIB as well as by comparing their retention indices and mass fragmentation patterns with those of the available references and with published data, (Adams, 2007). The percentage composition of components of the volatile was determined by computerized peak area measurements.

5. Preparation of formulated orange essential oil:

Concentrations (1000, 5000 and 10000 ppm) and (0.1, 0.5 and 1%) of formulated oil of balady orange were prepared by emulsifier (Triton-100).

6. Toxicity bioassays:

Laboratory bioassays were conducted to determine the bioactivity of formulated citrus oil of balady orange and summer oil against nymphs, adults and gravid females of *A. tubercularis* and *M. mangiferae*. The toxicity bioassay was conducted to evaluate toxicity of formulated citrus oil of balady orange to nymphs, adults and gravid females of the two scale insects *A. tubercularis* and *M. mangiferae* at three different concentrations of formulated citrus oil and summer oil (1.5%). Leaves were sprayed with 1ml for five seconds of the formulated citrus oil and summer oil, then, kept at room temperature until the leaves dry. Control insects were sprayed with Triton-x100 emulsifier alone (without oil). Three replicates were used and the experiment was repeated for three times and mortality was recorded after 1, 3 and 6 days

7. Statistical analysis

The percentages of mortality in population were calculated by using Stafford and Summers equation (1963) and corrected with Abbot Formula (Abbott, 1925). Data of all experiments were evaluated statistically using ANOVA and means compared using Duncan's Multiple Range Test at $P<0.05$). All statistical analyses were done using the software package Costat program.

Results and discussion

1. Toxicity bioassay:

The obtained formulated citrus oil of balady orange in this study was mainly conducted to investigate a relationship between the oil constituents and their potency towards nymphs, adults and gravid females of *A. tubercularis* and *M. mangiferae* compared to the reference product mineral oil (summer oil).

1.1. Toxicity of formulated orange oil and summer oil against *Aulacaspis tubercularis*:

The results of toxicity assays as represented in Table (1), showed that, essential oil of citrus peel exhibited toxicity rate with concentration and time dependent. Formulated peel essential oil achieved high mortality percentages against nymphs, adults and gravid females at the three different concentrations (1000, 5000 and 10000 ppm). The highest toxicity rates against nymphs, adults and gravid females were 90.00±0.0, 88.80±0.92 and 86.93±0.31%, respectively, at the maximum concentration 10000 ppm and after 6 days of treatment. The percentages of mortality achieved by summer oil (1.5%) were 84.59±2.45, 67.50±8.97 and 58.65±9.00% respectively, after 6 days of treatment.

1.2.Toxicity of formulated orange oil and summer oil against *Milviscutulus mangiferae* :

The results of toxicity assays as represented in Table (2), showed that, essential oil of citrus peels exhibited toxicity rate with concentration and time dependent. Formulated peel essential oil achieved high mortality percentages against nymphs, adults and gravid females at the different concentrations (1000, 5000 and 10000 ppm) than summer oil (1.5%). The highest toxicity rates of formulated citrus oil nymphs, adults and gravid females were 87.43±0.19, 85.48±1.97and 90.00±0%, respectively, at the maximum concentration 10000 ppm and after

6 days of treatment. While, the percentages of mortality of summer oil (1.5%) were 75.86±2.34, 73.83±3.00 and 80.97±0.95%, respectively, after 6 days of treatment.

The lowest mortality with the two scale insects was obtained with the lowest concentration (1000 ppm) and after one day of assay. The formulated citrus oil and summer oil were more potent against nymphs and adults than gravid females of *A. tubercularis* with the three treatments (after 1, 3 and 6 days).While, the citrus oil and summer oil were more potent against gravid females than nymphs and adults of *M. mangiferae* with the same treatments (1, 3 and 6 days). Generally, balady orange oil concentrations were more potent against the two scale insects than the reference product (summer oil 1.5%). There were significant differences in mortality between control and treated variants ($P< 0.05$).

Table (1): Toxic effect orange peel oil *Citrus sinensis* var balady and summer oil against nymphs, adults and gravid females of *Aulacaspis tubercularis* at different concentrations.

Conc. (ppm)	Corrected mortality(%)±SD								
	Nymphs			Adults			Gravid females		
	1 day	3 days	6 days	1 day	3 days	6 days	1 day	3 days	6 days
1000	87.94 ±0.83^{a}	89.91 ±006 ^{a}	89.64 ±0.24^{a}	69.49 ±13.11^{c}	82.48 ±9.88^{a}	86.75 ±1.26^{a}	57.72 ±15.64^{b}	77.39 ±9.74^{a}	85.51 ±0.99^{a}
5000	89.57 ±0.23^{a}	89.14 ±0.60^{a}	89.85 ±0.08^{a}	74.68 ±6.94^{b}	86.48 ±4.80^{a}	88.01 ±2.55^{a}	68.14 ±3.32^{a}	77.88 ±7.03^{a}	85.18 ±1.15^{a}
10000	89.79 ±0.22^{a}	89.06 ±0.00^{a}	90.00 ±0.0^{a}	78.87 ±7.02^{a}	86.26 ±6.37^{b}	88.80 ±0.92^{a}	68.32 ±11.30^{a}	80.13 ±8.37^{a}	86.93 ±0.31^{a}
S.O (1.5%)	60.09 ±9.34^{b}	76.20 ±7.22^{b}	84.59 ±2.45^{b}	54.78 ±9.55^{d}	55.05 ±9.46^{c}	67.50 ±8.97^{b}	38.80 ±12.35^{c}	47.37 ±8.94^{b}	58.65 ±9.00^{b}
Control	0	0	0	0	0	0	0	0	0
F value	10.79 **	0.32 Ns	0.83 ns	2.76 ***	10.72 **	12.394 **	23.11 ***	29.52 ***	22.30 ***
$LSD_{0.05}$	9.14	9.43	9.41	1.995	9.66	9.15	1.4	9.02	9.43
S.O= Summer oil									

Table (2): Toxic effect of orange peel oil *Citrus sinensis* var balady and summer oil against nymphs, adults and gravid females of *Milviscutulus mangiferae* at different concentrations.

Conc. (ppm)	Corrected mortality(%)±SD								
	Nymphs			Adults			Gravid females		
	1 day	3 days	6 days	1 day	3 days	6 days	1 day	3 days	6 days
1000	69.96 ±0.90^{b}	87.43 ±0.28^{a}	86.74 ±0.46^{a}	55.84 ±3.93^{c}	78.25 ±0.63^{a}	80.96 ±0.80^{a}	78.85 ±0.50^{b}	89.09 ±0.50^{a}	88.63 ±0.00^{a}
5000	79.02 ±0.13^{ab}	86.57 ±0.51^{a}	87.43 ±0.29^{a}	71.55 ±4.48^{b}	80.96 ±0.86^{a}	80.96 ±0.00^{a}	89.09 ±0.00^{a}	90.00 ±0.00^{a}	90.00 ±0.00^{a}
10000	81.77 ±0.07^{a}	87.11 ±0.33^{a}	87.43 ±0.19^{a}	81.46 ±10.38^{a}	83.22 ±6.93^{a}	85.48 ±1.97^{a}	88.63 ±2.51^{a}	89.09 ±0.00^{a}	90.00 ±0.00^{a}
S.O (1.5%)	48.48 ±7.32^{c}	58.93 ±6.98^{b}	75.86 ±2.34^{a}	44.85 ±6.55^{d}	58.93 ±3.67^{b}	73.83 ±3.00^{a}	50.65 ±4.45^{c}	77.02 ±1.29^{a}	80.97 ±0.95^{a}
Control	0	0	0	0	0	0	0	0	0
F value	27.31 ***	24.58 ***	1.10 Ns	21.90 ***	7.41 *	1.13 ns	20.72 ***	1.52 ns	1.29 ns
$LSD_{0.05}$	9.41	9.41	17.73	11.28	13.38	15.06	12.94	16.41	12.45
S.O= S.O=Summer oil									

Pumnuan *et al*. (2015) showed that, fresh peels essential oils of four citrus species recorded moderate toxicity at 2ml/L air (fumigation) and high toxicity at 2ml/L air against larvae of mealybug mealybug *Pseudococcus jackbeardsleyi* Gimpel and Miller (Hemiptera: Pseudococcidae) at 24h. These findings are confirmed by Karamaouna *et al*. (2013), they showed that the citrus peel essential oils of lemon (*Citrus limon*) and balady orange (*C. sinensis*) were the most toxic of all the tested essential oils against 3rd instar nymphs and females adults of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) . El-Badawy (2015) found that, all tested citrus oils especially balady orange achieved high insecticidal and repellent activities against mealybug *Icerya seychellarum* (Westwood) (Hemiptera: Monophlebidae).

1.3.Comparison of total mean mortality of citrus oil and summer oil against *Aulacaspis tubercularis*.

From the data of total mean values presented in Tables (3 and 4) it could be demonstrated that, at the three different concentrations (0.1, 0.5 and 1.0%) the formulated peel citrus oil was more nymphscidal and adultscidal effect than the summer oil (1.5%) against *A. tubercularis* and *M. mangiferae* after 1, 3 and 6 days of assay. The recorded total mean mortality values (69.62, 83.16 and 87.17%) of the lowest concentration (0.1%) of orange oil were higher than summer oil (1.5%) mortality values (52.46, 61.04 and 71.52%) against *A. tubercularis* after 1, 3 and 6 days of assay, respectively. The recorded total mean mortality values of the formulated orange oil at the other two concentrations (0.5 and 1.0%) against *A. tubercularis* were (77.17 and 77.62 %), (84.05 and 82.14%) and (87.21and 88.24 %), respectively, after the same time (Table, 3).

The mortality percentages values of the citrus oil against *M. mangiferae* after one day of assay ranged from 65.68 (conc. 0.1%) to 86.62% (conc. 1%), and after three days ranged from 87.09 to 95.68%, while, after six days ranged from 87.77 to 98.33%, respectively. Also, the lowest concentration (0.1%) of orange oil was higher than summer oil (1.5%) mortality values against *M. mangiferae* after the same time (Table, 4). The variation of the mortality values of citrus oil against *A. tubercularis* and *M. mangiferae* depending on the toxicity of the formulation of citrus oil, the mealybug and scale insect life stage.

These findings are confirmed by Karamaouna *et al*. (2013), who showed that, the LC_{50} values of citrus (*C. sinensis* and *C. limon*) oils ranged from 2.7 to 8.1mg/ml depending on the essential oil and the mealybug life stage. These LC_{50} values were significantly lower than the LC_{50} of the reference paraffin oil in the respective *P. ficus* life stages. Results of El-Badawy (2015), revealed that, the oil of balady orange achieved the highest toxicity against nymphs, adults and gravid females of mealybug *I. seychellarum*.

Table (3): Toxic effect comparison of *Citrus sinensis* var balady oil and summer oil against total mean of nymphs, adults and gravid female of *Aulacaspis tubercularis* at different concentrations.

Time (days)	Corrected mortality (%)			
	Conc. (%)			Summer oil (1.5%)
	0.10	0.50	1.00	
	Total Mean			Total Mean
1 day	69.62^{c}	77.17^{c}	77.62^{c}	52.46^{c}
3 days	83.16^{ab}	84.05^{b}	82.14^{b}	61.04^{b}
6 days	87.17^{a}	87.21^{a}	88.24^{a}	71.52^{a}
F value	3.54^{ns}	0.54^{ns}	18.95*	9.535*
$LSD_{0.05}$	16.91	19.02	18.95	10.69
Total Mean= Total mean of nymph, adult and gravid female				

Table (4): Comparison of toxic effect of balady orange peels oil and summer oil against nymphs, adults and Gravid females of *Milviscutulus mangiferae* at different concentrations

Time (days)	Corrected mortality(%)			
	Conc. %			Summer oil (1.5%)
	0.10	0.50	1.00	
	Total Mean			Total Mean
1 day	65.68[b]	76.73b[a]	86.62[a]	37.15[c]
3 days	87.09[a]	87.37[a]	95.68[a]	50.28[b]
6 days	87.77[a]	88.35[a]	98.33[a]	65.40[a]
F value	11.272**	3.851[ns]	1.54[ns]	14.39**
$LSD_{0.05}$	12.95	11.36	17.11	12.90

2. Chemical analysis of citrus peel essential oil:

The essential oil yield of fresh citrus peels of *C. sinensis* was (4.30%). The chemical compositions of the essential oil of citrus peels are presented in Table (5). The essential oil analysis by GC/MS revealed that, the presence of 12 peaks, all peaks were identified, representing 99.70 % of the essential oil of balady orange. The major constituents of this essential oil mainly belonged to two groups: monoterpene and oxygenated monoterpenes. Oxygenated monoterpenes hydrocarbons with contribution of 8.33% constituted the second major portion of the essential oil after monoterpenes hydrocarbons (89.84%) from peel oil. The chemical analysis of the citrus oil showed limonene as the main constituent (83.28%) for balady orange. The most abundant ingredients beside to limonene, were linalool (3.97%), β-myrcene (3.63%), β-Citral (1.97%), p-Cymene (1.73%) , α-Citral (1.64%) and Linalyl acetate (1.56%) in the citrus peels oil. The monoterpene hydrocarbons *α*-pinene, β-pinene and γ-Terpinene are present. Overall results indicated that the toxic effects of citrus oil balady orange on *A. tubercularis* and *M. mangiferae* could be related to the high content of limonene.

Table (5): Chemical composition of essential oil from balady orange peels .

No.	Components	RT(min.)	Ratio (%)
1	α-thujene	7.125	0.05
2	α-Pinene	8.718	1.34
3	β-Pinene	10.14	0.29
4	β-Myrcene	10.718	3.63
5	α-Terpinene	11.84	0.20
6	D-Limonene	12.343	83.28
7	γ-Terpinene	12.125	0.22
7	p-Cymene	12.163	1.73
8	β-Linalool	12.952	3.97
9	Linalyl acetate	13.066	1.56
10	α-cis-Citral	13.234	1.64
11	β-cis-Citral	14.477	1.97
12	Geraniol	14.739	0.69
	Monoterpene Hydrocarbons	-	89.84
	Oxygenated Monoterpene Hydrocarbons	-	8.33
	Other compounds		1.53
	Total	-	99.70

Our results of the chemical composition of citrus peel oil are in agreement with many other studies (Ahmad *et al*., 2006, Asekun *et al*., 2007 and El-Badawy, 2015). All these studies showed that, limonene was the main component with high variation in all citrus peel oils and also, there are considerable variations in the other constituents of the chemical composition of citrus oils. Such variation in chemical composition (Limonene

content and other constituents) in citrus peel oils may be related to the time of harvesting, the degree of freshness, genetic makeup and the size of the fruit. Also, geographical location, fruit variety and method of extraction (Ahmad *et al*., 2006).

Regarding to potency of citrus oil against nymphs, adults and gravid females of *A. tubercularis* and *M. mangiferae*, the data presented in Tables (1-4) indicated that the potency of the tested formulated oil was related to the major component limonene content of that oil. These results are confirmed by El-Badawy (2015), who showed that the toxic effect of five citrus oils on *I. seychellarum* could be related to the high content of limonene. Also, these results are in agreement with these obtained who showed that by Hollingsworth (2005). The best limonene mixture (1% limonene, 0.75% emulsifier APSA-80 and 0.1% surfactant Silwet) controlled from 69 to 100% of mealybugs and scale insects, depending on the species, insect stage and application method.

Citrus oil of balady orange was more toxic than summer oil against *A. tubercularis* and *M. mangiferae*, so, it can be used as an effective natural alternative to mineral oil (summer oil). It is recommended to expand such laboratory experiments to semifield and field conditions and determine the efficacy of balady orange oil against *A. tubercularis* and *M. mangiferae* and other mealybug and scale insect species.

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Influence of host plants on biological aspects of the citrus mealybug *Planococcus citri* (Hemiptera: Pseudococcidae) under laboratory

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Abstract:

Planococcus citri (Risso) (Hemiptera: Pseudococcidae)) is a highly polyphagous and has been reported on over 200 host plant species belonging to 191 genera and 82 families and can seriously damage many crops, particularly citrus and glasshouse tomatoes. Biological studies were carried out in Scale Insects and Mealybugs Laboratory, Plant Protection Research Institute, Kafer EL-Sheikh, Egypt during 2018 to study duration periods of immature and mature stages of *P.citri* on three different host plants [Two varities of potato (*Solanum tuberosum* var. spunta and *Solanum tuberosum* var. cara) and sweet potatoes (*Ipomoea batatas*)] and the effects of host plant on the development, longevity and reproduction of *P. citri* . This study showed that a highly significant difference between the mean duration of *P. citri* adult female and male on three host plant (spunta, cara and sweet potatoes). There was a highly significant difference between the mean number of eggs laid per female of *P. citri* on aforementioned host plants .

Introduction

The citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) is an important piercing sucking insect pest attacking several crops . It attacks new shoots and leaves of a wide range of crops including citrus, apple, avocado, ficus, gardenia, jasmine, oleander and persimmon (Angeles-Martinez *et al.*, 1991; Correa *et al.*, 2008 and Ahmed and Abd-Rabou, 2010). It has piercing sucking mouth parts that remove plant fluids so plant damage is caused by loss of sap extracted by high numbers of mealybugs resulting in wilted, distorted and yellowed (chlorotic) leaves, premature leaf drop, stunted growth and occasionally death of infested plants or plant parts. The sticky sugary fluid excreted by mealybugs is called honeydew which provides a medium for the growth of black sooty mold fungi. Black sooty mold fungi are detrimental to plants because they cover leaves, thus reducing photosynthesis and inducing plant stress (Hill, 1983; Al-Ali, 1996; Smith *et al*., 1997; Serrano *et al*., 2001 and Heinz *et al*., 2004). The citrus mealybug is also known as a vector of some important plant viruses (Kubiriba *et al*., 2001 and Watson and Kubiriba, 2005).

Its life cycle (egg to egg laying adult) duration ranges from 20 to 44 days (Ortu *et al.*, 2002). Asiedu *et al.* (2014) mentioned that, development, longevity and total

number of eggs laid by *P. citri* are influenced by yam variety and the order of preference of *P. citri* for development, longevity and oviposition is *Dioscorea rotundata* var. pona followed by *D. rotundata* var. labreko, *D. rotundata* var. muchumudu then *D. alata* var. matches and the least being *D. rotundata* var. dente.

The goal of this study, was to determine the relative susceptibility of the two varities of potato (*Solanum tuberosum* var. spunta and *Solanum tuberosum* var. cara L.) and sweet potatoes (*Ipomoea batatas* L.) to *P. citri* biology. The specific objectives were studied the determination of incubation period, development, longevity and reproduction parameters of *P. citri*.

Materials and Methods

The stock culture of the citrus mealybug *P. citri* used in this study was originally collected from citrus orchard citrus, Kafer EL-Sheikh Governorate, Egypt. Potato tubers, (*S. tuberosum* var. spunta and *S. tuberosum* var. cara) and sweet potatoes (*I. batatas*) were washed in water and put on moistened plastic dishes 30 cm. Water was sprinkled daily to keep the plastic dishes moistened to encourage sprouting. After 28-30 days potatoes produced sprouts of 5-7 cm long while the sweet potatoes produced sprouts of 25-35 cm. Then the insects were transferred with the aid of camel hairbrush to the potatoes sprouts and sweet potatoes reared in laboratory conditions. The mealybug females settled on potatoes sprouts started to egg laying. The crawlers emerged out and started feeding and developed to adults. The newly adult females were separated and placed on new potato sprouts kept with the help of fine camel hairbrush. Biological studies were started from the egg stage which was laying from the second generation females. A total of 40 eggs laid from different females on the same day were observed and followed to study the biological aspects. The crawlers were observed daily in the morning by a stereomicroscope (X 15) to determine the nymphal instars duration with checking for exuvia which were visible through the loose waxy filaments. The preoviposition, oviposition and postovipostion periods for female were calculated. Longevity, life cycle and generation periods were also registered. The eggs laid by mealybug females were examined under binocular microscope and counted for calculating fecundity. The number of males out of the total population that survived to adult stage was calculated. Data were statistically analyzed of variance (ANOVA) using (SAS Institute, 1998). Means were compared using least significant difference (LSD) test.

Results and discussion

The study was carried out in Scale Insects and Mealybugs Laboratory, Plant Protection Research Institute, Kafer EL-Sheikh, Egypt during 2018 to study duration periods of immature and mature stages of *P. citri* on three different host plants were two varieties of potatoes (*S. tuberosum* var. spunta and *S. tuberosum* var. cara) and sweet potatoes (*I. batatas*) and the effects of host plant on the development, longevity, reproduction and some biological parameters of *P. citri*. Effect of host plants on biological aspects of *Planococcus citri* showed that in Tables (1and 2).

1. Females:

1.1. Egg incubation period:

Results tabulated in Table (1) showed that the mean incubation periods of female eggs (eggs that developed into females) on potato tubers (spunta and cara) and sweet potatoes 10.00 ±0.88, 11.11±0.81 and 10.26±0.93 days, respectively. There was significant difference among the mean incubation periods of *P. citri* female eggs on sweet potatoes and potato sprouts.

1.2. Nymphal instars:

Results presented in Table (1) showed that the first nymphal instar duration of *P. citri* female was longer on potato sprouts cara (9.05±0.71days) then on sweet potatoes (8±0.82 days) while the shortage duration was on potato sprouts spunta (7.47± 0.90

days). There was a highly significant difference between the mean duration of *P. citri* female first nymphal instar on potato sprouts (spunta and cara) compared with sweet potatoes. The mean duration of *P. citri* second nymphal instar of female was shortage on potato sprouts spunta with values of (6.21± 0.85 days) after that on sweet potatoes (7.05± 0.97 days), potato sprouts cara (7.84±0.50 days), consecutively. There was significant difference among the mean duration of *P. citri* second nymphal instar of female on three host plants (spunta, cara and sweet potatoes). The duration of *P. citri* female third nymphal instar on potato sprouts (spunta and cara) and sweet potatoes the same trend of first and second instar nymphal stage.

There was a highly significant difference between the mean duration of *P. citri* female third nymphal instar on three host plant (spunta, cara and sweet potatoes). Immature nymphal stage also, showed that a highly significant difference between the mean duration of *P. citri* female on three host plant (spunta, cara and sweet potatoes).

1.3.Adult:

Results illustrated in Table (1) mentioned that the mean duration of adult female was shorter on potato sprouts cara (13.35±1.31days) and the highest on potato sprouts spunta (15.16±1.17days). There was a highly significant difference between the mean duration of *P. citri* adult female period on (spunta, cara and sweet potatoes). The mean number of eggs laid per female (fecundity) was a high number 497.58±14.95 eggs/female on potato sprouts spunta while the less number was 434.42±45.11 eggs/female on potato sprouts cara. There was a highly significant difference between the mean number of eggs laid per female of *P. citri* on (spunta, cara and sweet potatoes).

This result was similar with that obtained by Awmack and Leather (2002) who reported that host plant quality is a key determinant of the fecundity of herbivorous insects. Components of host plant quality such as carbon, nitrogen and defensive metabolites affect potential and achieved herbivore fecundity. The adult female postoviposition period ranged from 3 to 7 days and 4 to 7 days on pumpkin fruits and potato sprouts, respectively.

Table (1): Developmental durations (mean ± SE) in days of *Planococcus citri* female reared on potato sprouts (spunta and cara) and sweet potatoes under laboratory.

Biological parameter	Spunta	Cara	sweet potatoes	P	LSD
Egg incubationperiod	10.00 ±0.88b	11.11±0.81a	10.26±0.93a	0.0007	0.57
1st instar	7.47± 0.90b	9.05±0.71a	8±0.82b	0.0001	0.53
2nd instar	6.21± 0.85c	7.84±0.50a	7.05± 0.97b	0.0001	0.52
3rd instar	8.68± 0.58b	10.26±0.87a	9.16±0.83b	0.0001	0.50
Total immature	32.37± 2.01c	38.26±1.11a	34.47± 2.59b	0.0001	1.34
Adult female	15.16±1.17a	13.35±1.31b	15.11±0.66a	0.0001	0.70
Egg /female	497.58±14.95a	434.42±45.11c	461.21±31.58b	0.0001	21.43

2. Males:

2.1.Egg incubation period:

Results arranged in Table (2) showed that the mean incubation periods of male eggs (eggs that developed into males) on potato tubers (spunta and cara) and sweet potatoes 11.44 ±0.51, 11.70±0.72 and 11.04 ±0.81 days, respectively. There was significant difference among the mean incubation periods of *P. citri* male eggs on sweet potatoes and potato sprouts.

2.2.Nymphal instars:

Results presented in Table (2) showed that the first nymphal instar duration of *P. citri* male was longer on potato sprouts cara (8. 50±0.74days) then on sweet potatoes (8.00± 0.93 days) while the shortage duration was on potato sprouts spunta (7.09± 0.75 days). There was a highly significant difference between the mean duration of *P. citri* female first nymphal instar on potato sprouts (spunta and cara) compared with

sweet potatoes. The mean duration of *P. citri* second nymphal instar of male was shortage on potato sprouts spunta with values of (6.76± 0.54 days) after that on sweet potatoes (7.29± 0.64 days), potato sprouts cara (8.00±0.71 days), consecutively. There was significant difference among the mean duration of *P. citri* second nymphal instar of male on three host plant (spunta, cara and sweet potatoes).

2.3. Prepupa and pupa:

The duration of *P. citri* male prepupa on potato sprouts (spunta and cara) and sweet potatoes were (2.15± 0.37, 3.00±0.32 and 2.60± 0.50 days), respectively. There was a highly significant difference between the mean duration of *P. citri* male prepupa on three host plant (spunta, cara and sweet potatoes). Male pupal stage, showed that a highly significant difference between the mean duration of *P. citri* male on three host plant (spunta, cara and sweet potatoes).

2.4. Adult:

Results illustrated in Table (2) mentioned that the mean duration of adult male was shorter on potato sprouts cara (1.36±0.50 days) and the highest on potato sprouts spunta (2.47±0.51 days). There was a highly significant difference between the mean duration of *P. citri* adult male period on (spunta, cara and sweet potatoes).

Table (2): Developmental durations (mean ± SE) in days of *Planococcus citri* male reared on potato sprouts (spunta and cara) and sweet potatoes under laboratory.

Biological parameter	Spunta	Cara	sweet potatoes	P	LSD
Egg incubationperiod	11.44 ±0.51a	11.70±0.72a	11.04 ±0.81b	0.0001	0.45
1st instar	7.09± 0.75b	8. 50±0.74a	8.00± 0.93a	0.0001	0.50
2nd instar	6.76± 0.54c	8.00±0.71a	7.29± 0.64b	0.0001	0.41
Pre pupa	2.15± 0.37c	3.00±0.32a	2.60± 0.50b	0.0008	0.25
pupa	2.74± 0.45b	3.26±0.45a	2.89± 0.32b	0.0001	0.28
Adult male	2.47±0.51a	1.36±0.50c	1.84±0.37b	0.0001	0.30

This result was confirmed by the findings of **Asiedu *et al.* (2014)** who studied the biology of *P. citri* on five yam varieties (*Disocorea* species) and reported that adult male lived for two to four days after the final nymphal molt. Also, **Mahmoud *et al.* (2017)** mentioned that developmental time, longevity, life cycle and generation period of *P. citri* affected when fed on different host plants.

It is concluded that the results is useful information for mass rearing for this pest to be a host to the natural enemies mass production in designing a comprehensive pest management program.

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Oviposition deterring and antifeeding activities of certain essential oils of medicinal plants against *Pieris rapae* (Lepidoptera: Pieridae)

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Abstract:

Cabbage (*Brassica oleracea* var. capitata L.) is one of the most important cruciferous in Egypt. Cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae) consider a serious pest affecting cabbage production. The present study was conducted to evaluate the repellent, antifeedant and oviposition deterrent effects of eight essential oils on *P. rapae* under laboratory conditions. Data revealed that all tested oils affected on oviposition and feeding of *P. rapae* and their effects were concentration dependent. At 1000 µg/L, mint and fenugreek oils showed the highiest repellent rates with 73.89 and 77.83%, respectively, with insignificant differences between them. Also, the highest antifeedant effect was recorded in garlic oil (87.21%) in bar with mint oil (86.93%) and fenugreek oil (79.13%). At 1000 µg/L, mint oil reduced oviposition of *P. rapae* females by 91.97% followed insignificantly by thyme oil (84.26%) and garlic oil (76.71%) in choice test. In case of no choice test, the highest oviposition deterrence index was obtained in mint (69.36%), followed insignificantly by thyme (66.46%) and garlic (57.01%). So, the application of garlic, mint and thyme oils can be combined with different biological and agricultural methods in an integrated pest management program could reduce the use of synthetic insecticides, especially in the early stage of cabbage.

Introduction

Cabbage or headed cabbage (*Brassica oleracea* var. capitata L.) is one of the most important cruciferous crops which cultivated with the aim of using leaves as human food during the various seasons. Cabbage white butterfly *Pieris rapae* L. (Lepidoptera: Pieridae) is cruciferous specialist phytophagous and consider as a serious pest affecting cabbage production in Egypt, the feeding injury caused by its larvae may reduce production to zero (Awadalla *et al.*, 2013 and Embaby and Lotfy, 2015). In conventional agriculture, farmers use pesticides to control *P. rapae*, however, with the increasing popularity of organic farming in Egypt, there is a need to use environment-friendly tools to overcome the problem of insect pests. One of the most important and crucial event in the life cycle of Lepidoptera is the selection of a suitable site for

oviposition and larval feeding. The oviposition deterrents and antifeedants have attracted a lot of research in the recent years as the first line defense against insect infestation (Ikeura *et al.*, 2012; Kordan and Gabryś, 2013; Ali *et al.*, 2017 and Zhang *et al.*, 2017). Essential oils are secondary metabolic products of plant which are volatile, natural and complex compounds. Essential oils can act as toxins, antifeedants and oviposition deterrents to many insect pests (Pavela, 2005; Yazdani *et al.*, 2014 and Kumari and Kaushik, 2016). The present study was conducted to evaluate the effects of 8 essential oils on oviposition and feeding of *P. rapae* under laboratory conditions.

Materials and methods

All experiments were conducted in the Laboratory of Plant Protection, Shandweel Agricultural Research Station, Sohag Governorate at room conditions during November and December 2019, where ambient temperature ranged between 10 and 25 ºC, and RH.% varied from 44% to 50%.

1. Insect culture:

The stock culture of *P. rapae* was maintained under laboratory conditions. For this purpose, larvae were collected from the cabbage crop in the Farm of Shandaweel Agriculture Research Station and reared in plastic cups 15 cm diameter with 10 cm in deep and fresh cabbage leaves were provided daily to the larvae till pupation. After pupation, the pupae were transferred to a wooden rearing cage (1x1x1 meter). When adults emerged, a small cotton-wool wick soaked in 10% honey solution and placed in the cage as a source of carbohydrate for adults. Cabbage plants at eight to ten-leaf stage were placed inside cage for oviposition. Cabbage leaves with eggs collected and kept in petri dish (9 cm diameter) the filter papers. Newly hatched larvae were transferred to plastic cups.

2. Essential oils:

The mainline value in the selection of the tested essential oils was their commercial availability and their phylogenetic distance to Brassicaceae on the angiosperm. Oils were obtained as ready-made oil from El-Captain Company for Extraction of Natural oils, Plants and Cosmetics, Cairo, Egypt. The essential oils of garlic (*Allium sativum* L.: Liliaceae), mint (*Mentha piperita* L.: Labiatae), thyme (*Thymus vulgaris* L.: Lamiaceae), camphor (*Cinnamomum camphora* L.: Myrtaceae), colocynth (*Citrullus colocynthis* L.: Cucurbitaceae), cumin (*Cuminum cyminum* L.: Apiaceae), fenugreek (*Trigonella foenum graecum* L.: Fabaceae) and orange (*Citrus sinensis* L.: Rutaceae) at three concentrations were used for each oil (250, 500 and 1000 µg/L).

3. Repellent activity:

Repellent assay was conducted on two cabbage leaf disks (3 cm diameter) on petri dish (15 cm diameter), one of them was dipped for 10 seconds in oil solution at the required concentration and the second distilled in water only as a control. The 4th instar larva was placed between the two disks and the disc chosen by the larva was noted. The experiment replicated six times (replicate include 10 larvae). The repellent rate is expressed according to the formula of Ikeura *et al.* (2012) as follows: Repellent rate = 100 (T / (T+C)), where T and C are the mean number larvae choosing treated and control discs, respectively.

4. Antifeedant activity:

Antifeedant activity was evaluated using a leaf disc bioassay in no choice test. Leaf discs (15.40 cm^2) were cut from fresh cabbage leaves and soaked in solutions with three concentrations of different oils for 30 seconds (control discs received distilled water only), then dried at room temperature. The fourth instar larvae were starved for 8 h. and introduced singly into the center of each petri dish. Each treatment was repeated five times. After 24 hours, the area of feeding on the leaves was measured using LI-3000A Portable Area Meter. The antifeeding index (AFI%) was calculated using the formula: AFI% = [(C - T)/(C + T)] x 100, where C and T are the areas consumed by the control and

treated leaf disks, respectively (Zhang *et al.*, 2017).

5. Oviposition deterring activity:

Two different assays were used, choice and no choice tests were adopted to evaluate the effect of eight oils at three concentrations on the oviposition of *P. rapae*. The two experiments were conducted using wooden cage (60x60x60 cm). Each cage was supplied with a piece of cotton soaked in 10% honey aqueous solution to facilitate feeding. A replicate consisted of one cage with three graved females (five days old) and six replications were performed for each bioassay. In choice test, two cabbage plants of 6–8 leaves were put in each cage, one of them was sprayed with one of the eight studied oil at the required concentration and the other was sprayed with water as control. However, in no choice test, each cage contained one plant with same treatment. The eggs were counted after 24 hours and the oviposition deterrent index (ODI%) was calculated with formula of Huang *et al.* (1995) as follows: ODI% = 100 {(C-T)/(C+T)}, where C and T were the mean number of eggs laid on control and treated plants, respectively.

6. Statistically analysis:

The data of deterrence rate, antifeeding index (AFI) and oviposition deterrent index (ODI) were statistically analysis using one-way analysis of variance (ANOVA). The differences between concentrations were subjected by L.S.D. test, however, Duncan Multiple Range Test was used to find significant differences in the activity among the studied oils **(Snedecor, 1956)**.

Results and discussion

1. Repellent activity:

Data in Table (1) showed that the tested oils differed significantly in regardless to concentration. Also, the same results were obtained for concentration in regardless to oil type. The highest and the lowest repellent activity were recorded in 1000 µg/L and 250 µg/L concentrations, respectively. It is clear that the repellent activity was concentration dependent. At low concentration (250 µg/L), no repellent effects were observed in all oils (FDI < 50%). However, in 500 µg/L and 1000 µg/L concentrations, only mint and fenugreek oils showed repellent rates more than 50%, with 63.66% and 59.13%, respectively, at 500 µg/L and 73.89 and 77.83%, respectively, at 1000 µg/L, with insignificant differences between them. No significant difference was found between 1000 µg/L and 500 µg/L for the two oils. The previous results are in agreement with Ikeura *et al.* (2012) who found that spearmint have a notable feeding repellent effect against *P. rapae* larvae with 72%. Also, the repellent effect of fenugreek oil against insect was reported by Meghwal and Goswami (2012).

Table (1): Repellent and antifeedant activities of eight essential oils against *Pieris rapae* larvae.

Oil	Repellent rate%					Antifeedant index%				
	250 µg/L	500 µg/L	1000 µg/L	F. value	L.S.D.	250 µg/L	500 µg/L	1000 µg/L	F. value	LSD
Garlic	19.11**b BC**	39.09**ab B**	48.15**a B**	4.88	20.27	24.66**b B**	76.64**a A**	87.21**a A**	65.8	12.72
Mint	32.08**b AB**	63.66**a A**	73.89**a A**	8.67	22.31	27.49**b AB**	71.90**a A**	86.93**a A**	33.3	16.67
Thyme	12.22**b C**	27.51**ab B**	44.18**a B**	7.07	18.13	24.96**c B**	50.34**b BC**	66.11**a C**	31.5	11.53
Camphor	16.80**b BC**	23.60**b B**	37.42**a B**	7.48	11.58	35.46**b A**	64.09**a AB**	72.51**a BC**	19.7	13.42
Colocynth	18.58**b BC**	24.43**b B**	37.50**a B**	5.65	12.28	25.94**c AB**	46.47**b C**	73.76**a BC**	57.0	9.71
Cumin	20.73**b BC**	24.71**b B**	47.02**a B**	6.53	16.72	14.22**c C**	22.84**b D**	36.44**a D**	23.5	7.10
Fenugreek	36.51**b A**	59.13**ab A**	77.83**a A**	4.01	31.13	19.33**c BC**	51.79**b BC**	79.13**a AB**	74.4	10.73
Orange	18.19**b BC**	21.46**b B**	34.92**a B**	5.78	11.12	20.41**b BC**	26.91**b D**	41.71**a D**	14.9	8.81
F. value	**2.77**	**7.34**	**4.92**	---	---	**3.92**	**17.60**	**36.4**		

Mean in the same column sharing similar capital letters are not significantly different by Duncan Test at P-0.05
Mean in the same row sharing similar small letters are not significantly different L.S.D. Test at P-0.05

2. Antifeedant activity:

Data in Table (1) revealed that the differences between oils were significant in regardless to concentration. Also, the effect of oil concentration was significant in all tested materials. The highest and the lowest antifeedant activity were recorded in 1000 µg/L and 250 µg/L concentrations, respectively. It is clear that the antifeedant activity was concentration dependent. The present study showed that all tested oils decreased the leaf area consumed by single larvae at the three concentrations (Figure, 1). At low concentration (250 µg/L), no antifeeding effects were observed from all tested oils (AFI < 50%). With concentration increase (500 µg/L), high antifeeding effects were recorded in garlic (76.64%), mint (71.90%) and camphor (64.09%), with insignificant differences between them. Also, thyme and fenugreek gave 50.34% and 51.79%, respectively. At high concentration (1000 µg/L), only cumin and orange did not have antifeedant effects. The highest AFI was recorded in garlic oil (87.21%) in bar with mint oil (86.93%) and fenugreek oil (79.13%), followed significantly by colocynth oil (73.76%), camphor oil (72.51%) and thyme oil (66.11%).

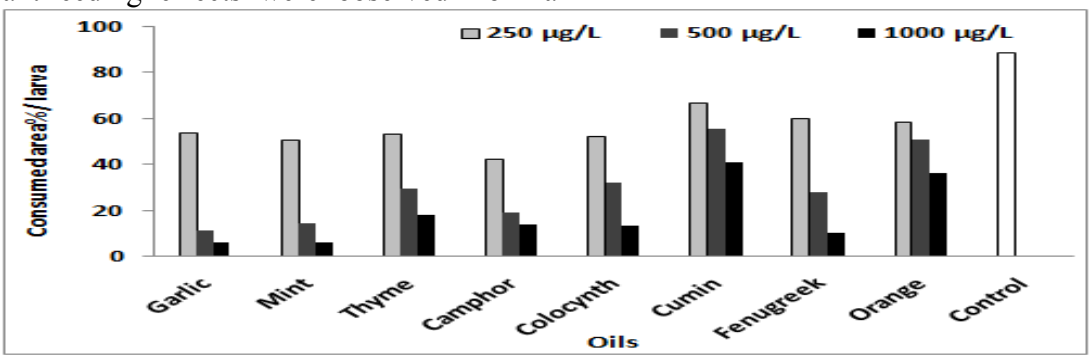

Figure (1): Leave area consumption percentages of *Pieris rapae* after the application of eight essential oils at three concentrations.

Depending on the consumed leaf area percent in Figure (1), garlic, mint and camphor strongly inhabited feeding of *P. rapae* larvae at 500 µg/L and 1000 µg/L, however, thyme, colocynth and fenugreek oils had a strong antifeeding effect at 1000 µg/L only. At 1000 µg/L, the larvae consumed approximately 13 times less food than in the control in case of garlic and mint oils. No significant difference was found between 1000 µg/L and 500 µg/L for garlic and mint oils, however, 1000 µg/L concentration differed significantly from 500 µg/L and 250 µg/L in fenugreek oil.

In previous studies, garlic and mint extracts decreased food consumption of *P. brassicae* (Khan and Siddiqui, 1994 and Ali *et al.*, 2017). Also, Sharaby and El-Nojiban (2015) reported that the garlic and mint oils exhibited antifeedant and starvation effects on *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) larvae. Many studies suggested that sulfide derivatives are the most active compounds in insect repelling in garlic oil (Dugravot *et al.*, 2004 and 2005 and Mann *et al.*, 2011). However, menthol and menthone may be the most active compounds in the case of mint (Gracindo *et al.*, 2006 and Tsai *et al.*, 2013). Also, Kordan and Gabryś (2013) showed that monoterpenes such as thymol in thyme oil had active deterrent effects against *Pieris brassicae* (Linnaeus) (Pieridae: Lepidoptera).

3. Oviposition deterrent activity:

3.1. Choice test:

Data in Table (2) showed that the differences between various tested oils were significant at the three concentrations used. It is clear that the highest oviposition deterrent index was obtained from the highest concentration, while the lowest one recorded in the lowest concentration. The recorded oviposition deterrent indexes were lower than 50% (7.16% to 31.85%) at 250 µg/L. Mint oil decreased the mean number of eggs by 59.24% followed insignificantly by thyme

(49.60%) at 500 µg/L, however, the rest oils showed low oviposition deterrent activity (ODI < 50%). At high concentration, mint oil reduced oviposition of *P. rapae* females by 91.97% followed insignificantly by thyme oil (84.26%) and garlic oil (76.71%). Also, the oviposition deterrent activities of camphor and colocynth oils increased with increase of concentration to record 68.14% and 57.56%, respectively. On the other hand, the oils of cumin, fenugreek and orange seem to be ineffective on *P. rapae* oviposition. At 1000 µg/L, the untreated plants received approximately 5, 26, 14, 5 and 3 folds more than the treated plants with garlic, mint, thyme, camphor, colocynth, respectively (Figure, 2).

Table (2): Oviposition deterrent activity of eight essential oils against *Pieris rapae* females in choice and no choice tests.

Oil	Oviposition deterrence index									
	Choice					No choice				
	250 µg/L	500 µg/L	1000 µg/L	F. value	L.S.D.	250 µg/L	500 µg/L	1000 µg/L	F. value	L.S.D.
Garlic	8.94**c B**	35.11**b BC**	76.71**a ABC**	25.2	20.541	4.74**c B**	27.20**b ABC**	57.01**a AB**	69.8	9.46
Mint	31.85**c A**	59.24**b A**	91.97**a A**	18.3	21.213	25.39**c A**	46.11**b A**	69.36**a A**	65.3	8.21
Thyme	25.50**c A**	49.60**b AB**	84.26**a AB**	19.3	20.262	20.74**c A**	40.49**b AB**	66.46**a A**	14.7	18.02
Camphor	9.36**c B**	30.88**b C**	68.14**a BC**	31.1	16.071	8.11**b B**	19.75**ab BC**	42.96**a BC**	4.25	25.94
Colocynth	8.16**c B**	38.97**b BC**	57.56**a CD**	37.4	12.303	6.01**b B**	25.07**ab ABC**	42.85**a BC**	4.47	26.28
Cumin	7.16**c B**	28.17**b CD**	39.76**a DE**	18.9	11.470	5.52**a B**	25.11**a C**	26.08**a CD**	3.06	N.S.
Fenugreek	11.41**b B**	13.83**b DE**	28.70**a E**	12.8	7.8894	6.11**b B**	12.56**ab C**	19.92**a D**	5.30	9.05
Orange	7.85**b B**	10.65**b E**	29.60**a E**	11.4	10.560	7.35**a B**	10.84**a C**	20.83**a D**	2.87	N.S.
F. value	6.87	9.76	14.6	---	---	18.45	3.40	10.16	---	---

Mean in the same column sharing similar capital letters are not significantly different by Duncan Test at P-0.05

Mean in the same row sharing similar small letters are not significantly different L.S.D. Test at P-0.05

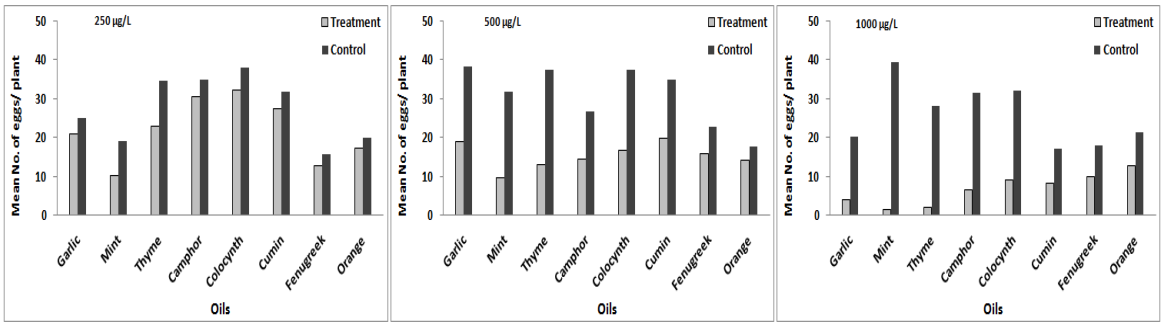

Figure (2): Mean numbers of eggs laid by females of *Pieris rapae* on cabbage plants treated with eight essential oils at three concentrations in choice test.

3.2. No choice test:

In the same line, the tested oils varied significantly at the three concentrations used. The differences between concentrations were significant in all essential oils, except in cumin and orange. The highest oviposition deterrent index was obtained from the highest concentration, while the lowest one recorded in the lowest concentration. At 250 µg/L and 500 µg/L, the eight tested oils showed ineffective oviposition deterrence by ODI less than 50%. The oils ranged between 4.74 to 25.39% at 250 µg/L. and between 10.84% to 46.11% at 50ko µg/L. Only at 1000 µg/L concentration, three oils were reduced oviposition of *P. rapae* females with ODI more than 50%. The highest effect was obtained in mint (69.36%), followed insignificantly by thyme (66.46%) and garlic (57.01%) (Figure, 3).

The present results are in agreement with Lundgren (2008) who found that extracts of thyme and onion decreased *P. rapae* oviposition on cabbage. Also, Ribeiro *et al.* (2015) reported that garlic, mint and thyme essential oils gave oviposition

repellence (IDO>80%) rates against *Anticarsia gemmatalis* Hubner (Lepidoptera; Noctuidae) , especially in the free-choice experiment. Magierowicz *et al.* (2019) found that the lowest number of *Acrobasis advenella* (Zinck.) (Lepidoptera, Pyralidae) eggs was observed for thyme treatment. Non host plant chemical compounds may play a significant role in the rejection of plants as hosts by female butterflies (Hussain, 2015), such as sulfide compounds in garlic oil (Mann *et al.*, 2011), menthol and menthone in mint oil (Tsai *et al.*, 2013) and thymol in thyme oil (Kordan and Gabryś, 2013).

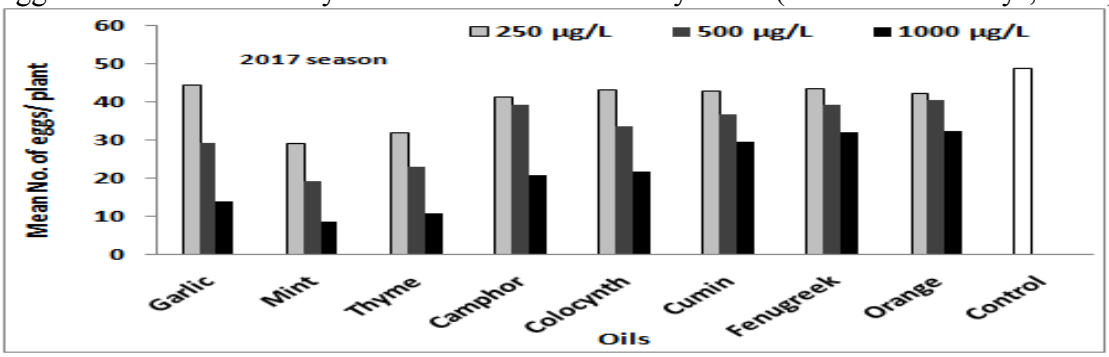

Figure (3): Mean numbers of eggs laid by females of *Pieris rapae* on cabbage plants treated with eight essential oils at three concentrations in no choice test.

From the present data, it is clear that the essential oils of garlic, mint and thyme reduced food consumption of *P. rapae* larvae and decreased the oviposition of its female. So, the application of these oils can be combined with different biological and agricultural methods in an integrated pest management programs could reduce the use of synthetic insecticides, especially in the earlier stage of cabbage. However, further research is needed to investigate the effect of plant essential oils that have the strongest effect in the field conditions.

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Nano bio pesticide for controlling cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract:

Nanoparticle compounds such as *Moringa oleifera* Lam. leaf powder, boiled white and brown eggshells powder for chickens and kapritia spring water form Siwa Oasis in Egypt, these natural materials were evaluated for first time under laboratory conditions against the 4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). The results investigated that new nanoparticles of all eggshells and *M. oleifera* powder and kapritia water affected on biological aspects of target pest such as, larval mortality were recorded that,١٠, ٨, ٦ and 4% for *M. oleifera*, kapritia water, brown and white eggshells, respectively, compared with control 1%, the intermediated shapes were recorded during this studied. Also adults moths emergency percentage of *S.littoralis* are decreased to 89 , 82, 73 and 65 % after treatment with white eggshells, brown eggshells , *M. oleifera* leaf powders and kapritia Siwa water, respectively, compared with control 93%. Subsequently, adults malformed increased after all treatments; fecundity (Eggs/ female), fertility and also were affected. These compounds considered as nanoparticle, these innovation technologies to enhance our country developmental strategies in order to achieved sustainable agriculture and integrated crop management by alternative insecticide, environmental protection form pollutant, increase natural enemies for insect and food safety for better human healthy for a new generation.

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is the most dangerous pests in Egypt and Africa, causing significant economic loss of cotton, tomato, lettuce, strawberry and other vegetables crop in both greenhouses and open fields (Abd El-Razik and Mostafa, 2013 and EPPO, 2014). *Moringa oleifera* Lam. is medicinal plant with many pharmacological properties, leaves are a nutrient-dense food with high concentrations of protein, carbohydrates, fiber and a good source of several vitamins and minerals such as vitamins A, B, C, D, and E; folic acid; nicotinic acid; iron; calcium; zinc; potassium; magnesium and copper (Singh and Singh, 2019 and Walia *et al*., (2019). The chemical constituents of *M.oleifera* stems, leaves, flowers, pods and seeds are alkaloids, phenolicacids, gallic,

chlorogenicacid, ferulicacid, glucosinolates, flavonoids, quercetin, vanillin and kaempferol, tannins, steroids, coumarins, saponins, quinones and resins such as which have nutritional, pharmaceutical and antimicrobial properties (Anwar *et al*., 2007; Mbikay , 2012 and Mensah *et al*., 2012). Siwa Oasis (29.12° N, 25.43° E) is an isolated location in the Western Desert of Egypt, approximately 330 km from Matrouh City situated in the Northern Mediterranean Coastal Zone, chemical and radioisotopic constituents of ground water resources were Cl-, SO42-, CO32-, HCO3-, Na+, K+, Ca2+ and Mg2+, Al, B, Ba, Cd, Co, Fe, Cu, Mn, Mo, Ni, Pb, Sr, V, Zn, Si and Cr, 226Ra, 232Th and 40K. (El-Sayed *et al*., 2017). Siwa Oasis is eco-geographically isolated and is a nature reserve, so using of "Good Agricultural Practice" (GAP) is essential for agriculture production there such as using of various bio fertilization techniques (Hamed, 2018). Chicken eggshell (ES) is an aviculture by product that has been listed worldwide as one of the worst environmental problems, the chemical composition consists of calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic matter (4%) such as type X-collagen, sulfated polysaccharides and other proteins (Bashir and Manusamy , 2015). ES chemical composition and availability makes ES a potential source of filler for bulk quantity, inexpensive, lightweight and low load-bearing composite applications. There have been several attempts to use eggshell components for different applications; adding ES into food supplements for people and animals, art projects galore include egg shells as an ingredient, mosaics, paints, paper making, dying and carving (Yi *et al.,* 2004). The aim of this research work is to study the control of *S. littoralis* by using a new natural nanoparticles like bio-pesticide compounds such as *M. oleifera* leaf powder, boiled white and brown eggshells powder for chickens and kapritia spring water form Siwa Oasis.

Materials and methods

1.Laboratory studies:

1.1. Sample preparation:

1.1.1. Eggshells:

The samples of boiled chicken eggshellswhite and brown color was collected,then rinsed with clean water, then eggshells drying under hot sun for 3 days to dry then crushing themby mini food chopper or a coffee grinder to make a powder.

1.1.2. *Moringa oleifera* Lam. leaf powder:

Sample of leaves were obtained from farmer on Ismalia Governorate during summer season, then dried and grinding to powder.

1.1.3. Samples of kapritia Swia water

Samples of kapritia Swia water collected during early summer seasons 2018, used as it is without any additives then dipping the castoroil leaves on solution then dray under room temperature and put all leaves in glass gar contain 50 4th instar larvae of target pest

2. Insect culture:

The fourth instar larvae of cotton leafworm *S. littoralis* were obtained from laboratory colony, Cotton Leafworm Department, Plant Protection Research Institute, ARC, under laboratory conditions at 25 ± 1ºC and 65–70 ± 5 RH.

3. Bioassay of nanoparticle against 4th instar larve of *Spodoptera littoralis*:

3.1. Experiments were carried out under laboratory conditions, ten larvae of 4th instar of *S. littoralis* perreplicate for each treatment, it was obtained from culture reared on castor oil leaves for several generations.

3.2. Castor oil leaves were dipping on 50 ml of kaperitia water without any additives (absolute), then dry the water from the surface of leaves.

3.3. Estimated weight with 0.5 gm/leave of *M.oleifera* leaf powder used for fogging castor oil leaves for each replicate.

3.4. Eggshells powder white and brown used as nanoparticles by 0.5 gm/leave for each replicate and putted leaves as a food material on glass jars contain ten 4th instar larvae of *S. littoralis* for five replicates, then kept at

incubator at 25 ± 2° C and 60 – 65% RH., as shown in Figure (1) . All biological parameters of tested *S. littoralis* stages, fed on treated leaves such as larval duration, pupation, pupal duration,pupal weight, % moth emergence, sex-ratio, fecundity (eggs/ ♀), and hatchability %.

3.5. Statistical analysis:

Statistical analysis was carried out using Analysis of variance (ANOVA) was conducted on all data (SAS Institute, 1996).

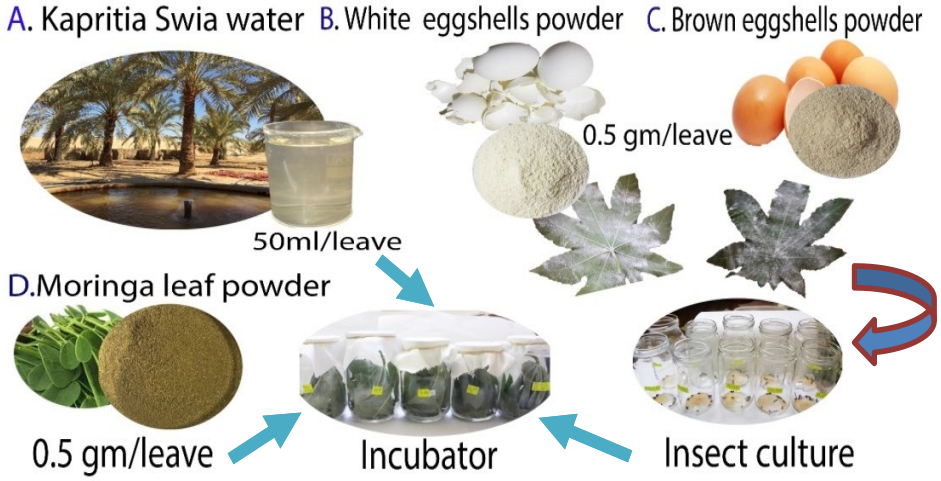


Figure (1): Novel natural bio-pesticide.

Results and discussion

1. Effect of nanoparticles material on the different biological aspects of the cotton leafworm *Spodoptera littoralis*:

1.1. Immature stages of *Spodoptera littoralis*:

The present results confirmed that, *M. oleifera* leaf powder, boiled white and brown eggshells powder for chickens and kapritia spring water form Siwa Oasis are considered a novel natural material such as green pesticide . Data as shown in Table (1) ,4th instar larval duration had impact after feeding on all treatments that caused larval mortality were 10, 8, 6 and 4%, respectively, compared with control 1%, pupal duration recorded highly significant between all treatments that caused highly reduction of pupation, it was recorded that 88% in case treatment kapritia water, while 90% after feeding on *M. oleifera* leaf powder, 92% brown eggshells powder and 96 % white eggshells powder. Both pupal weight of control and treated larvae on brown eggshells powder recorded no significant, while highly significant between other treatments. The maximam effect of kapritia water, *M. oleifera* leaf powder, brown and white eggshells powder on pupal stage induced 34, 26, 17 and 10% mortality, respectively, but 4 % only in control (Table, 1). Goswami *et al* . (2010) investigated that, the nanoparticles of SiO2 show nearly 100% mortality against *Sitophilus* oryzae L. (Coleoptera: Curculionidae). El-Sayed *et al*. (2017) recorded kapritia spring of Siwa Oasis content, major ions included chloride (Cl-)3702 mg/l, sulfate (SO42-)1800 mg/l, carbonate (CO32-), bicarbonate (HCO3-), sodium (Na+)1700(mg/l), potassium (K+), calcium (Ca2+) and magnesium (Mg2+590 mg/l)) and heavy metals included aluminum (Al), boron (B), barium (Ba), cadmium (Cd), cobalt (Co), iron (Fe), copper (Cu), manganese (Mn),molybdenum(Mo), nickel (Ni), lead (Pb), strontium (Sr), vanadium (V), zinc (Zn), silicon (Si) and chromium (Cr), that explain the highly mortality by treatment of kapritia water against cotton leafworm *S. littoralis.*

Table (1): Nanoparticles material affected on biological aspects of *Spodoptera littoralis* (immature stages) under laboratory conditions.

Biological aspect of 4th instar larvae of *Spodoptera littoralis*	Treatments					F.
	Kapritia Swia water	*Moringa oleifera* leaf powder	Brown eggshells powder	White eggshells Powder	Control	
Larval duration (days)	11±0.1 a	10 ±0.1 b	9.6 ±0.1 c	8.2±0.1 d	9.8 ±0.1 bc	75.08
% Larval mortality	6%	10%	8%	4%	1%	
Pupal duration(days)	12 ± 0.3 ab	13 ± 0.3 a	11.75± 0.2b	9.8 ± 0.1 c	9.5 ± 0.1 c	56.44
Pupal weight (mg)	326.05±11a	297.0±11b	260.6±7.7 c	291.09 ±5.1 b	244.0 ±8.1 c	11.48
% Pupation	88 %	90 %	92 %	96 %	99 %	
% Pupal mortality	34 %	26 %	17 %	10 %	4 %	

Means followed by different letters in each column are significantly different (P, 0.05).

The present results coincide with Dimetry *et al.* (2017) mentioned that acceptability and antifeedant effect of *M. oleifera* leaves as host plant towards 1st and 4th larval instars of the cotton leafworm *S.littoralis* in comparison with castor oil leaves as a control and leaf extract as organic insecticide reported by (Ndubuaku *et al*., 2015).

1.2. Mature stages of *Spodoptera littoralis*:

Adult emergence percentage as illustrate on (Table, 2) low percentage had recoded 65,73, 82 and 89 %, respectively, in all treatments, on the other hand control was 95%. Results revealed that the highly percentage of adults malformed 62, 46, 39 and 21 % after treatment with brown eggshells powder, kapritia water, white eggshells powder and *M. oleifera* leaf powder, respectively. On the other hand, the treatments of different affected on pupal stages induced a high mortality, male and female malformed also that as shown in (Table, 2) compared with normal pupae on control treatment. The results consistent with **Adenekan (2019)** who recorded that, a significant difference in the eggs laid of bruchid beetles in cowpea seeds, the number of adults that emerged and lowest mean number of eggs of 6.40 was laid when 0.5 g *M. oleifera* flower powder was applied.

Table (2): Nanoparticles material affected on biological aspects of *Spodoptera littoralis* (mature stages) under laboratory conditions.

Biological aspect of 4th instar larvae of *Spodoptera littoralis*	Treatments					F.
	Kapritia Swia water	*Moringa oleifera* leaf powder	Brown eggshells powder	White eggshells powder	Control	
% Moth emergence	65 %	73 %	82 %	89 %	95%	
% Moth malformed	46 %	21 %	62 %	39 %	10 %	
% ♀ Moth malformed	23 %	12 %	19 %	4 %	5 %	
% ♂ Moth malformed	23 %	9 %	43 %	4 %	5 %	
Sex ratio (Female: male)	1:1	1:1	1:1	1:1.3	1:1.6	
Fecundity (Eggs / ♀)	850±183 bc	710 ± 154 c	1100 ±168.8 bc	1630 ± 155 ab	2230 ± 301.0 a	6.9

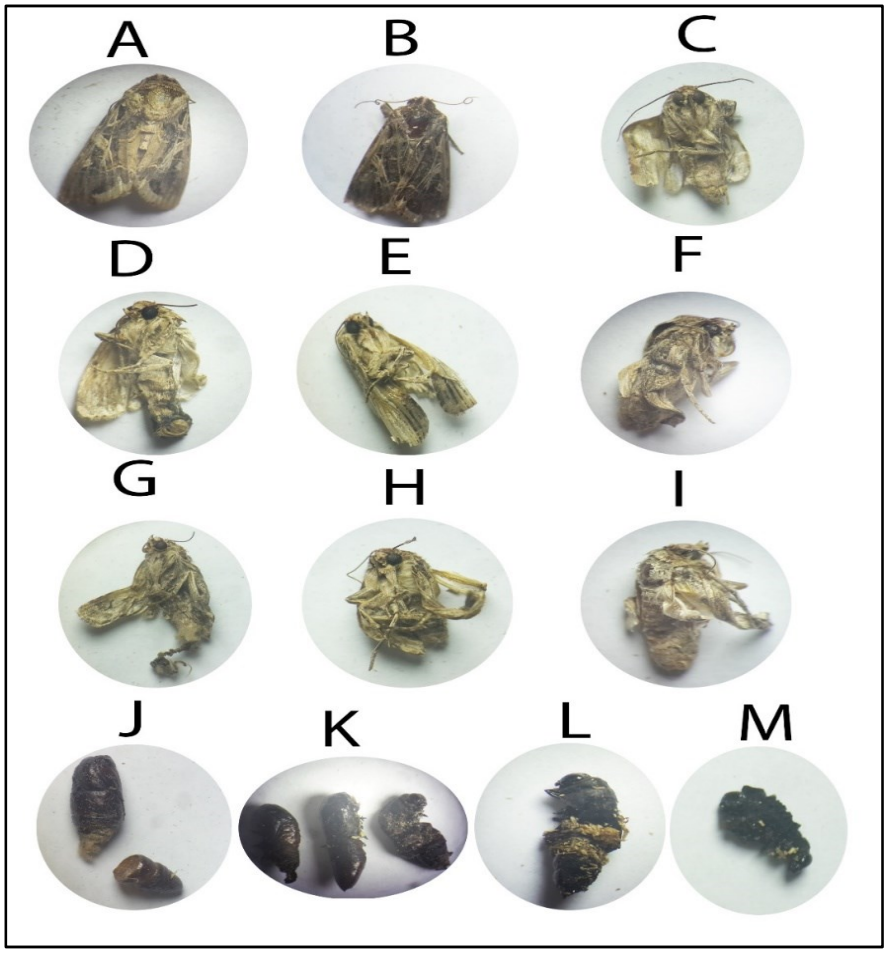
Means followed by different letters in each column are significantly different (P, 0.05).

As shown in (Table, 3 and Figures 2, 3 and 4) the different scores of malformed of the target pest after treatment with the new natural materials that highly affected so we need a lot of studies to improve this material for more efficiency and safety for used as a bio-insecticide to control insect pest and alternative the insecticides. This research work also will be helpful to use a new natural materials safe and useful on living organisms and safety on the environment and increase crop yield and sustainable agricultural.

Table (3): Scoring of larval – pupa – adult of *Spodoptera littoralis* after treated with nanoparticles material.

Scores	Characteristics	Kapritia Swia water	*Moringa oleifera* leaf powder	Brown eggshells powder	White eggshells powder
0	Adults seemed to be normal			+	+++++++
1	Adults with wings slightly curled	++++	++	+++++++	++
2	Adults wingless	+	++	+++++ +++++	++++++++
3	Adults severely curled	+++++++	++++++	+	
4	Adults attached with puprium	+	+	+	
5	Partial emergency (head and thorax)		+		
6	Partial emergency with head only		+		
7	Posteriorly partial emergency	+			+
8	Dead pupa	+++++ +++++ +++++	+++++ ++++	+++++ ++++	+++++
9	Larval pupal intermediate	+	++	+++	+
10	Dead larvae	++	++++	+	++

(+):Number of malformed adult moths



A: Normal Adults (Control), B: Adults seemed to be normal, C:Adults with wings slightly curled, D:Adults wingless, E:Adults severely curled, F:Adults attached with puprium. G: Adults wingless, H: Adults attached with puprium, I: Adults attached with puprium, J:Posteriorly partial emergency, K:Dead larvaeL:Larval pupal intermediate and M: Dead larvae.

Figure (2): Malformed of *Spodoptera littoralis* after treatments with kapritia Swia water.

A: Adults seemed to be normal, B: Adults with wings slightly curled, C:Adults severely curled, D:Adults severely curled, E:Adults wingless, F:Adults severely curled, G:Adults attached with puprium , H:Dead pupa and I:Larval pupal intermediate.

Figure (3): Malformed of *Spodoptera littoralis* after treatments with brown eggshells powder.

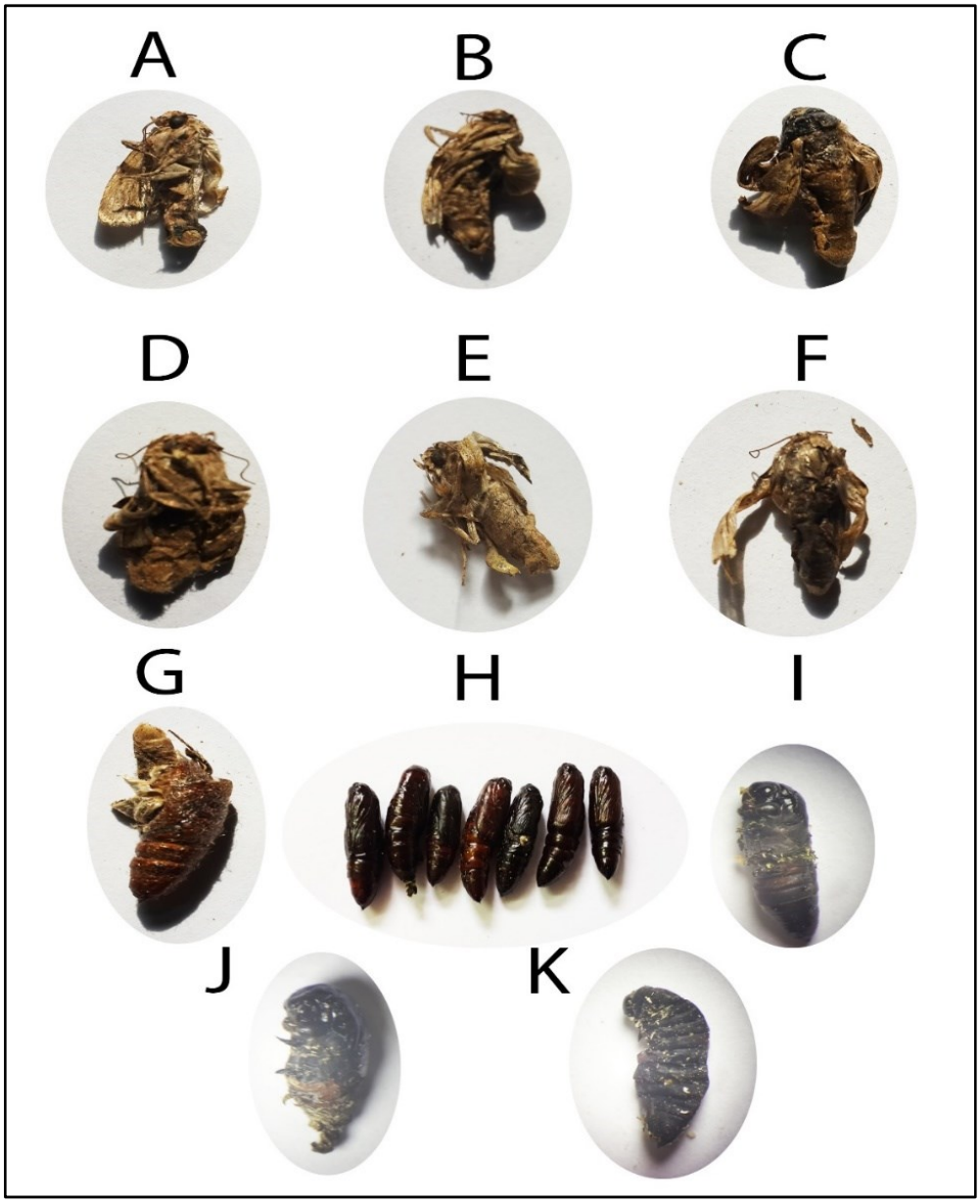


Figure (4): Malformed of *Spodoptera littoralis* after treatments with *Moringa oleifera* leaf powder.
A: Adults with wings slightly curled, B: Adults severely curled, C:Adults severely curled, D :dults severely curled , E:Adults wingless, F:Adults wingless, G:Adults attached with puprium, H: Dead pupa, I: Larval pupal intermediate, J: Larval pupal intermediate and K: Dead larvae.

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Acute toxicity of furosemide under laboratory condation and animals farm against *Rattus rattus* (Rodentia:Muridae)

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Abstract:

Acute toxicity of furosemide (40 mg) and its effect on black rats *Rattus rattus* L. (Rodentia:Muridae) under laboratory and animals farm condations. LD_{50} and palatability were determined under laboratory condation. The reduction of *R. rattus* were evaluated in Kafr EL-Zayat- Gharbiya Governorate. The results recorded that the mandatory feeding bait on furosemide bait caused 100% mortality for black rats (*R. rattus*) after four days. Free- alternative feeding bait (furosemide bait) gave 80 % mortality and acceptance was 51 %. The effect of furosemide bait investigated 89% mortality in animals' farm. The population reduction in *R. rattus* was very high in the farm. So, this compound may used to reduce *R. rattus* especially in animals farm or stores because the furosemide kept in black bags to prevent the degradation of compound by light exposure. Also, may be can using in rodents control as a safety compound.

Introduction

Rodents are important pests in Egypt. They cause translation of diseases to human, they damage crop plants and cause economic losses for agriculture (Meehan, 1984). Rodenticides play important role in rodents control. The use of rodenticides causes poisoning for farm animals and domestic animals (Vipin and Tripathi, 2006). Rodents acquired resistance due to repeated and wrong use of the rodenticide (Witmer *et al.,* 2007). So, some chemical compound products as drugs cause using to reduce the rodents and they are non toxic to human.

Mode of action of furosemide inhibits tubular reabsorption of sodium and chloride in the proximal and distal tubules, as well as in the thick ascending loop of Henle by inhibiting sodium-chloride co-transport system resulting in excessive excretion of water along with sodium, chloride, magnesium and calcium (Kidney Dis., 2010). In addition, the high dose of furosemide (Suki *et al.,* 2015) found that the acute treatment of hypercalcemia with furosemide recorded the sodium, potassium and magnesium decreased comparing with control in human. The loop diuretics furosemide, bumetanide and torsemide act from the lumen to inhibit the Na-K-2Cl cotransporter (NKCC2, encoded by SLC12A1) along the thick ascending limb and macula densa. As organic ions, they bind within the translocation pocket on the transport protein by interacting with the chloride-binding site. Because they are larger than chloride, they are not transported

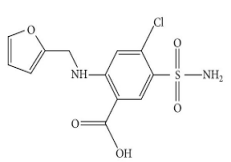
through the pocket and thereby inhibit the transporter (Ellison, 2019). In addition increasing urine flow, furosemide is a direct renal vasodilator and also increases tubular flow rates, results in greater contrast dilution within the renal tubule, decreases the metabolic workload of the kidney and subsequently decreases oxygen demand as well as prevents or ameliorates ischemic injury (Maretrio *et al.,* 2012). Furosemide causes hepatic necrosis and increase of alanine aminotransferase (ALT) activity in mice (Williams *et al.,* 2007). The oral LD_{50} of furosemide values were 2700 to 7537 mg\kg in rat (EMA., 2000).

The aim of this work is to study the LD_{50} determination of furosemide on *Rattus rattus* L. (Rodentia:Muridae) toxicity and palatability of the compound under laboratory conditions and apply it in animal's farm to reduce the population density of rats.

Material and methods

1. Compound:

- Chemical name : Furosemide (drugs)
- Synonyms: 5-(aminosulfonyl)-4-chloro-2[(2-furanylmethl)amino].
- Structure:



2.Test animals:

Adult wild black rats, (*R. rattus*) caught by rat traps from fields and stores. The rats were acclimatized individually in cadge for 15 days. They fed on free diet and water. The weight of rats ranged about from 150 to 200g. Total rats were 50 rats. The rats divided into six groups; the first group was to estimate oral dose of LD_{50} furosemide. The first group divided to 5 subdivides groups for 5 serial doses and the second group was as a control. The non-choice feeding determined with five rats (Third group) and the fourth group as a control. The fifth group was for free-choice feeding test and the sixth group was as a control.

3. Experimental laboratory:

3.1. LD_{50} determination of furosemide:

The acute lethal dose (LD_{50}) was determined on black rats (*R. rattus*) using oral dosage technique. Serial doses measured as mg/kg (b.w.). The rats were fasted before treatment for 12 hours. The rats were divided to six groups, each group contained five replicates. The first group was as a control. From second to six groups were treated with different doses. The LD_{50} value was evaluated according to Weil (1952).

3.2. Non-choice feeding bait:

The rats fed on challenge diet (50g) and water for four days the amount of consumed bait were weighted daily. Then the rats fed on treated crushed maize mixed with furosemide powder (25.12% active ingredient) and water. After that, the consumed amount was weighted daily for four days. Finally, the treated bait removed and the rats fed on challenge diet and water. During this the experimental, the mortality was recorded. This method was according to Shefte *et al.* (1982).

3.3.Free-alternative feeding bait:

Free-choice bait method is very important to determine the acceptance percentage for furosemide bait. In the first all, the rats fed on three dishes. Each dish contained of water, challenge diet and crushed maize mixed with furosemide bait (25.12% active ingredient) for four days. Then, the amount of challenge diet and furosemide bait in each dishes were weighted daily through four days. After that, the challenge diet and furosemide bait were removed. Finally, the rats fed on crushed maize only and observed for 15 days with the amount of diet was weighted daily according to Russell *et al*. (1989).

A parallel control test was used standard diet. From previous methods was carried out to increase the acceptance of furosemide bait. The acceptance bait was recorded using the following equation (Mason *et al.,* 1989).

$$\text{Acceptance \%} = \frac{\text{Treated bait consumption (g)}}{\text{Treated bait consumption (g)+challenge diet (g)}} \times 100$$

4. Test field:

The location was recorded in animal's farm in Kfour Belshay Village, Kafr EL-Zayat Centre, Gharbiya Governorate. The area was about 350 m^2 and the infection was with black rats, *R. rattus*. Furosemide bait was estimated under animals farm condition. The population density of *R. rattus* (dominant species) was evaluated. The method was conducted as follows; pretreatment challenge diet (small black bags) was put inside the plastic pipes and it distributed inside and outside the animals farm. Then, the consumed amount of challenge bags were weighted daily for four days. After that, the amount of pretreatment removed. Then, the treated bait (furosemide mixed with crushed maize) (25.12% active ingredient) distributed in and out the animal´s farm for four days and the consumed amount in bags was collected and weighted daily. Finally, posttreatment was challenge diet bags. These bags were distributed in and outside of animal´s farm for four days. Then, it collected and weighted daily. The percentage of population reduction was calculated according to **(Mason *et al.*, 1989).**

$$\text{Population reduction \%} = \frac{\text{Pretreatment consumption (g)} - \text{posttreatment consumption (g)}}{\text{Pretreatment consumption (g)}} \times 100$$

Result and discussion

Data in Table (1) indicated that the oral doses of furosemide were 3094, 3681, 4381 and 5200 mg/kg b.w. which gave 50,100,100 and 100 daily mortality % for four days for every groups compared with control. These results revealed that the LD_{50} value was 4745mg/kg b.w. These results were similar the oral LD_{50} of furosemide values were 2700 to 7537 mg\kg in rat (EMA., 2000). The oral LD_{50} of torasemide in the rat is 5 g/kg. When overdose occurs, there is a marked diuresis with the danger of loss of fluid and electrolytes which has been seen to lead to somnolence, confusion, hypotension, hyponatremia, hypokalemia, hypochloremic alkalosis, hemo-concentration dehydration and circulatory collapse. The mortality may be due to disorder of liver function, this result agreed with Williams *et al.* (2007) recorded that the metabolism and toxicity of furosemide in the Wistar rat and CD-1 mouse: a chemical and biochemical definition of the toxicophore which found the hepatotoicity of liver and caused toxicity in mice after 24h. This result observed the loss of weight and loss of water in urine may be due to the toxic in renal.

Table (1): LD_{50} determination of furosemide bait on *Rattus rattus* under laboratory condition.

Dosage mg/kg	Mortality %	LD_{50} (mg/kg)
3094	50	4745
3681	100	
4381	100	
5200	100	

1. Body weights of test animals:

The results in Table (2) observed that the lethal dose of furosemide bait caused loss in body weight of rats, this occurred may be due to the profound dieresis through four days the rats fed on excessive amount of furosemide bait. This observation similar the use of higher doses of furosemide was associated with higher net fluid and weight loss at the cost of an increased incidence of rising serum creatinine (Felker *et al.,* 2011 and Inomata *et al.,* 2017).

Table (2): Effect of furosemide bait on weight bodies of *Rattus rattus* through the feeding four days.

Compound	Body weight before treatment (g)	Body weight after treatment (g)
Furosemide bait	180	150
	200	180
	200	190
	200	170
	170	140

2. Mandatory feeding bait:

In Table (3) the results recorded that the non- choice feeding on furosemide bait cause 100% mortality for black rats (*R. rattus*) with the average bait consumption of 17g. Also, data indicated that the time required to death ranged between one to four days with average 0.5 day. Furosemide bait gave compelate mortality for the rats with non-choice may after four days be due to the rats fed only on mandatory food (furosemide bait) and excessive amounts daily from furosemide bait for four days. So, furosemide compound was more toxic on rats compared the other recommendeded compounds as anticoagulant and other compound. Kandil *et al.,* (2015) recorded that abamectine bait achieved 80% mortality and diphcinone caused 73.8 % mortality after three days treatment in non-choice test. This mortality was lesser than the mortality which caused with furosemide bait which gave 100% after 4 days of treatment. The effect of furosemide mortality on rats in non- choice feeding test similar the effect brodifacoum mortality on rats in mandatory feeding test. Lund (1981) recorded that 0.005% bredifacoum gave 100% mortality after one day feeding against *Mus musculus* L. , *R. rattus* and *Rattus norvegicus* (Berkenhout) (Rodentia:Muridae). In the no-choice laboratory feeding tests with brodifacoum, 100% animals died in all treated groups and 0% died in the control groups caused with dose of brodifacoum (Frankova *et al.*, 2019).

Table (3): Effect of furosemide bait on *Rattus rattus* with non-choice feeding method for four days under laboratory.

Feeding method	Average treated bait consumption(g)	Mortality %	Time of death	
			Range	mean
Non- choice	17	100	1-4	0.5

3. Free-alternative feeding bait:

Data in Table (4) showed that the free-choice feeding on furosemide bait gave 80% mortality with the average time to death one day and half. The average of treated bait consumption was 4.93g. While, the average of challenge bait consumption was 4.7g. From the previous result, the acceptance % of furosemide was 51 %. The furosemide bait proved to be attractive for black rats in free-choice test more than the challenge bait in consumption may be due to furosemide forms of chlorobenzoic acid that is 4-chlorobenzoic acid substituted by a (furan-2-ylmethyl) amino acid and a sulfamoyl group. So, the results were explaining the bait of furosemide very attractive to rats. Furosemide bait was very attractive compared with the challenge bait for *R. rattus*. So, the acceptability of furosemide was higher than other compound, although, the black rats prefer the vegetable but it prefer the furosemide mixed with crushed maize in feeding compared with the challenge diet.

Table (4): Effect of furosemide bait on *Rattus rattus* with free-choice feeding method for four days.

Feeding method	Average treated bait consumption(g)	Average challenge bait consumption(g)	Mortality %	Acceptance %	Time of death	
					Range	mean
Free- choice	4.93	4.70	80	51	2-4	1.5

The other compound as potassium tartrate which added for bromadiolone in free choice test caused acceptability 10.55% (ELgohary and Abou EL-Kheer, 2018). The effect of furosemide bait cause acceptability 51.1% and the level of mortality was 80%. It is very important to reduce of rats. While palatability and efficacy of bromadiolone rodenticide black rat previously exposed to environmental conditions (Nakagawa *et al.,* 2015) revealed that the mortality was considered to be satisfactory, showing 75% in the two choice food trial and 100% in non-choice food trial. The high palatability of the bait may result in repeated feeding for several days; nevertheless, exclusive feeding on the

bait is uncommon under natural conditions, as mice are diffuse and sporadic feeders, regularly visiting many feeding points during their feeding activities (Crowcroft, 1959). Moreover, recently showed that anticoagulant bait has the potential to substantially decrease food intake shortly after the initial consumption of the bait, which shortens the activity period of the over-dosed individuals (Frankova *et al*., 2017).

4. Field experimental:

The evaluation of furosemide baits against black rats *R. rattus* in animal's farm were explained in Table (5). The results showed that the average consumption of crushed maize in pretreatment was 3190g from 3200g. Also, the results revealed that the bait consumption of posttreatment was 352 g from 3200g while the consumption of treated bait was 3156g from 3200 g. From the previous results, the population reduction was 89% and the consumption rate was decreased comparing with the consumption rate before using furosemide bait. Furosemide bait was very toxic on rats in animals farm comparing with other results which traditional rodenticides compound. Although furosemide is drugs for human, it is highly toxic and caussed death for rats in animals farm.

Table (5): Effect of furosemide bait on *Rattus rattus* for four days under (animal's farm).

Treatment	Total weight of consumption (g)	Bait consumption (g)			Population reduction %
		Pretreatment	Treated bait	Postreatment	
Furosemide	3200	3190	3156	352	89

Diphacinone anticoagulant caused 61.7 % population reduction and added the population reduction in *R. rattus* with abamectine biocide was 70.5%. (Kandil *et al*., 2015). Efficacy of rodenticide baits with decreased concentrations of brodifacoum: Validation of the impact of the new EU anticoagulant regulation. Frankova *et al.* (2019) recorded that the low-dose baits (25 ppm) were consequently tested under field conditions in two populations showing 95.7% and 99.8% efficacy. So, may be furosemide caused the high mortality in animals farm compared the previous resultant with other authors. It can be concluded that furosemide may be can use as a safety compounds to reduce the rodents in animals farm or stores, and it can be use in integrated rodent management.

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Potential antimicrobial activity of different types of Libyan honeys

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Abstract:

Honey exhibits antimicrobial activity against a wide range of bacteria. The aim of the present work was to evaluate the antimicrobial effects of the Libyan honeys harmal (*Peganum harmala* L.) , red camphor (*Cinnamomum camphora*), white camphor (*Eucalyptus globule*), sarou (*Cupressus sempervirens*), athl (*Tamarix aphylla*) and kharoub (*Ceratonia silique*) on *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeuroginosa, Escherichia coli, Bacteriods* spp., *Sarcina* spp. and *Candida albicans.* Pathogens exhibited different sensitivities towards the honey samples. The results showed that *C. camphora* inhibited seven out of the nine tested microorganisms followed by *T. aphylla* honey, which inhibited six of them. The lowest effects were shown by *P. harmala* and *C. semperviren* honeys, where they only inhibited four different types of the tested microorganisms.

Introduction

Honey is a complex natural food produced from the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) feeding on plant nectar of blossoms, exudates of trees and plants or from honey bees feeding on honeydew produced by hymenoptran insects. Honey is a saturated solution of sugar of 31% glucose and 38% fructose and its colour and flavor vary considerably depending on it botanical and geographical origin (Gheldof *et al*., 2002) and of a moisture content of about 17.7% (Nagai *et al.*, 2006). In addition to minor component of phenolic acids, flavonoids, glucose oxidase, catalase, ascorbic acid, carotenoids, organic acids and α-tocpherol (Ferreres *et al*.,1993). Honey contains at least 181 components (White, 1975).

The use of honey for the treatment of diseases and wounds has been mentioned since ancient time (2100-2000 BC), where Aristotle (384-322 BC) described pale honey for sore eyes and wounds (Mandal and Mandal, 2011 and Vallianou *et al.* , 2014). Microorganisms such as *Staphylococcus aureus, Staphylococcus epidermis*, *Micrococcus luteus, Streptococcus uberis, Enterococcus faecalis, Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia* are frequently isolated from

human and animal skin wounds (Vuong and Otto, 2002; Nasser *et al.*, 2003; Halcon and Milkus, 2004; Altoparlak *et al.*, 2005 and Basualdo *et al*., 2007).

The healing effect of honey could be due to its physical and chemical properties (Rusell *et al.*, 1990 and Snow and Manley-Harris, 2004) and to its antioxidant and antimicrobial activity (Martos *et al.*, 2000; Escuredo *et al.*, 2012; Vandamme *et al.*, 2013; Isidorov *et al.*, 2015; Francine *et al*., 2016; Almasaudi *et al.*, 2017 and Leyva-Jimenez *et al*., 2019). Honey acts as an effective thermal insulator and a protective biofilm (Black and Costerton, 2010). Its antimicrobial activity is connected with its osmotic pressure which draws fluid from wounds, decreasing tissue edema (Molan, 2001). A possible reason for its activity depends on its ability to generate hydrogen peroxide by the bee derived enzyme glucose dehydrogenase (Saleh *et al.*, 2011). The strength of honey hydrogen peroxide is much lower than pharmacologic hydrogen peroxide, causing no damaging to the healing environment of a wound (Bang *et al*., 2003). Wound size is affected by pH value, Gethin *et al.* (2008) found that honey reduces the wound's pH and every 1 % reduction in pH is associated with a 1 % reduction in wound size. For wounds contaminated by methicillin-resistant *Staphylococcus aureus*, Manuka honey supported better wound healing than antibiotics (Gethin and Cowman, 2008). There are several studies indicating the effectiveness of honey in treating burns (Molan, 2001; Subrahmanyam, 1991 and Subrahmanyam *et al*., 2001).

Sukur *et al.* (2011) studied the effectiveness of Tualang honey in healing full-thickness burn wounds in rats. They found that topical application of honey on burn wounds contaminated with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* give better results of healing compared with other treatments.

The finding that the bacterium, *Helicobacter pylori* is a cause of stomach ulcers and the causative agent in many cases of dyspepsia has raised the possibility that the therapeutic action of honey for symptoms of dyspepsia may be due to Manuka honey's antibacterial properties. Somal *et al.* (1994) demonstrated that after an incubation period of 72 h.,5% Manuka honey completely prevent the growth of *H. pylori* (the causative organism of stomach ulcers). Similar invitro antimicrobial results of Manuka honey was reported against *Campylobacter* spp. (Lin *et al.*, 2009).

The aim of the present work was to evaluate the antimicrobial effects of the Libyan honeys Harmal (*Peganum harmala*), red camphor (*Cinnamomum camphora*), white camphor (*Eucalyptus globule*), sarou (*Cupressus sempervirens*), athl (*Tamarix aphylla*) and Kharoub (*Ceratoniasilique*) on *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeuroginosa, Escherichia coli, Bacteriods* spp., *Sarcina* spp. and *Candida albicans.*

Materials and Methods

The present investigation was carried out at the Beekeeping Research Section, Plant Protection Research Institute, Giza, Egypt.

1.Honey samples:

Six types of Libyan honeys of mono and multi-floral source were collected from selected beekeepers during the harvesting periods and from local markets in Western Libya. The honeys of mono-floral source were harmal (*P. harmala*, red camphor (*C.camphora*), white camphor (*E. globule*), sarou (*C. sempervirens*), athl (*T. aphylla*) and kharoub (*C. silique*). Honey samples were kept in dark at room temperature prior to analysis. The samples were investigated microscopically to determine their containing of pollen grain types (Table, 1).

2.Bacterial strains:

Bacterial strains and *C. albicans* were kindly donated by the Microbial Genetic Department, Genetic Engineering and

Biotechnology Division, National Research Center, Giza, Egypt.

3. Assay of antimicrobial activity:

Antimicrobial activity of honey samples was determined by the disc diffusion method (Collins *et al.*, 1995). A concentration of 20% of each kind of honey in distilled water was prepared in clean sterile test tube and kept in refrigerator at 4^{o}C to be used for microbiological test.

4.Preparation of the microbial culture:

The tested organisms were inoculated in the appropriate liquid media and incubated at 37 ^{o}C for 24 h. The microbial culture was used for the preparation of seed layer by inoculating the agar medium with 2% (v/v) of the microbial culture, thoroughly mixed and immediately used as the seed layer of plates.

5.Preparation of plates:

The appropriate agar medium was distributed at the rate of 7 ml portion in petri dishes. After solidification 5 ml of the seeded agar was distributed over the surface of the base layer and left for 15 min to solidify. The previously prepared filter paper discs (each disc was moistened with exactly 0.05 ml of the diluted honey) placed side down on the seeded agar and gently pressed with a tip of sterile forceps. Discs were placed symmetrically around the center of the dish. Plates were incubated at 37 ^{o}C for 24 hours. for *P. aeruginosa* and *M. leutus*, plates were incubated at 30 ^{o}C. Antimicrobial activity was determined measuring the diameter of inhibition zones around the discs to the nearest mm (Table, 2).

Three replicates were prepared for each honey sample. As a positive control method, the antibiotic tetracycline (30 µg) was used, while sucrose sugar solution (20%) was used as a negative control method.

6. Statistical analysis:

Results are expressed as mean $\pm$ standard deviation. ANOVA were applied at a confidence level of 95%.

Results and discussion

The results of inhibition effects of different honey samples in comparison to control are shown in Table (2). It was observed that all honey samples inhibited the growth of *C. albicans* with different degrees, where P<0.001. *Bacteroids* spp. was the most resistant bacteria, where it was only inhibited by *C.camphora* honey with an inhibition zone of only 5.0$\pm$0.67 mm. Except *C. silique* honey all honey samples affect the growth of *E. faecalis* with different degrees. *B. subtilis* was moderately inhibited by *T. aphylla* and *C. silique* honeys with inhibition zones of 11.33$\pm$0.57 and 11.66$\pm$0.57 mm, respectively. *C. camphora* inhibited seven out of the nine tested microorganisms followed by *T. aphylla* honey, which inhibited six of them.The lowest effects were shown by *P.harmala* and *C. semperviren* honeys, where they only inhibited four different types of the tested microorganisms. *Escherichia coli, P. aeruginosa* and *Bacteriods* spp. were found to be resistant to the antibiotic tetramycine (+ve control), while 20% sucrose sugar solution (-ve control) had no inhibitory effect on all bacterial strains.

The antimicrobial activity of honey is mainly contributed to the high osmolarity and acidity. In addition, hydrogen peroxide, volatiles, organic acids, flavonoids, phenolic compounds, wax, pollen, propolis are important factors that provide antimicrobial properties to honey. Shin and Ustunol (2005) stated that the sugar composition of honeys from different floral source are responsible for the inhibition of various intestinal bacteria. According to Moumbe *et al.* (2013) the minor components of honey including proteins, minerals, phytochemicals and antioxidants are responsible for the antimicrobial activity of honey in the treatment of infections, burns, wounds and ulcers.

Our results are in agreement with other published studies, showing that some kinds of honey have an inhibitory effect against the fungus *C. albicans* and the bacteria *S. aureus, B. subtilis, K. pneumoniae, E. faecalis, P. aeuroginosa, S. coli, Bacteriods* spp. and

Sarcina spp. (Basualdo *et al.*, 2007; Mercan *et al*., 2007; Al-Haj *et al.,* 2009; Sherlock *et al,*. 2010; Francine *et al.*, 2016; Almasaudi *et al.*, 2017 and Leyva-Jimenez *et al.,* 2019).

The results of this study are similar to the results obtained by Mohapatra *et al.* (2011), who reported that honey was effective against gram-positive bacteria *S. aureus, B. subtilis, E. faecalis* and gram-negative bacteria *E. coli* and *P. aeruginosa.*

The inhibitory effect of honey against *S. aureus*, *E. coli* and *K. pneumonia* is of great importance due to the fact that *Streptococcus* species and coliforms are recognized pathogens. In this work the growth of *Pseudomonas aeruginosa* was inhibited by 3 honey samples (*C.camphora*, *E. globule* and *C. sempervirens*). This type of bacteria is always found in wounds, especially those related to burns causing a variety of systemic infections, particularly in victims with severe burns (Yau *et al.*, 2001). Irish *et al.* (2011) noted that temperature, the time of storage, and the nature of flower's nectar may explain the different antimicrobial activities of different honeys. Our data are in agreement with the findings obtained by McCarthy (1995), who reported that, honey from different floralsources varies greatly in their antibacterial activity. Rybak and Szczęsna (1996) found that the minimum concentrations of honey which inhibit the growth of *B.subtilis* were 5-10%. Molan *et al.* (1988) reported significant differences between different kinds of floral honey in their activities on *S. aureus* at dilutions of 1/4, 1/8 and 1/16 original strength. Radwan *et al.* (1984) reported that honey from *Acacia mellifera* inhibits the growth of *E.coli*. Molan and Russell (1988) found that pollen present in honey could be the source of the antibacterial aromatic acids, which causes the component to act individually or synergically to prevent bacterial resistance (Cooper *et al.*, 2010). In addition to pollen, propolis is also found in honey. The antimicrobial and anti-inflammatory activity of European propolis is associated with the presence of flavonoids, flavones, and phenolic acids and their derivates (Bankova, 2005).

Table (1):Types and floral sources of Libyan honeys.

No. of samples	Local name of honey	Floral source
Sample 1	Harmal	*Peganum harmala*
Sample 2	Red camphor	*Cinnamomum camphora*
Sample 3	White camphor	*Eucalyptus globulu*
Sample 4	Sarou	*Cupressus sempervirens*
Samples 5	Athl	*Tamarix aphylla*
Sample 6	Kharoub	*Ceratonia silique*

Table (2): The diameter and standard deviation (in mm) of inhibition zones of different bacterial strains by honey samples compared to control.

Bacterial strains	*Peganum harmala*	*Cinnamomum Camphora*	*Eucalyptus globulu*	*Cupressus Sempervirens*	*Tamarix aphylla*	*Ceratonia Silique*	Tetracycline	Sucrose solution
Escherichia coli	10.33±0.57^{b}	11.00±0.00^{b}	11.66±0.57^{b}	0.00	0.00	0.00	0.00	0.0
Enterococcus faecalis	21.0± 0.0^{c}	23.66±1.52^{c}	5.33±57a	24.0±0.00^{c}	12.0±1.00^{b}	0.00	23.66±1.52^{c}	0.0
Staphylococcus aureus	0.00	0.00	0.00	21.33±1.15^{c}	6.0±0.5^{a}	5.66±1.15^{a}	22.0±0.1.5^{c}	0.0
Pseudomonas	0.0	10.33±0.57^{b}	10.66±0.57^{b}	6.66±0.57^{a}	0.00	0.00	0.00	0.0
Bacillus subtilis	0.00	0.00	0.00	0.00	11.33±0.57^{b}	11.66±0.57^{b}	21.33±1.15^{c}	0.0
***Bacteroids* spp.**	0.00	5.0±0.67^{a}	0.00	0.00	0.00	0.00	0.00	0.0
***Sarcina* spp.**	22.0 ±0.67^{c}	10.0± 1.12^{b}	0.00	0.00	11.0±0.00^{b}	11.0±0.00^{b}	23.66±1.52^{c}	0.0
Klebsiella pneumoniae	0.00	6.0±.00^{a}	11.0.± 0.00^{b}	0.00	6.0±0.50^{a}	5.0.±0.00^{c}	6.0±0.00^{a}	0.0
Candida albicans	20.33±0.57^{c}	20.66±0.57^{c}	10.66±1.15^{b}	11.0±0.00^{b}	20.66±1.15^{c}	21.33±0.57^{a}	22.0±67^{c}	0.0

Different letters indicate significant difference ($P < 0.001$).

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Field trials to control *Thrips tabaci* (Thysanoptera: Thripidae) infesting onion crop

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Abstract:

Thrips tabaci Lindeman (Thysanoptera: Thripidae) attack many vegetable crops specially onion causing grate economic damage on onion crops. Individuals of thrips are protected between the inner leaves of the plant where the pupal stage is spent in the soil. Field trial were conducted during two seasons 2017 and 2018 at Moshtohor, Toukh district, Qalubiya Governorate, to evaluat the efficacy of entomogenous fungus bioranza (*Metarhizium anisopliae*), two insecticide tracer 24% Sc (Spinosad) and marshall 25% EC (Carbosulfan), agriculture soap (potassium salts os fatty acids 49% Liquid) and two releasing rates (i.e. 3000 and 1000 pred./20m^2) of phytoseiid predatory mites, *Neoseiulus californicus* (McGregor) and *Neoseiulus arundonaxi* Metwally and Sanad (Acari*:* Phytoseiidae) for controlling *T. tabaci* infesting onion crop. Two applications were carried out during March and April during the two seasons. The results showed that all treatments reduced thrips populations compared to control. The maximum reduction in thrips population 73.42% for the predatory mite *N. californicus* at rate 3000 pred./ 20m^2 followed by *N. arundonaxi* at rate 3000 pred./ 20m^2. However, the minimum reduction in thrips population 57.27% for soap at rate 500ml/ 100 liter of water. Reduction percentage of thrips was highly in the second season 69.11% and 65.42% in the first year. The results indicate the potential of using *N. californicus*, *N. arundonaxi*, *M. anisopliae* and soap for the control of *T. tabaci*, there are safe enough to be used in an integrated pest management programs (IPM).

Introduction

Onion, *Allium cepa* L. (Alliaceae), is an important cash crop in Egypt for local consumption and exportation. Onion plants are infested with different insect pests during their growing season. *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), attack many vegetable crops specially onion causing grate economic damage on onion crops, which feeds on onion plants during their vegetative growth and fruit filling (Mahmoud, 2008). Both nymphs and adults cause severe damage to the crop, which can reach 40-60% in foliage damage and can lead to 10-20% yield losses annually (Waiganjo *et al.*, 2008).

Management of *T. tabaci* has proved to be difficult, due to its minute size and its thigmotactic behavior (Lewis, 1997). Usually, controlling thrips is conducted via the usage of chemical applications, which may explain the widespread chemical-resistance development in onion thrips (Jensen, 2000). Chemical control methods and alternative methods including biocontrol have been assayed against thrips (Cloyd and Sadof, 2000; Shelton

et al., 2008; Shan *et al.*, 2012 and Asghar *et al.*, 2018).

In previous studies, the applications of the biopesticide bioranza which is dependent upon the entomopathogenic fungi *Metarhizium anisopliae* was the most efficacious fungal formulation tested because it caused the highest reduction in mite counts in the hives. Mites are susceptible to entomopathogenic fungi (Chandler *et al.,* 2000). Some predators recorded here are known to readily accept *T. tabaci* as prey and may play an important role the integrated pest management of onion thrips (Lewis, 1973). The predacious mites of family Phytoseiidae are used as a biological control agent of thrips, which is a major pest of greenhouse crops (Gillespie, 1989). *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) is a potential biological control agent of spider mites and tarsonemid mites, and is commercially mass-produced for sale in various countries of the world (Cooping, 2001). Generally, control the thrips with phytoseiid predatory mites has established to be very success. The potential of *Neoseiulus arundonaxi* Metwally and Sanad (Acari*:* Phytoseiidae) in control western flower thrips Frankliniella occidentalis (Pergande) were assessed (Sanad and Hassan, 2019).

The present study was carried out during two successive seasons 2017 and 2018 at Moshtohor, Toukh district, Qalubyia Governorate, to evaluate the entomogenous fungus *M. anisopliae*, two insecticides tracer and marshall, soap and two predacious mite, *N. californicus* and *N. arundonaxi* as an ecofriendly tools in an IPM strategy to control infestation of thrips of onion production in Egypt.

Material and methods

1. Study sites:

Field trials to control were conducted at Moshtohor, Toukh district, Qalubiya Governorate, during 2017 and 2018 seasons. The site has an annual rainfall during December to February. The minimum and maximum temperatures during study are between 15.84˚C and 17.2˚C and between 25.8˚C and 28.46˚C, respectively.

2.Endomophagous fungi:

Commercial formulations of entomogenous fungus *M. anisopliae* is bioranza was used in this investigation; this was manufactured and produced by Plant Protection Research Institute, Agricultural Research Center. The active ingredient of bioranza is *M. anisopliae* (10% WP) and the recommended application concentration is 200g/100 liter of water.

3.Predacious mites:

3.1.Mass rearing of the predatory mite *Neoseiulus californicus*:

The predatory mite *N. californicus* was collected from different plants especially strawberry and cucumber plants. The colonies were maintained at room temperature under laboratory conditions in large plastic boxes (70x30x40 cm). Water was added when needed. Excised bean leaves highly infested with *T. urticae* were provided every day as prey source for the predatory mite.

3.2.Mass rearing of the predatory mite *Neoseiulus arundonaxi*:

N. arundonaxi was extracted from giant reed plants, using modified Tullgren funnel and was reared constantly on a mixture of all stages of the acarid mite, Tyrophagous putriscentiae (Schrank) (*Acari*: *Acaridae*) under controlled conditions of 25 ± 2°C in top vented plastic containers (6 cm diameter and 10 cm depth). All units were kept in large boxes provided with NaCl saturated solution for keep up RH inside the boxes as 75% (Winston and Bates, 1960). The acarid mite Tyrophagous putriscentiae (Schrank) (*Acari*: *Acaridae*) was reared on mixture of wheat bran and bakery dry yeast at 25°C in top vented plastic containers (6 cm diameter and 3 cm depth) (Sanad et al., 2007).

3.3.Release of the predacious mite:

Releases were carried out before sunset. It was released at the rate of 1000 and 3000 predator per treatment. The two predators were released twice per season on early March and April during 2017 - 2018 seasons. Releasing of phytoseiid mite was conducted by using main rearing medium mixed with means of a carrier, consisted of a mixture of wheat bran and vermiculate material by 1:1 ratio.

4.Field plots and treatments:

Onion cultivar, ‘Red Creole’ was transplanted in a complete randomized block

design (1/2 feddan) with four replicates; each replicate consisted of four rows (10-m-long). Plants were spaced 10 cm within rows and 30 cm between rows, and a 1-m-long distance was used to separate each block. The seedlings of onion were sown in the early of December 2017 and 2018, using the recommended agriculture practices.

The experimental area was divided into nine treatments including control. Two pesticides were evaluated Tracer 24% Sc as 30 ml/100 liter and marshall 25% EC as 100 ml/100 liter of water), an entomogenous fungus bioranza ($5x10^6$ conidia/ml of water), soap as 500ml/ 100 liter of water two predacious mite, *N. californicus* and *N. arundonaxi* at rate of 1000 and 3000 predators/20-m^2 (Table, 1).

Table (1) : Treatments and their application rates.

Trade name	Active ingredient	Rate of application
Bioranza	*Metarhizium anisopliae*	200 g/100 liter
Tracer 24% Sc	Spinosad	30 ml/100 liter
Marshall 25% EC	Carbosulfan	100 ml/100 liter
Soap	(Potassium salts of fatty acids 49%Liquid)	500 ml/100 liter
Neoseiulus californicus	-	1000 predators/20-m^2
Neoseiulus californicus	-	3000 predators/20-m^2
Neoseiulus arundonaxi	-	1000 predators/20-m^2
Neoseiulus arundonaxi	-	3000 predators/20-m^2

Two applications were conducted, the first in early March and the second in early April during two seasons. Samples of 5 plants/ replicate were collected randomly at early morning. Insect pests and their predators were sampled weekly. The thrips number on plants was counted immediately before treatment and 1st week, 2nd week, 3rd week and 4th week and shaking them over a white sheet and motile stages were counted.

Materials were sprayed using a highly volume motor sprayer of 20 liters capacity. Pre-count was conducted before spraying. Percentage reduction in population was estimated using Henderson and Tilton (1955) equation.

% population reduction= 100 * (1 - (Ta * Cb)/(Tb * Ca), where:

Ta= number of mite after spray; Tb= number of mite before spray;

Ca =number of mite in the control after spray; Cb = number of mite in the control before spray.

5.Statistical analysis:

Reduction percentage of thrips was analyzed by one-way ANOVA and means were compared by using student's least significant difference. Significance level was $P< 0.05$. Analysis was conducted using SAS statistical software (SAS Institute, 2003).

Results and discussion

1.In the first season 2017:

The first application in early March 2017, the number of nymphs were counted in a sample of 20 plants collected from different treatments randomize. From the data demonstrated in (Table, 2), it was found that the highest reduction percentage were 62.68, 62.62, 59.77 and 56.50 for entomogenous fungus bioranza (*M. anisopliae*), release of *N. californicus* at rate 3000/20m^2, *N. californicus* at rate 1000/20m^2 and *N. arundonaxi* at rate 1000/20m^2 after four weeks of first application, respectively. Efficiency of insecticide treatments decreased with increase in data collecting interval. While, the efficacy of bioranza, soap and the two predacious mites increased with increase the time after application (Figure, 1 and Table, 2).

After second application in season 2017, data in Table, 2 and Figure,1 illustrated that, significant differences between reduction percentages of treatments, it can be divided into three categories. The highest reduction percentages ≥75% of *T. tabaci* were recorded at treatments; *N. arundonaxi* at rate 3000/20m^2, *N. arundonaxi* at rate 1000/20m^2, *N. californicus* at rate 1000/20m^2 and *N. californicus* at rate 3000/20m^2, they averaged 78.66, 76.04, 76.56 and 76.04% after four weeks, respectively. The moderate

reduction percentages (≤75 to 70>) were recorded at treatments of bioranza and marshall 25% EC averaged 71.29 and 71.16%, respectively. The lowest reduction percentages (< 70%) of *T. tabaci* were recorded at treatments of tracer 24% Sc and soap with averaged 69.5 and 66.44%, respectively.

2.In the second season 2018:

The population of thrips nymphs per plant a day before application of treatments in different treatments was uniform which ranged from 22.2 to 26.2 nymphs/ plant in the second season 2018 (Figure,2).

Data presented in Table (3) showed that the reduction percentage of *T. tabaci* population as a result of spraying four products of fungi, marshall, tracer and soap and releasing the two predators at two rates in season 2018 after two application. Highest reduction percentage of *T. tabaci* nymphs were recorded at end of experiment at treatments; *N. californicus* at rate 3000/20m^2 (84.86%), *N. arundonaxi* at rate 3000/20m^2 (84.77%), marshall (84.77%), *N. californicus* at rate 1000/20m^2(82.04%), bioranza (79.74%) and *N. arundonaxi* at rate 1000/20m^2 (79.22%) without nonsignificant differences. The lowest reduction percentage was recorded at tracer (69.99%) and soap (65.96%).

Table (2): Reduction percentage of individuals Thrips tabaci infesting onion as a result after first and second application of different treatments under field conditions first application season 2017.

Treatments	Rate of	Reduction percentage of individuals/ 20 plants after First application				Reduction percentage of individuals/ 20 plants after Second application			
		First week	Second week	Third week	Fourth week	First week	Second week	Third week	Fourth week
Neoseiulus californicus 1000	1000/20m^2	52.99 b	61.22 ab	65.78 abc	59.77 a	72.93 b	71.39 bc	79.46 a	76.56 ab
Metarhizium anisopliae	5 x10^{6}spores/1ml	47.78 b	66.20 a	67.94 ab	62.68 a	74.53 ab	75.85 ab	78.33 a	71.29 bc
Neoseiulus californicus 3000	3000/20m^2	61.68 ab	73.28 a	73.56 a	62.62 a	76.46 ab	76.55 ab	74.17 a	76.04 ab
Tracer 24% Sc	30 ml/100 liter	71.37 a	59.11 abc	60.78 bcd	44.39 c	81.03 a	77.81 a	71.91 a	69.50 c
Neoseiulus arundonaxi 1000	1000/20m^2	29.39 c	49.18 bcd	54.09 cde	56.50 ab	71.31 bc	72.76 ab	73.01 a	76.76 ab
Marshall 25% EC	100 ml/100 liter	72.55 a	44.54 cd	46.87 e	39.79 c	81.31 a	77.39 ab	75.36 a	71.16 bc
Neoseiulus arundonaxi 3000	3000/20m^2	45.52 bc	63.21 ab	68.94 ab	52.28 b	74.16 ab	73.26 ab	75.43 a	78.66 a
Soap	500 ml/100 liter	60.63 ab	42.69 d	49.00 dc	52.07 b	65.56 c	65.69 c	63.16 b	66.44c
F value		5.94	4.05	5.8	12.32	4.25	3.73	3.58	4.75
Probability		0.0007	0.00059	0.0008	0.0001	0.0046	0.0089	0.0108	0.0025
LSD at level 5%		17.36	15.86	5.8	6.99	7.33	6.15	7.77	5.79

Different letters in same column denote significant difference (P < 0.05).

Table (3): Reduction percentage uction of individuals *Thrips tabaci* infesting onion as a result after first and second application of different treatments under field conditions first application season 2018.

Treatments	Rate of	Reduction percentage of individuals/ 20 plants after First application				Reduction percentage of individuals/ 20 plants after Second application			
		First week	Second week	Third week	Fourth week	First week	Second week	Third week	Fourth week
Neoseiulus californicus 1000	1000/20m^2	48.30 bc	62.01 bcd	71.63 abc	62.57 ab	71.71 bc	75.58 b	79.60 a	82.04 a
Metarhizium anisopliae	5 x10^{6}spores/1ml	54.73 b	65.09 cd	75.78ab	60.67 ab	67.60 c	73.48 cd	77.64 ab	79.43 a
Neoseiulus californicus 3000	3000/20m^2	60.66 b	70.27 abc	80.94 a	67.15 ab	77.61 ab	76.33 a	82.27 a	84.86 a
Tracer 24% Sc	30 ml/100 liter	78.76 a	73.27 ab	72.47 abc	58.20 bc	81.03 a	71.38 bc	71.96 b	69.99 b
Neoseiulus arundonaxi 1000	1000/20m^2	41.69 c	58.22 cd	65.80 c	49.12 cd	57.09 d	68.48 a	76.43 ab	79.22 a
Marshall 25% EC	100 ml/100 liter	77.67 a	76.30 a	70.60bc	64.50 ab	82.90 a	73.35 bc	71.86 b	84.77 a
Neoseiulus arundonaxi 3000	3000/20m^2	58.97 b	66.47abcd	78.76 ab	70.17 a	79.91 ab	84.25 a	81.40 a	84.77 a
Soap	500 ml/100 liter	39.50 c	57.99 d	65.90 c	42.16 d	57.75 d	56.83 d	64.57 c	65.96 b
F value		12.42	2.61	2.86	6.21	11.25	10.8	6.16	9
Probability		0.0001	0.0416	0.0293	0.0005	0.0001	0.0001	0.0001	0.0001
LSD at level 5%		12.36	12.27	9.6	11.09	9.01	6.98	7.07	7.22

Different letters in same column denote significant difference (P < 0.05).

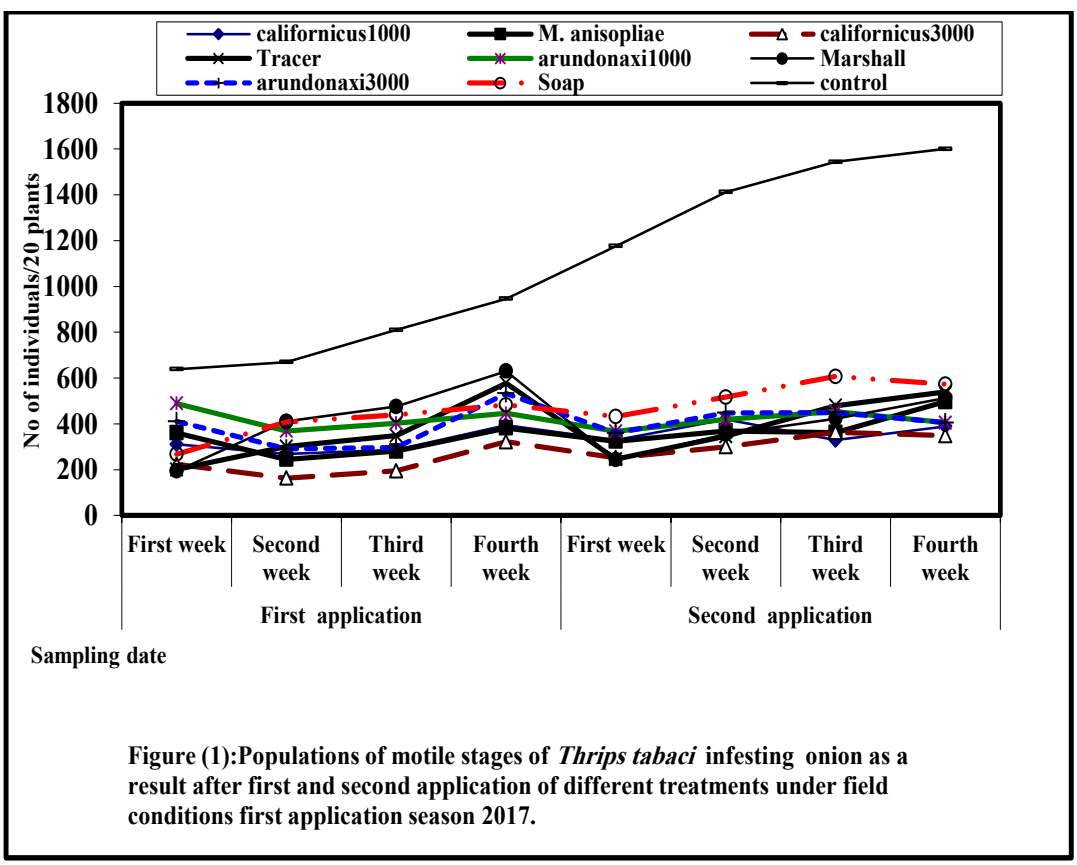

Figure (1):Populations of motile stages of *Thrips tabaci* infesting onion as a result after first and second application of different treatments under field conditions first application season 2017.

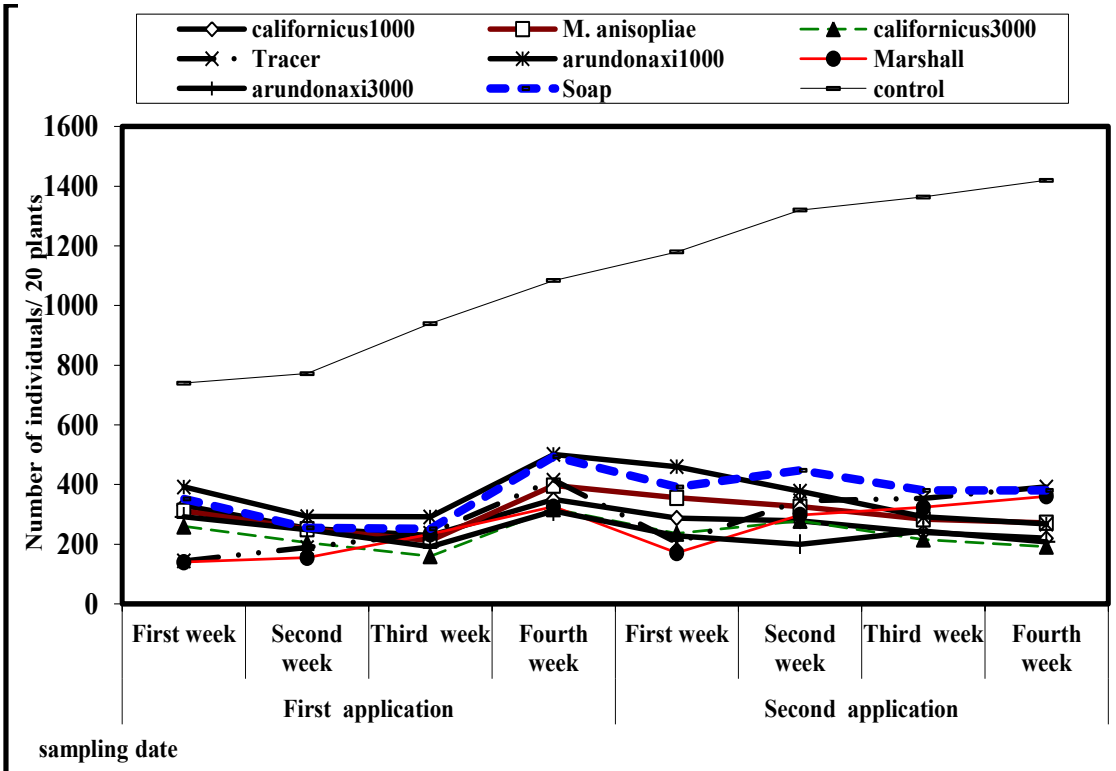

Figure (2):Populations of motile stages of *Thrips tabaci* infesting onion as a result after first and second application of different treatments under field conditions first application season 2018.

Generally, the results showed that all treatments reduced thrips populations compared to control. The maximum reduction in thrips population 73.42% for the predatory mite *N. californicus* at rate 3000 pred./ 20m^2 followed by *N. arundonaxi* at rate 3000 pred./ 20m^2. However, the minimum reduction in thrips population 57.27% for soap at rate 500ml/ 100 liter of water. Significant differences between two seasons, the percent reduction of thrips was highly in the second season 69.11% and 65.42% in the first year (Table, 4 and 5).

The results indicate the potential of using *N. californicus*, *N. arundonaxi* , *M. anisopliae* and soap for the control of *T. tabaci*, there are safe enough to be used in an integrated pest management programs (IPM).

Table (4): Reduction percentage of *Thrips tabaci* during two years 2017-2018 .

Treatments	Reduction percentage of two years 2017-2018
***Neoseiulus californicus* 1000**	68.37 b
Metarhizium anisopliae	68.71 b
***Neoseiulus californicus* 3000**	73.42 a
Tracer 24% Sc	69.56 ab
***Neoseiulus arundonaxi* 1000**	61.22 c
Marshall 25% EC	68.54 b
***Neoseiulus arundonaxi* 3000**	71.03 ab
Soap	57.27 c
F value	12.60
Probability	0.0001
LSD at level 5%	4.14

Different letters in same column denote significant difference (P < 0.05).

Table (5): Comparison between two seasons reduction percentage.

Season	%R	F value	Prob.	LSD
2017	65.42 b	12.10	0.0005	2.08
2018	69.11 a			

Different letters in same column denote significant difference (P < 0.05).

Entomopathogenic fungi are widely distributed with both restricted and wide host ranges which have different biocontrol potentials against arthropods insects and plant pathogenic fungi. These fungi have a lower risk on the environment and humans. These findings are in a close agreement with that presented by Goettel *et al*. (1990) showed that some commercial formulations of the entomopathogenic fungi can control aphids and thrips with low impact on non-target insects. Cloyd and Sadof (2000) indicated that both spinosad and acephate are efficacious against thrips in a commercial situation. Greenhouse managers should consider the presence of natural enemies out-doors when implementing pest management strategies because natural enemies may provide supplemental control of western flower thrips. Maniania *et al.* (2003) indicate that the potential of using *M. anisopliae* for the control of *T. Tabaci* while protecting biodiversity in the onion agroecosystem. Shelton *et al.* (2008) showed that acetamiprid, dimethioate, spinosad and imidacloprid performed better than lambda - cyhalothrin against thrips on cabbage. Arthurs *et al.* (2009) evaluated two species of phytoseiid mites as predators of chilli thrips, *Scirtothrips dorsalis* Hood. Gravid females of *Neoseiulus cucumeris* and *Amblyseius swirskii* both fed on *S. dorsalis* at statistically similar rates. Larvae were the preferred prey for both species, consuming on average 2.7/day, compared with 1.1–1.7 adults/day in no choice tests. Rahmani *et al.* (2009) reared the predatory mite, *N. californicus* on *T.tabaci*. The development, survivorship and life-history parameters of the boku strain of *N. californicus* feeding on first instar larvae of *T. tabaci*. Total prey consumption by protonymphs, deutonymphs and adult were 3.85 and 3.50 and 65.1, respectively. Arthurs *et al.* (2013) applied the commercial strains of entomopathogenic fungi were evaluated for control of chilli thrips, *S. dorsalis* Hood on pepper plants Spinosad reduced populations by 94–99%, *M. brunneum* F52 by 84–93%. Jafari *et al.* (2013) indicated that *Neoseiulus barkeri* Hughes is an indigenous biological control agent in west of Iran on cucumber and maize that preys on spider mites and *T. tabaci* and can prevent the outbreak of them. Asghar *et al.* (2018) indicated that the insecticides reduced thrips populations compared to controls. The maximum reduction in population of thrips and highest onion bulb yield was obtained with dimethoate 40EC followed by bifenthrin 10EC and the minimum onion bulb yield was obtained in the control.

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Food consumption, utilization and biochemical impacts of some insecticides on the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract:

This experiment was conducted on the 4th instar larvae of *Spodopetra littoralis* (Boisd.) (Lepidoptera: Noctuidae) to investigate the impact of some pesticides belonging to different groups in the laboratory to evaluate their antifeedant activity, relative growth rate and utilization of ingested food . Moreover the effect of the tested compounds on the total protein, total carbohydrate, total lipids and acetylcholinesterase were recorded. The obtained results clear antifeedant index for owner 5%EC seems to be the most powerfull tested compounds followed by dimilin 48%SC. Also, the relative growth rate (RGR) of the 4th instar larvae of *S.littoralis* fed on castor leaves treated with owner 5% was decreased clearly often one day exposure followed by strong 30% SC. After three days exposure emafel 45% ME achieved the lowest utilization of ingested food (FCI). Over and above the biochemical studies explained the four tested insecticide represented significant decrease in the amount of total carbohydrate, totalprotein, total lipids and acetylcholinesterase enzyme. Owner 5% caused the highest change percentage in total lipids (45.12%). Whereas, dimilin 48%SC cased the highest change percentage in total protein and total carbohydrate (33.07 and 54.6) respectively. Dimilin was the most inhibitive of acetylcholinesterase activities 28.06 change percentage.

Introduction

Cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the major pests in Egypt and other countries in Africa and Asia. It causes considerable damage to cotton and other cultivated crops and vegetables causing severe damage and consequently reduction in the obtained yield (Nasr *et al*., 1984; Ahmed, 1988 and Korrat *et al*., 2012) makes it a model of serious polyphagous pests.

Emamectin benzoate (1.9% EC) (Methylamin :Avermactin) belongs to Avermactin group of chemicals produced by the soil-dwelling actinomycete, *Streptomyces avermitilis*. It represented a second generation of abamectin in avermactin family which acts as nerve poisons stimulate (Fritz *et al*., 1979) which block the post synaptic potential of neuro muscular junction leading to paralysis and finally to the death.

Indoxacarb has a good toxin effect as a new class of oxidiazine insecticide against Lepidoptera pest with nearly no effect on non target insects by blocking the movement of sodiumion and cause stop feeding and paralysis (Dinter and Wiles, 2000).

The use of the insect growth regulators (IGR,s) for the control of insects of economic importance have been widely acclaimed, either as juvenile or other compounds (Smagghe *et al.,* 1995), these compounds interfere with the normal growth for development of insects and their effect could extended to affect the insects reproduction potential as well as other effects on the physiology of treated insects (Abdel-Aziz, 2012).

The aim of this work is to present the evaluation of the effect of owner 5% EC, dimilin 48% SC, emafel 4% ME and strong 30% SC on the feeding activity and food consumption and utilization of *S. littoralis*. Also, the evaluation of the latent effect of the formercompounds on the total carbohydrate, total protein, total lipids and acetylcholinesterase activity as main components of insects was conducted.

Materials and methods

1. Rearing of *Spodopetra littoralis* :

Collected egg-masses of *S.littoralis* from the field were allowed to hatch and the larvae were fed on fresh leaves of castor bean. The rearing was carried out under laboratory conditions 27cº and 55-65 % RH. The 4th instar larvae were selected on the basis of weight. The chosen larvae were starved for about 4 hours before feeding on leaves of castor bean which were treated by the followed compounds by using dipping method. The remaining living larvae were allowed to fed on castor bean leaves until the pupation period and emergence.

2.Insecticides used:

2.1.Lufenuron (Owner 5% EC) producd by: El-moneer for Agricultural .

2.2.Diflubenzuron (Dimilin 48%) producd by: Eristia life co.

2.3.Emamectin benzoate (Emafel 4% ME) producd by: Al-Qawafel Technical Ind. Agr.Co.

2.4.Indxocarb (Strong 30% SC)producd by: Spire for Agricultural.

3. Effect of compounds on food consumption and utilization:

The effect ofthe former compounds on the food consumption and utilization by the fourth instar larvae was investigated . one hundred larvae (4th instar) of *S.littoralis* was starved 4hours and then weighed fresh castor bean leaves, *Ricinus communis* were weighed then leaves were dipped for 10 second in the different compound solution . The treated leaves were left in shade to air dried. Twenty leaves for each treatment plus control treatment were divided into 4 replicates each one with 5 larvae each kept in glass containers with treated leaves. Another 4 replicates of larvae were kept in similar containers with untreated leaves as check .the larvae were daily individually weighed for 5day. The amount of consumed food was calculated, the antifeedant index (AFI) was calculated from the formula of Sadek (2003).

AFI = [(C - T) / (C + T)] x 100 according to

C: Food consumption of control leaves

T: Food consumption of treated leaves

Also, the faces were weighed and consumed was determined. The nutritional indices of growth rate (RGR) were calculated by Farra *et al*. (1989) as follows :

Relation growth rate (RGR) = ΔB/BaT.

Efficacy of conversion of ingested food (ECI) = (ΔB / I) x100

Where:

I: Weight of food consumed .

Ba: Mean of insect weight during the experiment.

T: Feeding period in day.

ΔB: Chang in body weight.

F: Weight of faces produced during the feeding period.

Data were subjected to analysis of variance (ANOVA), (F test) and the least significant differences (LSD) were calculated (Litchfield and Willcoxon, 1949).

4. Biochemical effect :

4.1. Sample preparation:

Fifteen to twenty treated larvae were homogenized in achilled glass Teflon tissue homogenizer (ST-2 Mechanic-preczyina, Poland). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. Supernatants were kept in a deep freezer at -20ºc till use for biochemical assays. Double beam ultraviolet/visible spectrophotometer was used to measure absorbance of colored substances or metabolic compounds. Which is referred as enzyme extract, can be stored at least one week without appreciable loss of activity when stored at 5°C.

4.2.Total carbohydrates:

Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubios *et al.* (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Sample (1 gm) was homogenized in 0.3N HClO4 (5 ml) at ºC for 1 min. The homogenate was kept in ice for further 10 min. Insoluble matter was removed by centrifugation for 3 min.at 2000 r.p.m. and washed twice in ice–cold HClO4 (5ml) by redispertion and centrifugation. The three supernatant combined into acid extract. Hundred microliters of the acid extract were added into a colorimetric tube to 0.5 ml of phenol (20 percent w/v). Then 5 ml of concentrated sulfuric acid were added rapidly with shaking .

The tubes were allowed to stand 10 min, then they were shaken and placed for 10-20 min in water bath at 25 to 30 ºC before readings. Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow –orange color is measured at 490 nm against blank. Total carbohydrate is expressed as : µg glucose / gm fresh weight.

4.3.Total proteins:

Total proteins were determined by the method of Bradford (I976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol. To this solution 100ml 85% (W/V) phosphoric acid were added . The resulting solution was diluted to a final volume of 1 liter. Sample solution (50µl)or for preparation of standard curve 50µl of serial concentrations containing 10 to 100µg bovine serum albumin were pipetted into test tubes . the volume in the test tube was adjusted to 1 ml with phosphate buffer (0.1M, pH 6.6). Five millimeters of protein reagent were added to test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1 hr against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent. Total proteins was expressed as mg/g-b-wt.

4.4.Total lipids :

Total lipids were estimated by the method of Knight *et al*. (1972) using phosphovanillin reagent prepared by dissolving of 0.6 gm pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water.Then 400 ml conc. Phosphoric acid were added .

4.5.Procedure :

250 ul of sample were added to conc. sulphuric acid (5 ml) in a test tube and heated in aboiling water bath for 10 min . After cooling to room temperature , the digest was added to phosphovanillin reagent (6 ml). After 45 min , the developed color was measured at 525 nm against reagent blank .Optical density was compared to that of a reference standard and results expressed as mg lipids/ ml heamolymph .

4.6.Acetylcholinesterase activity:

The reaction mixture contained 200 µl enzyme solution , 0.5 ml 0.067 M phosphate buffer (pH7) and 0.5 ml acetylcholine bromide (3 mM). The test tubes were incubated at 37 ºc for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes . Then 0.5 ml of Hcl (1 part of conc. Hcl and 2 parts of ΔH_2O) was added. The mixture shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M $Fecl_3$ in 0.1M

Hcl) was added and mixed well. The decrease in acetylcholine bromide resulting from hydrolysis by acetylcholinesterase (AchE) was read at 515 nm .

5. Statistical analysis :

All experiments contained 3-4 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one-way analysis of variance (ANOVA) using Costat statistical software (Cohort software , Berkeley). When the ANOVA statistics were significant ($P < 0.01$), means were compared by the Duncan's multiple range test.

Results and Discussion

1. Estimation of antifeedant index value of different formulation:

Antifeedant effects and toxicity of owner 5% EC , dimilin 48% SC, emafel 4% ME and strong 30% SC, formulations at 0.25, 0.125and 0.0625 field recommended rates against 4th *S.littoralis* instar larvae were estimated and calculated after 24, 48 and 72 hours from the beginning of the experiment though the consumption of tested leaner of castor been leaves Table (1).

Table (1): Effect of different concentration of owner , dimilin, emafel and strong on antifeedant activity of 4th instar larvae of *Spodoptera littoralis*.

Treatments	Does (Field/rate)	Antifeedant index (AFI) % Time exposure(day)			Mean
		1	2	3	
Owner	0.0625	25.6	41.2	64.8	43.9
	0.125	47.1	65.6	71.7	61.5
	0.25	56.2	69.5	72.3	66.0
Dimilin	0.0625	20.6	31.7	52.9	35.1
	0.125	40.5	44.3	56.8	47.2
	0.25	50.2	56.5	60.8	55.8
Emafel	0.0625	14.6	27.3	61.3	34.4
	0.125	20.6	34.9	65.2	40.2
	0.25	29.2	44.9	66.5	46.9
Strong	**0.0625**	**18.1**	**24.7**	**39.1**	27.3
	0.125	26.1	30.7	42.4	33.1
	0.25	31.2	39.4	45.1	38.6

The obtained data, clearly, indicated AFI values increased with the increase of tested concentration material. The rate of increase was recorded by elapse of time after treatments showing 18.1-56.2, 247-69.5 and 39.1-72.3 % for 1, 2 and 3 days, respectively. The greatest antifeedant were achieved by owner at 0.25 field rate being 56.2, 69.5 and 72.3% after elapsed time 1,2 and 3 days, respectively.

On the contrary, strong at 0.0625 field /rate had the lowest antifeedant value among all formulation acting 18.1, 24.7 and 39.1% after 1,2 and 3 days, respectively.

2. Food consumption and utilization:

At the same conditions, the treatments owner, dimilin , emafel and strong reduced the nutritional indices RGR and ECI of the fourth instar larvae. The data presented in Table (2) clearly showed that the majority of previous insecticide treatments decreased the relation growth rate (Food consumption) and also, the efficiency of conversion of ingested food (food utilization) of 4th instar *S. littoralis* larvae. The ECI and RGR were reduced by the high concentration and increased with increasing the time elapsed of the treatment Table (2) these results were agreement with those obtained by Barrania (2013) who found that feeding the 4th instar *S. littoralis* larvae on treated cotton leaves at different rates of the lost compounds decreased the food consumption. Also the relative growth rate of 4th instar larvae of *S. littoralis* fed on cotton leaves treated with chlorantraniliprole , thiamethoxam and

novaluron at 1,1/2 and 41/4 field rates was decreased.

Table(2): The effect of different treatments on nutitional indices,related by food consumption and utilization of the 4th instar *S.littoralis* larvae.

Nutritionnal indices	Feeding period	Relative growth rate (RGR) and efficiency of conversion of ingested food (ECI)Mg/gm												
		control	Owner			Dimilin			Emafel			Strong		
			0.0625	0.125	0.25	0.0625	0.125	0.25	0.0625	0.125	0.25	0.0625	0.125	0.25
RGR	1day	0.056	0.63	0.701	0.6	0.644	0.801	0.6	0.79	0.7	0.631	0.526	0.537	0.5
	2day	0.058	0.547	0.593	0.513	0.206	0.331	0.271	0.632	0.601	0.538	0.049	0.108	0.213
	3day	0.193	0.172	0.181	0.151	0.201	0.31	0.27	0.259	0.231	0.195	0.049	0.041	0.037
	4day	0.201	0.02	0.029	0.017	0.188	0.201	0.199	0.232	0.2	0.193	0.033	0.039	0.013
	5day	0.656	0.008	0.011	0.004	0.036	0.152	0.1	0.037	0.023	0.029	0.027	0.036	0.003
ECI	1day	19.41	20.75	23.81	9.81	42.5	49.81	33.21	37.291	36.361	31.311	41	39.39	31.3
	2day	20.86	20.32	22.7	18.55	39.67	41.61	30.91	14.997	14.011	13.691	13.75	14.81	19.47
	3day	22.42	11.91	17.11	11.91	21.82	24.71	18.75	8.997	10.321	8.673	13.48	14	13.48
	4day	26.32	10.96	15.71	8.88	17.69	20.83	15.52	5.271	3.316	3.711	13.44	13.21	7
	5day	68.51	9.88	14.19	7.91	8.72	11.01	7.33	3.289	2.217	2.001	12.28	12.11	5.12

3.Biochemical effects:

3.1. Effects on total carbohydrates, total protein, total lipids and acetylcholine esterase:

3.1.1.Effects on total carbohydrates:

Data in Table (3) indicated that emafel gave the highest decrease in the total carbohydrate activity lower than control, were 45.6%.in the owner was recorded the lowest decrease, being 3.19% . According to activity ratio represented in the same table, the obtained values 0.54, 0.62, 0.71 and 0.97 time less for emafel, dimilin, strong and owner with the control value , respectively.

3.1.2.Effect on total proteins :

In Table (3) showed that a significant changes in the activity of total protein resulted from the treated 4th instar larvae Emafel and Owner caused significant reduction than control were 33.07 and 24.99%, respectively .but ,it was obvious that there was no significant different between the efficacy of dimilin treatment on the total protein activity was 2.49% than control. Depending on the activity ratio as shown in Table (3) emafel was 0.69 but the activity ratio of owner, strong and dimilin were 0.77, 0.86 and 1.03, respectively.

3.1.3. Effect on total lipids:

Data in Table (3) indicated that owner gave the highest decrease in the total lipid activity lower the control,was 45.12%. While, the remaining treatments were recorded lower decrease than control, they gave 23.75,8.29 and 7.71% change by dimilin, emafel and strong the control which cased 6.87 as mean of total lipids. According to activity ratio represented in Table (3) the obtained value 0.55, 0.76, 0.92 and 0.92 the less for owner, dimilin, emafel and strong with the control, respectively.

All used compounds were caused significantly decrease in the total carbohydrate, total protein and total lipids. There results are in harmony with those obtained by Anwar and Abdel-Mageed (2005) who found that reduction in carbohydrate content, total protein and total lipid of the *S.littoralis* when treated with castor oil, gossypol, Diflubenzuron, tebufenozide, hexaflunuron ,flufenoxuron ,chlorfuazuro and lufenuron. Also, these results are in agreement with Abdel-Salam *et al.* (2018) they found the effect of protecto, viruset ,cascade and ataborn caused significant decrease in the amount of total protein ,total carbohydrate and total lipids in 4thinster of *S. littoralis* larvae.

3.1.4. Effects of acetylcholinesterase activity:

Table (3) showed AchE activity in the 4thinster of *S.littoralis*. The obtained results indicated that. dimilin caused the highest significant decrease in AchE enzyme activity

(28.06%) reduction than control. In the contrary strong was caused the little increase in the AchE enzyme activity by 11.8% than control, the remaining treatment were achieved decreasing than control as 15.99 and 8.45% by owner and emafel, respectively.

Depending on the activity ratio value dimilin was the highest decrease (0.72 time) less than control while, the remaining treatments obtained values 0.92, 0.88 and 0.84 time less for emafel, strong and owner,respectively .

Table(3): Means of total lipids, total proteins, total carbohydrates and acetylcholinesterase percentage change and activity ratio in 4th instar larvae of *Spodoptera littoralis* after treatments by tested compounds .

Treatment	Acetylcholinesterase			Total lipids			Total proteins			Total carbohy		
	Mean ± SE (gm/g.b.wt.)	Change %	Activity ratio	Mean ± SE (gm/g.b.wt.)	Change%	Activity ratio	Mean ± SE (gm/g.b.wt.)	Change %	Activity ratio	Mean ± SE (gm/g.b.wt.)	Change %	Activity ratio
Owner	353±3.51 $_b$	15.99	0.84	3.77±0.15 $_c$	45.12	0.55	31±0.64 $_{bc}$	24.99	0.77	22.46±0.73 $_a$	3.19	0.97
Dimilin	302.3±4.33 $_c$	28.06	0.72	5.24±0.18 $_b$	23.73	0.76	40.3±1.17 $_a$	2.49	1.03	14.5±0.76 $_{bc}$	37.5	0.625
Emafel	384.67±7.86 $_a$	8.459	0.92	6.3±0.19 $_a$	8.29	0.92	27.66±0.73 $_c$	33.07	0.69	12.6±0.19 $_c$	54.6	0.54
Strong	370.67±6.06 $_{ab}$	11.89	0.88	6.34±0.18 $_a$	7.71	0.92	34.56±0.59 $_b$	19.85	0.86	16.53±0.80 $_b$	28.75	0.71
Control	420.22±9.28 $_c$			6.87±0.22 $_a$			41.33±1.30 $_a$			23.2±0.95 $_a$		
F.value	37.01	----		49.58	----		39.85	----		41.76	----	
L.S.D.	29.44			0.815			4.17			3.29		

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Physiological effects of some pollen substitutes diets on caged honey bee workers *Apis mellifera* (Hymenoptera: Apidae)

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Abstract:

Nutritional value of four proteinaceous diets and its physiological effect on honey bee workers were evaluated under laboratory conditions. The tested diets were as follows, diet 1 (Date syrup, skimmed milk powder and dried brewer's yeast), diet 2 (Turmeric, fenugreek powders and dried brewer's yeast), diet 3 (Chick pea flour, wheat germ and dried brewer's yeast) and diet 4 (Soybean meal, skimmed milk powder and dried brewer's yeast). *Apis mellifera* L.(Hymenoptera: Apidae) carnica was used in small groups of caged honey bee workers. The consumption rate, longevity, hypopharyngeal glands development degree (HPG) and rectal contents were determined. The greatest consumption rate was recorded for the control group which fed with bee bread and the lowest one recorded for diet 4. Feeding bees on diet 3 gave the longest LT_{50} (27.0 days) after the controlled bees, which gave LT_{50} (29.0 days). On the other hand, the honey bee workers that fed on diet 4 gave the lowest LT_{50} (20.5 days). The highest degree (3.78 HPG degree) of gland development was recorded at 9 days old in bees fed on bee bread followed by those fed on diet 3 (3.24 HPG degree). Whereas, the lowest developed HPG (2.14HPG degree) was obtained in bees fed on diet 4. The results of rectal content weight of honey bee workers reflect the suitability of diet 3, diet 1 and diet 2 for honey bee workers which recorded 13.43, 16.03 and 16.12 mg/bee/3 days, respectively.Accordingly, the diet 3 had good nutritive value for honey bee colonies which help bees to establish healthy colonies with good production.

Introduction

Pollens are indispensable food for honey bee colonies and their shortage intermittent periods cause several problems for the colonies. Moreover, different pollen types collected from different plant origins could be had differently effects on the physiological conditions of worker honey bee (Amro *et al*., 2015). Completely absence of protein sources in the hive cause starvation for honey bees and consider main reason for colony collapse disorder (CCD) (Seitz *et al*., 2015). So, provide colonies with protein source all over the year, especially during dearth periods of pollen is critical

matter.Recently, several studies gave more attention to formulate supplementary diets or substitutes to compensate the lake ofthe natural protein source (pollen) (Zheng *et al*., 2014; Amro *et al*., 2016; Negri *et al*., 2017; GamalEldin *et al*., 2018 and Gregorc *et al*., 2019).

The presented pollen substitute showed have special specification, i.e. Palatable (Saffari *et al*., 2010), consumption (Doull, 1973), attractiveness (Abd El-Wahab *et al*., 2016) and have a good physiological effectson honey bees (Amro *et al*., 2016). Also, the useful pollen substitute should be stimulating colony growth and support aspects of worker quality, such as large brood areas and the long length of adult stage (Winston *et al*., 1983). Several studies documented many food materials as a suitable pollen substitutes for honey bees. However, Soybean meal (Abbasian and Ebadi, 2002), yeast (Abd El-Wahab and Gomaa, 2005), skimmed milk (Amro *et al*., 2016) and wheat gluten (Nutter *et al*., 2017) can be used as a basic material for preparing pollen substitute cakes. Its high content of protein encourages HPG to stimulate royal jelly secretion and promote honey bee workers to rearing moor brood. However, the roll of these materials to enhance workers physiological condition still lacking. Some pollen substitutes were tested for feeding honey bee workers by Amro *et al*. (2016) and GamalEldin *et al*. (2018). They considered consumption of pollen substitute in the period after worker emergence, development of HPG, longevity and rectal content weight are important criteria for estimating the suitability of proteins diets for honey bee workers.Younis (2006) found that, the Wheat Germ is the best pollen substitute, as it increases the bee's activities, especially in the lack of pollen grains sources. Followed by Dried Brewer's yeast, then Soybean flour and the Palm Date come last. Al-Ghamdi *et al.* (2011) found significant differences in the degree of HPG development when the bees were fed on bee bread, followed by pollen loads and a mixture of yeast, gluten and sugar (1:1:2). The longevity of honey bee workers appears to be directly associated with level of body protein (Sagili *et al*., 2005). De Groot (1953) concluded that protein feeding increases the length of life emerged bees in cages, compared with those fed sugar syrup only. Al-Qarni (2006) consider honey bee workers rectal content weight directly reflects the food suitability. It used to test the variability of food demonstrated to honey bee and its utilization by honey bee colonies. Also, Amro *et al.* (2016) found that, the heist rectal content weight was recorded in caged honey bee fed with corn gluten, while the lowest one was recorded for FeedBee®. These finding reflect the good digestion and full benefits reward from FeedBee® to the workers.

Recently, some commercial pollen substitutes as Feedbee® and Bee-pro® were offered for application. The suitability ofFeedbee® was tested by Omar *et al*. (2017) and recorded that it promotes HPG development better than a protein-free diet but not as good as the pollen mixture or mono-floral pollen from *Asparagus*sp. or *Castanea* sp. Also, Amro *et al.* (2016) concluded that Feedbee® was able to enhance brood rearing activity in despite of present it under isolation condition in full absence of pollen. However, its high price consider barrieragainst its application.

This study was done to evaluate the physiological effects ofsomeproteiounes diet based on soybean meal, chick pea flour wheat germ, dried brewer's yeast, skimmed milk powder, date syrup, turmeric and fenugreek powders. Diet consumption, longevity, HPG development and rectal content weight of caged honey bee workers were used to evaluate nutritive values of the tested pollen substitute diets.

Materails and methods

The experimental research was carried out under laboratory conditions at Department of Apiculture Research, Plant Protection Research institute, Agriculture

research Center, Egypt, during summer season of 2018.

1.Proteinaceous materials :

Seven materials (Table, 1) that are rich in their protein content and are available in local area were selected for testing them as pollen substitute. Total protein% of these raw materials was determined by Kjeldahl method (Kirk, 1950). Four proteinaceous mixtures were prepared from the raw materials (Table, 2). Diet 2 is a pollen substitute tested by Abd El-Wahab *et al.*(2016) and consisting of (10g brewer's yeast + 1g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A,D and E vitamins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup).Beebread was used as a control diet in the tested cages.

Table (1): Total protein percentage of raw materials used for pollen substitutes.

Raw materials for pollen substitutes*	Total protein %
Soybean meal (*Glycine max*	39.88± 0.13 b
Brewer's dried yeast	40.57 ± 0.19 a
Skimmed milk powder	28.82 ± 0.19 d
Date syrup (*Phoenix dactylifera*)	7.55 ± 0.26 g
Wheat germ (*Triticum aestivum*)	31.58 ± 0.27 c
Chick pea flour (*Cicer arietinum*)	22.24 ± 0.20 f
Fenugreek powder	23.02 ± 0.21 e

***Means followed by the same letter do not differ significantly at the 5% level of probability.**

Table (2): Description of mixed proteinaceous diets administrated to honey bee workers.

Materials	Composition of the diets / 1 Kg.			
	Diet 1	Diet 2	Diet 3	Diet 4
Soybean meal (*Glycine max*)				252
Chick pea flour (*Cicer arietinum*)			154	
Date syrup (*Phoenix dactylifera*)	280			
Diet 2*		1000		
Wheat germ (*Triticum aestivum*)			154	
Dried skim milk	91			84
Brewer's yeast	91		62	84
Sugar powder	457		315	420
Honey (ml)	17		251	17
Water (ml)	64		64	143
Total	1000	1000	1000	1000

*** Pollen substitute tested by Abd El-Wahab, *et al.* (2016)**

2. Honey bees:

The present study was carried out in experimental cages using newly emerged honey bee (*Apis mellifera carnica* poll.) workers. Sealed brood combs free from bee bread were placed in an incubator at 32 ± 1 °C, 65 ± 5 RH. in screen cages to obtain newly emerged bees (0-12 hours) to prevent the emerging bees from consuming pollen or honey in the brood comb, all areas of comb containing these materials were covered with aluminium foil wax or both (Standifer *et al*., 1960).

3.Experimental cages:

Experimental wooden cages of 15×15×5 cm. dimensions with a glass side and other side were covered with black muslin. Every cage was provided with a vial of tap water and other vial of sugar solution 1:1 (w/v) and a piece of wax comb was attached to the top of each cage to imitate natural conditions experienced by honey bees (Williams *et al*., 2013). Four cages were used for every treatment; each contains 100 workers.The above mentioned pollen substitutes were introduced to each cage, into a small plastic feeder (1 cm. height and 3 cm.

diameter) covered with a small sheets of polyethylene to avoid water evaporation and any food loss which could be occurred at free access of honey bees to the whole (Sticking on the legs, wings, the small hairs on the body, etc.). Each feeder contains an average amount of 5 g. Pollen substitutes stock are kept into the refrigerator at 4 °C until administration. The diets were changed in each cage every 3 days. All cages were kept in the dark in an incubator at 32 ± 1 °C and 65 ± 5 RH. Additionally, the weight loss by one sample of diet without bees was measured to determine the daily evaporation rate. For this calculation, the percentage of evaporation was subtracted from the recorded amount of the protein diet consumed (Pernal and Currie, 2000).

4. Measurements:

The experiment was carried out in two groups, each one consists of twenty cages, daily food consumption of nurse bees and worker longevity were measured in the first group. However, the degree of HPG development and rectal content were measured in the second group.

5. Food consumption:

Daily food consumption was calculated every 3 days by mg./bee/3days until 15 days old. The amount of diet/cage was compared with the number of live bees existing in each cage during the investigation (Schmidt *et al*., 1987).

6. Workers longevity:

Dead bees in each cage were counted and removed every 3 days interval until half of the initial number of honey bee were died (Standife *et al*., 1960). The LT_{50} was estimated. Values in days of bee workers fed with different diets were determined by a computerized probit analysis program using SAS 9.1.3 programme (SAS Institute, 2004).

7. Hypopharyngeal glands development:

Development of HPG was determined on honey bee workers of 3, 6, 9, 12 and 15 days old. Ten honey bees were used to assimilate each age from each treatment. The heads of honey bee workers were dissected under a binocular in physiological saline. The degree of gland development was determined according to Maurizio (1954). An arbitrary scale (I to IV) was used to determine the degree of development grade I, represented undeveloped glands and IV, represented complete development glands.

8.Weight of rectal content:

The same ten bees, used for measuring HPG development were used to determine the weight of rectal content by extracting the rectum with a fine forceps, and placing it on a cover glass, previously weighted and then reweighed on an analytical balance **(Al-Qarni, 2006)**.

9. Statistical analysis:

The experimental design for the all experiments mentioned above were completely randomized design (CRD). ANOVA was performed and means were compared by using Duncan's multiple range tests at 5% level of probability (Duncan, 1955) with the SAS 9.1.3 programme (SAS Institute, 2004).

Results and discussion

1.Food consumption:

The consumption rate of tested pollen substitutes is illustrated in Figure (1). The consumption patterns were quietly similar in all tested diets. During the first six days measurements recorded the greatest rate of consumption especially during the second period (4-6 days).The consumption rate decreased sharply till the appearance of the of the minimum value after 15 days at low level by the 15^{th} days.These results confirmed the findings of Crailsheim *et al*. (1992) who's reported that protein diets were mainly consumed by caged honey bees aged between 1 and 8 days old.Wherever, the same age bees in a colony performed the same brood care behaviour, with the highest consumption observed during day 3. The total amount of pollen substitute consumed per bee throughout 15 days were 10.5 > 8.3 > 7.8 > 6.3 > 4.2mg./bee/3days for bees fed on bee bread, diets 2, 1, 3 and 4respectively.The obtained results revealed significant

differences between all treatments under the experimental condition. Although, the main row material of pollen substitutes was differed in the protein percentages, the pollen substitute consumption did not affect by the protein content of these materials. Schmidt and Johnson (1984) found weak correlation between bees feeding preference and/or the protein level of pollen diets. These findings agreed with present results whereas bees do not increase consumption to compensate the reduction in dietary protein. Also, they suggested that consumption may be influenced by physical or chemical factors that are unrelated to diet quality.

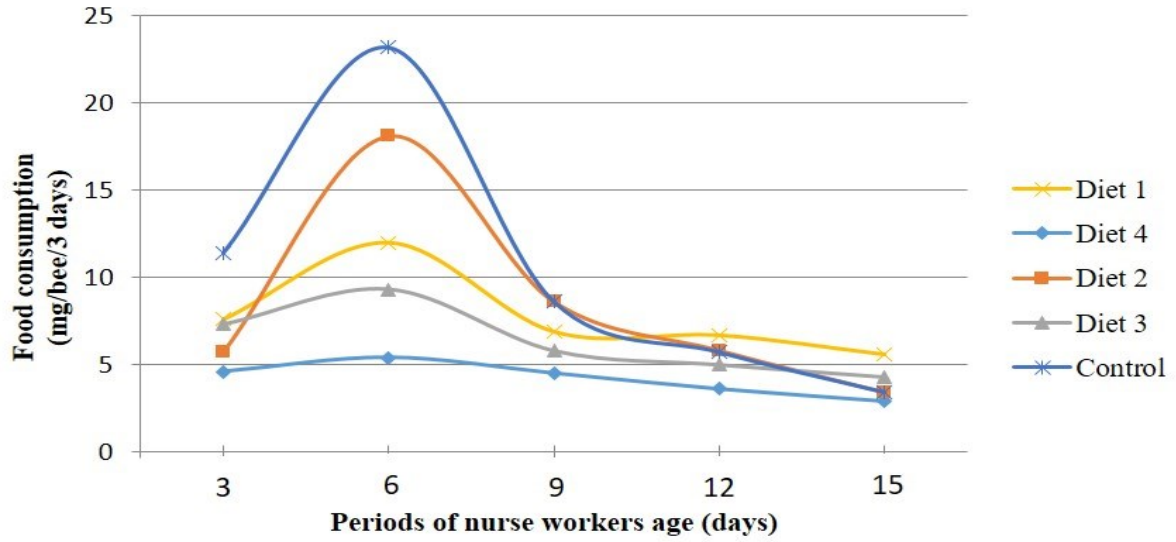

Figure (1) : Rate of food consumption by Carniolan honey bee workers fed on tested pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

2. Workers longevity:

The mortality percentages and LT_{50}were illustrated in Figure (2). Data revealed that feeding bees on pollen substitutes contained soybean fell short the longevity of honey bee workers (LT_{50}, 21.0 days) in comparison with those fed on other pollen substitutes. The results indicated that the life span of honey bee workers fed on diet 1 (date syrup) and diet 2 (Fenugreek powders) was quietly similar and recording 24.2 and 25.1 days respectively. Bees fed on diet 3 (chick pea flour+ wheat germ) presented LT_{50}reached to 27 days. Finding of Younis (2006) which proved that, feeding with Wheat Germ gave the longest bees average life. While the bees fed on Soybean flour gave the shortest average life, Wallace *et al*. (2016) showed that, chick pea flour contains 21g protein, 53g carbohydrates, 10g crude fibre, 6g fat and 356 calories, may be reflect the high longevity of workers fed on diet 3 during the present work. The mortality rates of newly emerged workers fed on different protein sources was found to be related with the material type and their contents of protein. From the obtained results, it appeared that the commonly accepted protein sources used as pollen substitute for bees is diet 2. This diet can be used as protein source for feeding honey bee colonies mixed with other ingredients such as yeast, skimmed milk and pollen. On the other hand, the lowest survival was observed in caged bees which fed on soybean meal.In this approach, Manning *et al*. (2007) studied soybean effect on bees longevity. They reported that, soya bean flour had the lowest oleic acid concentration and was the best in giving bees greater longevity, but it was still worse than pollen diets.

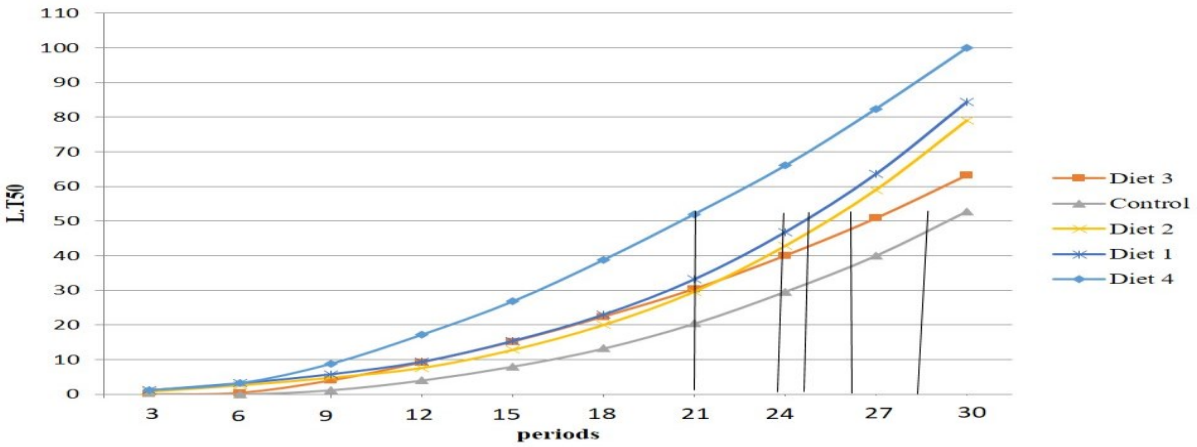

Figure (2): Cumulative mortality percentage and LT50 of Carniolan honey bee workers after feeding on tested pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

3. Hypopharyngeal glands development:

Hypopharyngeal glands (HPG) development of worker bees fed on different pollen substitutes are illustrated in Figure (3). The result clearly showed significant differences in the development of HPG between the workers fedon different Proteinaceous diets.The acini reached their maximum size when the bees are 2 to 9 days old and then became smaller. This confirms previous findings in colonies or cages (Altaye *et al*. , 2010). The highest development of HPG was recorded in bees received bee bread (control) (3.78 HPG development degree) and the lowest one was recorded by bees received diet 4 which containedsoybean meal (2.14 degree). The present results indicated that the general means of HPG development degree were 3.24>2.70> 2.36>2.14HPG development degree for bees fed diets 3, 2, 1 and 4 respectively.According to Huang (1990) the HPG organs secreting enzymes and royal jelly, quickly respond to changes in the nutritional value of feed protein. This means that the development of HPG was strongly correlated with amount of protein consumed by honey bee workers from presented diets (Pernal and Currie, 2000). Therefore, crude protein is an essential dietary component for the development and will being of bee colony. In the same line, Sagili *et al*. (2005) recorded that bees fed 1% soybean trypsin inhibitor (SBTI) had significantly reduced HPG protein content. They also concluded that nurse bees fed a pollen diet containing at least 1% SBTI would be poor producers of larval food, potentially threatening colony growth and maintenance. The supply of soybean meal caused an abrupt reduction in pupae emergence, attributing this to the lack of niacin amino acid in soybean meal (Haydak, 1949).

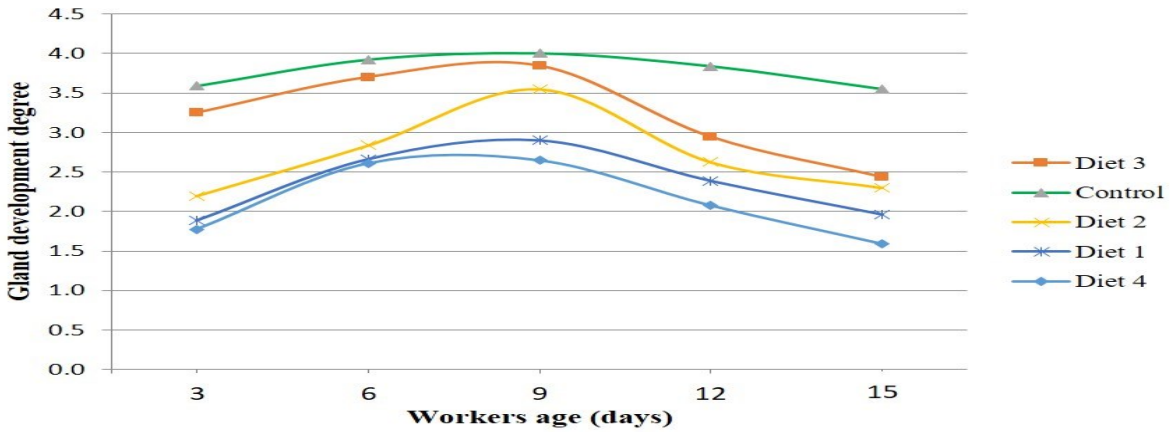

Figure (3): Hypopharyngeal gland development of Carniolan honey bee workers fed on different pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

4.Weight of rectal content:

Figure (4) showed that for most of the tested diets the rectal contents started with low weight on the first inspected age (3-days), then grew up to reach the highest weight at the eldest age (15 days). This result explain the progress of workers appetite with the age progressing. The lowest means of workers rectal contents weight showed that values of bee bread as a most suitable food for honey bees. The highest general mean was presented by the bees fed on diet 4 (soybean meal) with an average of 18.25mg./bee/3 daysand on diet 2 (Fenugreek powders) 16.12mg./bee/3 days with significant differences from the means of honey bee workers fed on other diets. Diets 3 contains chick pea flour+ wheat germ ranked the second after control cages followed by date syrup diet recording 13.43 and 16.03 mg./bee/3 days, respectively. As honey bees do not defecate in the cage, the accumulation of waste material in the rectum, depending on other components present in each protein source, may lead to the premature death of caged workers (Maurizio and Hodges, 1950). This may contribute to the higher rectal content weigh in bees fed diet (4). These results were in the same line with those obtained by Amro *et al.* (2016) which recorded the highest rectal content weight in bees fed with soybean meal.

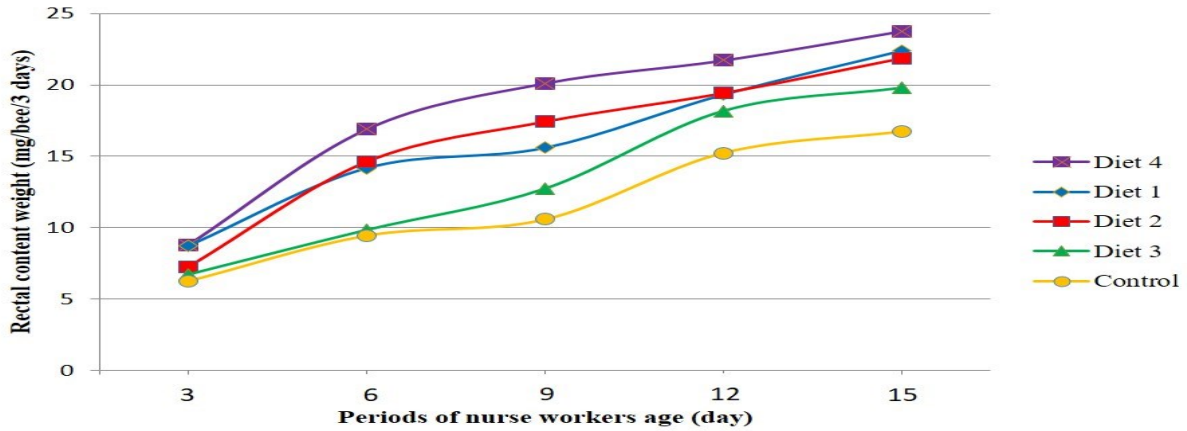

Figure (4):Variation of rectal contents of Carniolan honey bee workers fed on different tested pollen substitutes in cages placed in an incubator at 32 ± 1 °C, 65 ± 5 RH.

Since providing honey bee colonies with protein is very important, especially when no natural protein sources (pollens) are available for them. Using diet 2 was found tobe consumed rapidly other than tested diets. Also, itis successful to enhance some physiological characteristics of honey bees better than date syrup. Although, diet 3 was not consumed rapidly by the bees, it had the highest HPG development, pest longevity and the lowest rectal content weight compared to the other tested diets. While,diet 4 was not good in regard to the investigated parameters, and this diet is not recommended. Beekeepers are advised and diet 3 when no or few natural pollen sources are available for their bee colonies.

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Efficiency of certain insecticides and their histological effects against sugar beet beetle *Cassida vittata* (Coleoptera: Chrysomelidae) in sugar beet field

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Abstract:

Sugar beet beetle *Cassida vittata* (Vill.) (Coleoptera: Chrysomelidae) is a serious pest on sugar beet causes losses in root yield and sugar content in Egypt. The present work dealt with the efficiency of four compounds representing different classes of insecticides against sugar beet beetle *C. vittata* larvae under field conditions at Dakahlia and Behira Governorates in 2015 season. The tested compounds were imatrade (35% SC) (Imidacloprid), flagtra (25% WP) (Thiamethoxam), agriflex (18.6% SC) (Thiamethoxam + Abamectin) and dora (48% EC) (Chloropyrefos). In each field, the results revealed that, all treatments were able to suppress the larval population comparison to the untreated control. The suppression varied according to the tested compound, dora caused 93.51% reduction after ten days post spray in the larval population followed by flagtra (87.15%). Generally, the highest reduction was recorded in dora treatment at two regions. The mean Reduction percentage were 89.64 and 91.09 % in Dakahlia and Behira Governorates, respectively. The histological examination of mid gut of 3rd instar larvae of *C. vittata* showed that all tested insecticide lead to several damages occurred in all the cells layers in the mid gut as separation of the basement membrane. In addition, imatrade (35% SC) possessed many mid gut cells with a weakly stained cytoplasm as well as a nucleus with decondensed chromatin and evident nucleoli. Agriflex (18.6% SC) due to increase proliferation of columnar cell. While, dora (48% EC) caused increase most of columnar cell lining mid gut were necrosis. Also, flagtra (25% WP) lead to increase in number of goblet cell, most of columnar suffer from necrosis.

Introduction

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops in the world. It considered as an important source of feed for livestock and pectin production from the pulp of sugar beet (Fouad *et al.*, 2011). The Egyptian Governments encourage sugar beet growers to increase the cultivated area with sugar beet for decreasing the gap between sugar production and consumption (Al-Habshy, 2013). Sugar beet quality is of great

economic importance. Sugar beet is strategic sugar crops in Egypt because of its lower consumption of irrigation water and its shorter growing season. In Egypt, sugar beet is cultivated in 555.6 thousand faddan produce 909.9 Ton with an average production of 16.7 tons per faddan (Anonymous, 2016)

Sugar beet plants attack by numerous insect species during growing season such as beet fly *Pegomia mixta* (Vill.) (Diptera: Anthomyiidae) , the green beach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Cassida vettata* Vill. (Coleoptera: Chrysomelidoe) caused considerable damage in its yield (Sherief *et al.*, 2013). The sugar beet beetle *C. vettata* is one of the most destructive pest of sugar beet plant (El-Zoghbey *et al*., 2003). Both sugar beet beetle larvae and adults feed on the lower side of the sugar beet leaves, where, they eat the lower epidermis and inner tissue, but the upper epidermis remains intact looking like a glass. In addition, adults feed on leaves tissue, causing regular circular holes (Abo El-Ftooh *et al.*, 2013). Crop loss occurs due to leaf feeding and reduction in sugar content of infested plants (Ali *et al.* , 1993).

There are two important methods of insect control, biological and chemical methods. This pest is controlled in Egypt by conventional chemical insecticides. Use of insecticides has several disadvantages. It reduces population of predators and parasitoids of insect pests, leads to environmental pollution and development of pesticides resistant biotypes of insects (Abo El-Ftooh *et al*., 2013). Some conventional insecticides, i.e. Pirimiphos-methyl, monocrotophos, profenofos, methomyl were comparatively more effective against *C. vittata* under field conditions (Shaheen *et al*., 2011).

The main purpose of the current investigation is selecting the most suitable four compounds to contribute the integrated control operations to *C. vittata* larvae under the field condition and their effect on histology of this insect.

Materials and methods

The experiments were conducted at the farms of Dakahlia and Behira Governorates during 2014-2015 sugar beet growing season. Sowing dates were the 15th and 18th of October in Dakahlia and Behira, respectively. All normal agricultural practices were performed and no insecticide treatments were applied. These experiments were carried out to study the following to:

1. Efficiency of certain insecticides on the larvae of *Cassida vittata* :

The experiment was carried out to evaluate the efficacy of different compounds against the tortoise beetle *C. vittata* on sugar beet. An area of experimental was about 875 m^2 was cultivated with cable variety. The area was divided with randomized complete block design to 20 plots. The tested treatments are four compounds in addition to the control (untreated) as shown in Table (l). Each compound was applied with the recommended rate when the numbers of *C. vittata* were high. Spraying was directed to the plants using a knapsack sprayer was used for sprays at the morning hours during the two regions. Random samples were taken just before spraying the field and those for posttreatment counts were taken 1, 3, 7 and 10 days after application. Larvae were counted directly at random on 5 plant / plot.

2. Statistical analyses:

Reduction percent in *C. vittata* larval population after treated with four insecticides calculated according to **(Henderson and Tilton, 1955).**

Table (1): Tested insecticides, dosages, common name and trade name during 2014 - 2015 season.

Trade name	Common name	Rate of use/100L water
Imatrade (35% SC)	Imidacloprid	100 cm^2
Flagtra (25% WP)	Thiamethoxam	20 g
Agriflex (18.6% SC)	Thiamethoxam (3.3 2g/l) +Abamectin (15.24 g/l)	80cm^2
Dora (48% EC)	Chloropyrefos	330 cm^2
Untreated (control)	----	----

3. Histological technique for examination of mid gut, integument of larvae:

The 3rd instar larvae treated and untreated were obtained from the field after 24 hour of the treatment with recommended doses of all the tested compounds and transferred to the laboratory. Larvae were individually dissected in petri dish containing Ringer's solution to obtain mid gut by using fine entomological needles under a binocular dissecting microscope at 40X magnification and fixed in alcoholic Bouins solution for 12 hour and dehydrated through a graded series of ethanol and embedded in paraffin wax (42-48 Cº M.P) for half hr then changed 3 times in paraffin wax of melting point 58-60 ºC. 30 minutes each transverse sections of 6 microns thickness of the mid gut were stained with heidenhain's haematoxylin and eosin according to **Junqueira and Carneiro (1980).**

Results and discussion

1.Effect of certain insecticides on *Cassida vittata* larvae:

The efficiency of certain insecticides was examined against the *C. vittata* larvae in the sugar beet field in Dakahlia and Behira Governorates during 2015 season. The percent of reduction infestation is presented in Tables (2 and 3). The results showed that, all compounds were able to suppress the levels of infestation of *C. vittata* larvae to different degrees in comparison to that of untreated control. Generally, the highest reduction was recorded in dora treatment at two regions. The mean reduction % was 89.64 and 91.09 % in Dakahlia and Behira Governorates, respectively.

1.1. In Dakahlia Governorate:

The percentage reductions of *C. vittata* larvae population were showed in Table (2). All treatments declined alive larvae at 1, 3, 7 and 10 days post spraying comparing with control. The first day after treatment, dora was the most effective achieving 88.09% reduction in larvae. Three days after post spray, the highest reduction were with dora which recorded 89.52% followed by agriflex, imatrade and flagtra which recorded 83.92, 83.48 and 83.34%, respectively. Also the reductions after 7 and 10 days recorded the highest value with dora 90.95% followed by agriflex flagtra and imatrade which were 84.87, 84.29 and 83.93%, respectively.

Table (2): Reduction percentage in *Cassida vittata* larvae population after treated with certain compounds in the field sugar beet during 2015 season in Dakahlia Governorate.

Compounds	Rate of use/100L (recommended)	Reduction Post spraying (days)				Mean
		1	3	7	10	
Imatrade	100 cm^2	4.16	83.48	84.93	83.68	64.06
Agriflex	20 g	2.60	83.92	85.34	84.87	64.18
Flagtra	80cm^2	1.90	83.34	84.76	84.29	63.57
Dora	330 cm^2	88.09	89.52	90.00	90.95	89.64

1.2. In Behira Governorate:

The data in Table (3) did not differ from Behira Governorate. A significant decline in alive larvae was observed at 1, 3, 7 and 10 days post spraying with all tested insecticides. After one day post spray, dora showed the highest present of reduction (89.35%) while imatrade, agriflex and flagtra

were recorded the lowest reduction. However, the reduction percentage reached 90.28, 86.70, 86.51 and 86.17% for dora, flagtra, imatrade and agriflex after three days post spray. Also the same trend, after ten days from application, dora caused highly decrease in the larval population where the percent reduction were 93.51% at recommended rate followed by flagtra was 87.15%, while agriflex and imatrade were the least effective (86.92 and 86.84%, respectively.) at recommended rate.

Table (3): Reduction percentage in *Cassida vittata* larvae population after treated with certain compounds on the field sugar beet during 2015 season at Behira Governorate.

Compounds	Rate of use/100L (recommended)	Reduction Post spraying (days)				Mean
		1	3	7	10	
Imatrade	100 cm^2	4.74	86.51	87.04	86.84	66.28
Ariflex	20 g	3.66	86.17	87.15	86.92	65.97
Flagtra	80cm^2	3.66	86.70	87.61	87.15	66.28
Dora	330 cm^2	89.35	90.28	91.20	93.51	91.09

The obtained results are in a harmony with that recorded by Asmahan and Qasem (2004) evaluated the efficiency of chlorfenapyr against eggs, larvae, pupae and adults of the tortoise beetle *C. vittata*. Results indicated that, chlorfenapyr was highly toxic effect. El-Kouly (1998) reported that the profenofos was the most efficient compound against all stages of *C. vittata* under field. Abdou (2009) mentioned that, Marshal the most effective to control the adult of *C. vittata,* marshal caused 82.3% decrease in the adult population followed by achook 68.5% while (Bancol, alkanz and chess) had moderate efficient against this pest. Abo El-Ftooh *et al.* (2013) who evaluated the effect of three pesticides (Radiant 12% SC, dursban 48% EC and mospilan 20% SP) against sugar beet beetle *C. vittata* at Behira Governorate. They found that, radiant SC 12% pesticide was more toxic against *C. vittata* (Larvae and adults). But the dursban EC 48% was superiority during some periods of experiment.

2.Effect of tested insecticides on histological structure of mid gut of *Cassida vittata* larvae:

The mid gut is the main origin for digestion and absorption of ingested food. Insects mid gut wall comprises of two muscular layers and an epithelial layer lining the lumen. The intestinal epithelium contains four types of cells; digestive, regenerative, endocrine and goblet cells.

2.1. In the control group:

The mid gut of 3rd instar larvae of *C. vittata* consists of two layers of muscle fibers, the outer longitudinal fibers and the inner circular one. The circular muscle fibers are very close to the basement membrane of the epithelial cells. There is wide space between the longitudinal fibers. The peritrophic membrane is followed by the epithelial layer which lining the cavity of the mid gut. The mid gut epithelium consisted of a single layer of digestive cells exhibiting a well developed brush border and cytoplasm with acidophilic regions. (Figure, 1)

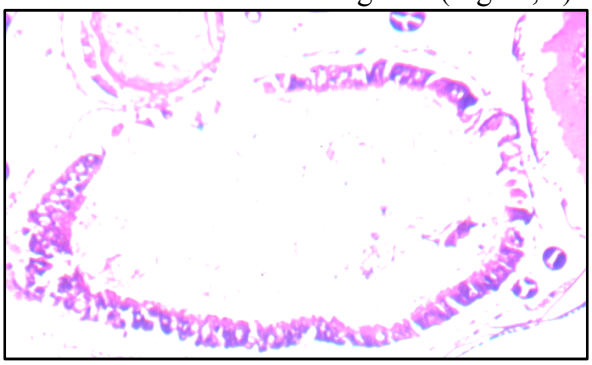

Figure (1): Transverse section in the mid gut of the control 3rd instar larvae of *Cassida vittata*.

2.2. Imatrade treatment:

Microscopic examination of the transverse section of the mid gut of 3rd instar larvae of *C.vittata* treated with imatrade (35% SC) (Figure,2) showing increase in number of goblet cells as well as necrosis of some columnar cells lining it. In addition, several damages occurred in all the cells layers in the mid gut as separation of the basement membrane. Imatrade treated larvae possessed many mid gut cells with a weakly stained cytoplasm as well as a nucleus with decondensed chromatin and evident nucleoli.

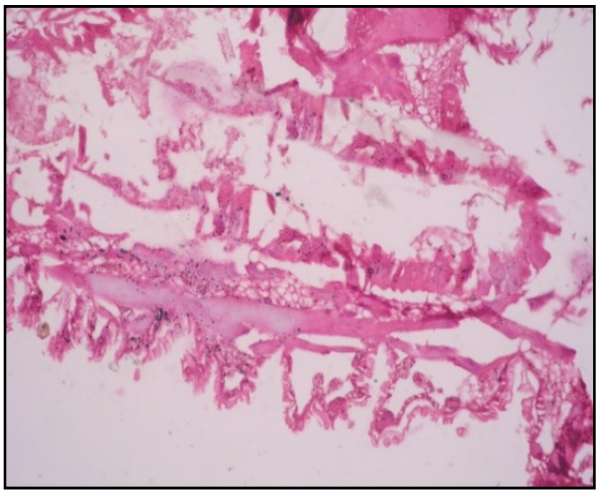

Figure (2): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with imatrade (35% SC).

2.3. Flagtra treatment:

The most affected tissue was the mid gut epithelium when compared with the untreated mid gut. Microscopic examination of transverse section in the mid gut on the 3rd instar larvae of *C.vittata* with flagtra (25% WP) (Figure, 3) illustrated the increase in no. of goblet cell, most of columnar suffer from necrosis.

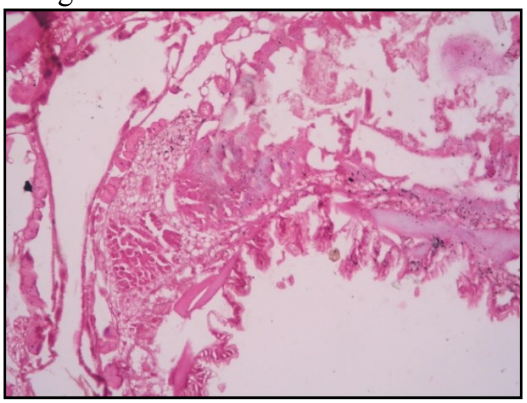

Figure (3): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with flagtra (25%WP).

2.3. Agriflex treatment:

Transverse section in the mid gut on the 3rd instar larvae of *C.vittata* which treated with agriflex (18.6SC%) (Figure, 4) showed that severe damage effects on the mid gut layers and increase proliferation of columnar cell. Epithelia cells separated and collected in groups.

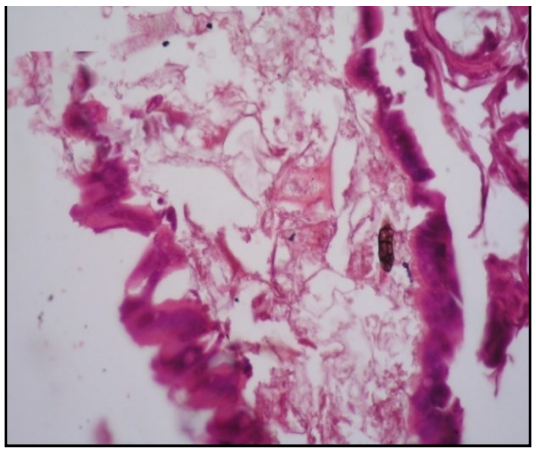

Figure (4): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with agriflex (18.6% SC).

2.4. Dora treatment:

Severe effects were found in the mid gut of the larval treated with In case treated the 3rd instar larvae of *C. vittata* by dora (48% EC) (Figure, 5). The mid gut showing increase most of columnar cell lining mid gut were necrosis. In addition destruction in the longitudinal, epithelial and columnar cells.

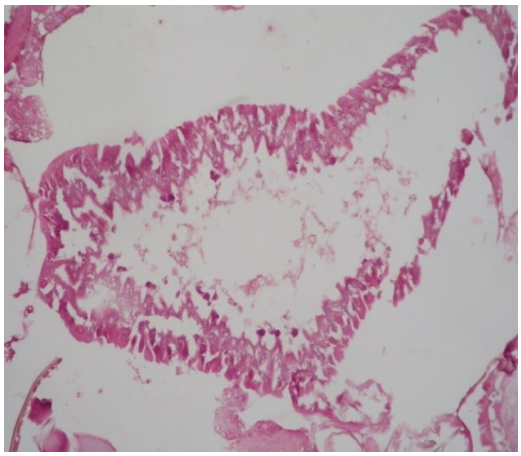

Figure (5): Transverse section in the mid gut of 3rd instar larvae of *Cassida vittata* treated with dora (48% EC)

These results are similar to that obtained by Sayed and Abd El- Aziz (2014) who reported that, microscopic examination of transverse sections in the mid gut of 3rd instar larvae of *C. vittata* treated with robust and marshal showed sever histological effects in all layers of the mid gut. And it showed separation of both basement and peritrophic membranes. Sharaby and El-Nujiban (2016) found that, the most affected tissue was the mid gut epithelium when compared with the untreated mid gut of *A. ipsilon* larvae. The epithelium possessed deeply stained nuclei, the regenerative cells were not pronounced and could not been identified in some areas at the base of the epithelial cells due to the sever destruction of the epithelium, goblet cells increased their secretion. And they added, Epithelial cells of the treated larvae were destroyed, large vacuoles were found between the epithelium and the muscular layer. Yasmeen and Amir (2018) who cleared that, the insecticide chlorpyrifos was provided in food to 3rd instar larvae *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) causing loosely attached epithelial layer in sections which became filled with vacuoles at higher concentrations in mid gut. In addition epithelial cells became more or less circular

in shape and highly separated from each other but on increasing the concentration, significant elongation in epithelium cells and decrease in lumen of the mid gut were seen. The number of epithelial cells has been reduced.

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Impact of aphid infestations and yellow rust infection on some wheat cultivars Egypt

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Keywords: Wheat (Puccinia striiformis f. sp. tritici), aphid infestations, adult-plant resistance and yield losses**,**

Abstract:

This study was carried out to evaluate the susceptibility of six wheat varieties, sids 12, gemmeiza 11, misr 1, misr 2, sakha 94 and giza 171 to infestation with cereal aphids especially the oat bird-cherry aphid, *Rhopalosiphum padi* L., the greenbug aphid, *Schizaphis graminum* (Rondani) and the English grain aphid, *Sitobion avenae* Fabricius (Hemiptera: Aphididae) and the yellow rust disease in grain yield under field conditions at Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt during two growing seasons and successive seasons 2017/2018 and 2018 / 2019. Data showed that the presence of aphids on six varieties was highly concentrated during the period was extended from the first week of January till the end of March during both tested seasons. The highest value was 69.8, 61.9, 73.1 and 30 individuals/ 10 plants on misr 1, sids 12, misr 2 and sakha 94 at 2nd week of March. While, it was reported at the first week of March by 69.7 and 51.8 individuals/ 10 plants on gemmeiza 11 and giza 171, respectively. A significant difference was reported between the tested wheat cultivars.

The field experiment with nirtire of races yellow rust *of Pucciniastrii formis f. sp. tritici* physiologic races as a source of inoculam. Yellow rust infection significantly reduced grain yield of all inoculated cultivars as compared to the protected ones Disease severity was recorded weekly, and area under disease progress curve was estimated and ranged from 41,30 to 558.00 in wheat cultivars Sakha 94 and Sids 12 during season 2017/18 while, 60.00 to 925.00 in wheat cultivars Giza 171 and Sids 12 during season 2018/19. The loss in 1000 kernel weight of the different wheat cultivars was variable according to the varietal response. The 1000 kernel weight of the protected plants of all wheat cultivars were higher than the infected treatment and also significant differents were found between infected and protected wheat cultivars under the study. according yield losses ranged from 10.54 to 30.73 in wheat cultivars sakha 94 and Sids 12 during season 2017/18 7.53, 73.44 wheat cultivars giza 171 and sids 12 during season 2018/19.

Introduction

Wheat crop (*Trirticum aestivum*) is one of the most important strategic cereal crops in Egypt and also in the world. It provides about 20% world food calories and food

for nearly 40% of the world's population. The cereal is grown on 23% global cultivated area is for great importance in bread, diet, pharmaceutics and other industries. Wheat is important product of international trade for worldwide market. Many pests attack wheat causing yield damage and leading to great losses in quality and quantity of the wheat crop. The major insect pests in cereal crop were the oat bird-cherry aphid, *Rhopalosiphum padi* L., the greenbug aphid, *Schizaphis graminum* (Rondani) and the English grain aphid, *Sitobion avenae* Fabricius (Hemiptera: Aphididae) in Egypt. The damage caused by cereal aphids is direct through feeding by sucking the plant sap and indirect through the effects of honeydew in combination with fungi, which reduce rate of photosynthesis and available leaf area (Martin and Johnston, 1982; Ryan *et al*., 1987). Wheat yield loss due to cereal aphid infestation was estimated by 7.5 to 18.7% (Tantawi, 1985), up to 23% (El-Heneidy *et al*., 1991) and 17.83% (Abdel-Rahman, 2005).The most important and economic cereal aphid species in Egypt were; R. padi, *S. graminum*, *Rhopalosiphum maidis* (Fitch) and *S. avenae* (El- Heneidy and Adly, 2012).

Yellow rust (*Puccinia striiformis f. sp. tritici)* is one of the wheat rusts that cause severe losses throughout the world. Losses of 50-70% have often been reported under field conditions. The actual amount of loss caused by rust can range from slight to complete destruction of the crop. Grain from infected crops is shrivelled and light in weight, and therefore has reduced quality (Stubbs *et al*., 1986 and Zadoks, 1961). Epidemics occur when environmental conditions during the growing season are favourable (Leonard and Szabo, 2005).

Varietal resistance to stem rust has generally provided adequate protection without the need for fungicides. Wheat stem rust caused by the fungus *Puccinia graminis f. sp. tritici* is a heterocious obligate biotroph with a macrocyclie lifecycle feating five distinct spore stages. The full stem rust lifecycle begins with an infected plant, with elongated blister like pustules (uredinia) full of losses browinshred uredinia spores found on the leaf sheaths, awns, glumes, stem tissue and leaves (Singh *et al*., 2008). As the growing season progresses and the infected plant matures, the uredinia convert into telia and teliospores are black in color, and give forth the name black rust (Leonard and Szabo, 2005). The infected plant attack all of the above ground parts of the wheat plant and causes losses by reducing grain yield and affecting grain quality (El-Daoudi *et al*., 1996). The infection plant usually produces fewer tillers, set fewer seeds per head and the kernals are smaller in size and weight. For this reason, wheat genotypes have been discarded due their susceptibility for the disease (Singh *et al.,* 2002). Many other main factors i.e. virulent, pathotypes, susceptible genotype are involved in disease incidence and development in any wheat growing area of Egypt (Abd El- Badeea, 2015). The appearance of virulent and aggressive pathotypes of the causal organism is one of the most dangerous factors in the occurrence of any disease epidemic. Breeding for adult plant resistance is still the most economic and desirable mothed for controlling the disease. Therefore, the present study was conducted to evaluate the susceptibility of six wheat varieties, sids 12, gemmeiza 11, misr 1, misr 2, sakha 94 and giza 171 to infestation with cereal aphids especially the oat bird-cherry aphid, *R. padi*, the greenbug aphid, *S. graminum* and the English grain aphid, *S. avenae* and the yellow rust disease in grain yield under field conditions at Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt during two growing seasons during two successive seasons 2017/2018 and 2018 / 2019 .

Materials and methods

The seeds of the wheat cultivars were provided by Wheat Res. The experimental area was about 1050 m^2. This area was

divided into 18 plots. The experimental plots were arranged in randomized complete block design with three replicates for each variety. Samples of 10 wheat tillers were randomly chosen from each variety. Numbers of aphids/tiller were recorded and percentages of its infestation were calculated under a binocular stereomicroscope in the same day of the inspection. Direct inspection of wheat tellers were used to study the abundance and distribution of cereal aphids. The total number of aphid individuals (adults and nymphs) were counted and recorded during the two studied seasons. The individuals of aphids were recorded according to El-Heneidy and Adly (2012).

Weekly means of maximum and minimum temperature (ºC) and maximum & minimum of relative humidity (R.H.%) were obtained from the Central Laboratory for Agriculture Climate, and then recorded to correlate with the mean number of aphids and thrips in the three studied planting dates in both studied seasons. The differences between mean number of aphids were analyzed by using SAS program computer (SAS Institute, 2003) The experimental plots received the standard cultivation practices of that area and mechanical control was applied to remove weeds..

The impact of yellow rust infection on grain yield of six Egyptian wheat cultivars, sids 12, gemmeiza 11, misr 1 , misr 2, sakha 94 and giza 171 (Table, 1) bread wheat cultivars used in the study. The effect of yellow rust infection on grain yield 1000 kernel weight was determined in an experiment for two years (2017/2018-2018/2019). The main treatments were infected and protected plots. All plants were surrounded by a susceptible disease spreader (Morocco and *Triticum spelta saharinsis*). In addition, the plants under the study were inoculated with a mixture of yellow rust races at booting stage; To provide and maintain the rust inoculated by injection method twice in a week during the growing season, whereas, the other treatments were protected by the effective fungicide Tilt 25% EC 5EC(CE)-1-(2,4- Propiconazole)1-4,4-dimethyl 1-2-(1,2,4-triazol-1-y1) Pent -1-en -3-0L) at the rate of 75cm /300 litter water per Fadden at the early dough stage. The nursey was sown fifteen day after the regular sowing data (the first haff of December) to expose the plants to suitable environments of rust incidence, and development, all cultivar practise recommended in the commercial filed i.e. fertilization irrigation and other managent. Data on rust incidence were scored as response and severely infection to gather every week from rust appearance to final rust severity along with the stage of plant growth for each plot using the modified cobs scale (Paterson *et al*., 1948) and the infection response scale described in (Roelfs *et al*., 1992). The area under disease progress curve (AUDPC) was calculated for each variety according to the equation adopted by (Pandey *et al*., 1989). At 1000 kernel weight were determined for each cultivar. Yield loss was estimated as the difference among the protected and infected plots using simple equation adopted by (Calpouzos *et al.*, 1976)

Loss (%) = $1-y_d/y_h \times 100$

Where: y_d = yield of diseases plants.

Y_h = yield of healthy plants.

All data obtained were statistically analyzed for each season individually was used to compare yield components according to (Snedecor, 1957). Correlation coefficient was also used to detect the relationship between yield losses and area under disease progresses curve (AUDPC).

Table (1): Name, pedigree and year of release of six Egyptian wheat cultivars.

No.	Cultivar	Pedigree	Year of release
1	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//CMH74A.630/4*SX SD7096-4SD-1SD-1SD-0SD	2007
2	Gemmeiza 11	BOW"S"/KVZ"S"//7C/SER182/3/GIZA168/SAKHA61 GM5820-3GM-1GM-2GM-0GM	2011
3	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMSS00Y01881T -050M-030Y-030M-030WGY-33M-0Y-0S	2011
4	Misr 2	SKAUZ/BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S	2011
5	Sakha 94	OPATA/RAYON//KAUZCMBW90Y3180-0TOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S	2004
6	Giza 171	SAKHA 93 / GEMMEIZA 9 S.6-1GZ-4GZ-1GZ-2GZ-0S	2013

Results and discussion

1. Susceptibility of some wheat varieties to infestation by certain aphids:

This study was carried out in (Sakha Research Station), to evaluate the susceptibility of six wheat varieties (Sids 12, gemmeiza 11, misr 1, misr 2, sakha 94 and giza 171) to infestation with certain cereal aphids especially the oat bird-cherry aphid, *R. padi*, the greenbug aphid, *S. graminum* and English grain aphid, *S. avenae*.

1.1.During 2017/ 2018 season:

Data tabulated in Tables (2) showed that the population fluctuation of aphid individuals (*R. padi*, *S. graminum* and *S. avenae*) / 10 plants at Sakha Research Station during 2017/ 2018 season as indicated by weekly aphid counts throughout the whole period of wheat plant growth. Presence of aphids on six varieties was detected throughout most of the whole period of plant growth that extended from the second week of December, 2017 until the late week of March, 2018.

The infestation started from the 2 nd week of December with few aphid numbers on sids 12 when the plants were still in the seedling stage. Presence of aphids on wheat plants continued up to the end of March. The highest peak of aphids' abundance was always detected during the third inspection and the second week of March on all cultivars with average numbers 42.7, 82.5, 51.7, 62.8, 35 and 36.0 individuals/10 plants of misr 1, sids 12, misr 2, gemmeiza 11, sakha 94 and giza 171, respectively (Table, 1).

From data in Table (2), it could be stated that the highest general infestation rate by aphids occurred on sids 12 with seasonal mean numbers of 27.14 individuals/10 plants. Whereas, misr 2, gemmeiza 11 and misr 1 had medium aphid infestation with seasonal mean counts of 21.26, 21.91 and 16.13 individuals/10 plants, while lowest general infestation rate by aphids occurred on giza 171 and sakha 94 with seasonal mean numbers of 12.80 and 11.72, respectively. there were significant difference between the tested cultivars.

Table (2): Weekly mean counts of *Rhopalosiphum padi*, *Schizaphis graminum* and *Sitobion avenae* / 10 wheat plants on different wheat varieties during 2017/ 2018 wheat season in Agricultural Sakha Research Station,

inspection weeks	Wheat cultivars						Climatic factors		
	Misr 1	Sids 12	Misr 2	Gemmeiza 11	Sakha 94	Giza 171	Max. Temp.	Min. Temp	Average RH
Dec., 2nd 2017	0	0.5	0	0	0	0	19.75	11.50	70.63
3rd	0.3	0.1	0	0.6	0.3	0.4	20.83	11.50	62.67
4th	0.9	0.9	1.2	0.8	0.7	0.9	20.22	11.11	65.22
Jan., 1st 2018	3.3	6.3	4.7	2.5	0.4	0.5	18.43	11.00	44.71
2nd	4.4	10.2	8.6	6.9	1.4	1.7	19.88	11.13	64.13
3rd	6.5	18	12.3	13.5	2.9	3.9	18.00	9.50	50.67
4th	8.7	24.6	14.5	10	11.5	13	15.50	7.70	68.00
Feb., 1st	18.8	28.8	25.9	17.9	15.6	15.5	20.29	10.57	59.71
2nd	30.3	28.6	17.3	24	13	14.6	22.13	11.63	48.25
3rd	42.3	47.1	30	42.2	23	25.4	27.00	16.67	51.17
4th	25.2	54.8	28.9	36.9	26	28.7	25.38	14.38	52.50
Mach., 1st	40.9	47.2	38	61	25	26.4	26.43	16.14	49.14
2nd	42.7	82.5	51.7	62.8	35	36	27.88	16.88	39.88
3rd	14.5	59.3	61.5	42.7	18	21	24.33	14.83	51.33
4th	3.1	1.8	24.3	6.9	3	4	26.20	16.00	44.40
Mean	16.13 ab	27.38	21.26 ab	21.91 ab	11.72 b	12.80 b			
±SE	4.17	6.64	4.82	5.65	2.99	3.16			
F value	1.61								
LSD	13.371								

1.2. During 2018/ 2019 season:

Data tabulated in Table (3) showed the aphid incidence on the tested wheat varieties during 2018/2019 season as indicated by weekly inspections throughout the experimental period. Presence of aphids on six varieties was concentrated during period which extended from the fourth week of Jan. 2019 till the third week of Mar. 2019 (Table, 3). The highest value was 69.8, 61.9, 73.1 and 30 individuals/ 10 plants on misr 1, sids 12, misr 2 and sakha 94 at 2nd week of Mach. While, it was reported at the first week of March by 69.7 and 51.8 individuals/ 10 plants on gemmeiza 11 and giza 171, respectively.

Similarly, Abou-Elhagag *et al*. (2001) in Egypt evaluated the susceptibility of ten wheat cultivars to cereal aphids (*R. padi, S. graminum* and *R. maidis*) infestation. sids 9, sids 7, sids 5 and gemmeiza 1 showed the lowest population of *R. padi* and *R. maidis*, while sids 5, sids 7 and sids 9 were the least preferred cultivars by *S. graminum*. Sids 9, sids 7 and sids 5, aside from obtaining the highest yields, were the least susceptible to infestation by all cereal aphids studied. Saleem *et al.* (2009) fund that aphid infestation started during the last week of Dec., remained low during January with a peak in the 1 st week of March. Wains *et al.* (2010) revolved that a peak of aphids population was recorded during the beginning of the third week of March. Khan *et al*. (2011) in Pakistan recoded that the recorded that the 4th of February was found to be very favorable for aphids in wheat fields. Barbec *et al*. (2014) in

Pakistan stated aphids' population grow quickly and increased at the last period wheat plants. Ullah *et al*. (2014) showed that aphids attack wheat plants started in the 1st week of January and increased with the vegetative growth of plants and reached at peak level in the 3rd week of March.

Table (3): Weekly mean counts of *Rhopalosiphum padi*, *Schizaphis graminum* and *Sitobion avenae* / 10 wheat plants on different wheat varieties during 2018/ 2019 wheat season in Agricultural Sakha Research Station.

inspection dates	Wheat cultivars						Climatic factors		
	Misr 1	Sids 12	Misr 2	Gemmeiza 11	Sakha 94	Giza 171	Max. Temp	Min. Temp	Average RH
Dec., 2nd 2018	0	0	0	0	0	1.6	19.63	12.38	56.25
3rd	0.1	1.5	0.4	0	0	0.2	18.50	10.67	54.83
4th	3.2	5.9	2.4	5.7	0	0.8	17.11	10.44	53.33
Jan., 1st 2019	5.6	13.5	5.2	8	0	0.5	17.86	9.00	52.86
2nd	2.5	12.3	0.6	4.4	0.4	1.2	17.50	9.13	53.38
3rd	7.5	7.6	7	10.3	1.9	9	19.43	9.71	69.57
4th	13	18.5	13.7	19.2	8.5	14.3	18.33	11.00	52.67
Feb., 1st	12.8	24.3	14.2	33.2	12.6	10.6	19.57	9.00	60.86
2nd	23.7	35.7	28.1	40.3	10	17.7	19.50	12.00	55.88
3rd	46	32.7	49.4	44.5	20	27.6	17.00	8.33	61.00
4th	45.5	47.9	45.7	46	21	35.3	23.43	11.71	62.57
Mach., 1st	30.2	56.7	25.9	69.7	20	51.8	22.14	13.71	63.14
2nd	69.8	61.9	73.1	67.7	30	29.5	23.38	15.13	45.63
3rd	25.3	44	31	31.5	13	18.4	23.00	14.43	51.00
4th	0	9.5	5.9	0.7	0	0.3	26.11	15.78	50.33
Mean	19.01 ab	24.80 a	20.17 ab	25.41 a	9.16 b	14.59			
±SE	5.43	5.28	5.65	6.23	2.57	4.04			
	1.53								
	14.106								

2.Effect of yellow rust infection on 1000 kernel weight and its components of six Egyptian wheat genotypes.

Effect of yellow rust severity on 1000 kernel weight and its components of six Egypt wheat genotypes i.e. sids 12, gemmeiza 11, misr 1, misr 2, sakha 94 and giza 171 was estimated under field condition at Sakha Agricultural Research Station for two growing seasons (2017/2018-2018/2019).The reaction of wheat cultivars to yellow rust at adult plant stage under field condition were recorded on the treatment plants, whill the fungicide protected plants remained almost free from yellow rust during the two growing seasons of this study. The 1000 kernel weight due to yellow rust infection was estimated in the tested wheat cultivars, which showed different disease severity. However, the wheat cultivars which showed high yellow rust disease severity exhibited maximum values of AUDPC and 1000 kernel weight. while the wheat cultivars which showed low disease severity exhibited

minimum values of AUDPC and 1000 kernel weight the six tested wheat cultivars reacted differentially to yellow rust infection during the two growing seasons of the study indicated that the Yellow rust epidemic (FRS%) in 2018/19 was more severe than that in the other one growing seasons (2017/2018).

2.1.The first growing season 2017-2018:

Data presented in Table (4) revealed that yellow rust severity was higher on the high level of susceptible wheat cultivars i.e. sids 12 (56.67%), gemmeiza 11 (53.33%), misr 1(36.67%) and misr 2 (26.67%) for the infected treatment. On the other hand the rust severity was lower on the low level of susceptible wheat cultivars i.e. sakha 94 (2.47%) and giza 171 (6.67%) for the infected treatments.

Data in Table (4) revealed that AUDPC run in parallel line with disease severity. the values of AUDPC that found in the high level of susceptible wheat cultivars were sids 12 (558.00), gemmeiza 11 (520.00), misr 1 (308.00) and misr 2 (248.00) for the infected treatments. On the other hand, the values of AUDPC were lower in the low level of susceptible wheat cultivars i.e. Sakha 94 (41.30) and giza 171 (142.00) for the in infected treatments. In general, data presented in Table (4) showed that the increasing in 1000 kernel weight losses (%) with the increasing of yellow rust severity (%) and AUDPC values were clearly noticed in all the tested wheat cultivars. The thousand kernel weight (gm) of the healthy plants (protected treatment) of all wheat cultivars was higher than that of the infected ones. The loss% of the thousand kernel weight (gm) ranged from 13.32% to 30.73% The cultivars sids 12, gemmeiza 11, misr 1 and misr 2 gave the highest values of losses% of the on thousand kernel weigh (gm.) (30.73, 21. 82, 14.95 and 13.32%), respectively, followed by sakha 94 (10.54%) and giza 171 (12.55%) (Table, 4)

Table (4): Effect of yellow rust infection on grain yield 1000 kernel wheat of six wheat cultivars under field conditions at Sakha Agriculture Research Station in 2017/2018 growing season.

Cultivar	Rust induce		Mean grain 1000kernelweight(gm.)		
	a FRS.	b AUDPC.	Infected	Protected	Losses (%)
Sids 12	56,67	558,00	32,60	47,06	30,73
Gemmeiza 11	53.33	520,00	38,70	49,50	21,82
Misr 1	36,67	308.00	40,90	48.09	14.95
Misr 2	26,67	248,00	39,88	46.01	13.32
Sakha 94	2,47	41,30	41,60	46,50	10.54
Giza 171	6,67	142,00	42,50	48,60	12.55

a- (FRS). Final rust Severity. **b- (AUDPC). Area under disease progress curve**

2.2.The second growing season 2018-2019:

Data presented in Table (5) revealed that the yellow rust severity in the second growing season (2018-2019) was higher in all the tested wheat cultivars than that in the first growing one (2017-2018) which was relatively low. The rust severity (%) was higher on the high level of susceptible wheat cultivars Sids 12 (83.33%), Gemmeiza 11 (76.67), Misr 1 (56.67%) and Misr 2 (43.33%) for the infected treatments. On the other hand, the cultivars Sakha 94 and Giza 171 exhibited lower values of yellow rust severity % (4.33 and 2.67%) respectively for the infected treatments.

The same trend was found in case of area under disease progress curve AUDPC. It was found to be higher in all cultivars compared

with the previously season. However, the values of AUDPC were as following sids 12 (925.00), gemmeiza 11 (865.00), misr 1 (534.00) and misr 2 (400.00) for infected treatments, respectively. Whereas, in the low level of susceptible wheat cultivars, the values of AUDPC were recorded in sakha 94 (208.00) and giza 171 (60.00) for the same treatments. In general, data in Table (5) show that the increasing in thousand kernel weigh losses% with the increasing of yellow rust severity (%) and AUDPC values, were clearly noticed in all the tested wheat cultivars. Thousand kernel weights (gm.) of the healthy plants (protected treatment) of all wheat cultivars were higher than that of infected ones. The loss% of the 1000 kernel weigh (gm.) ranged from 15.61 % to 73.44%. The cultivars sids 12, gemmeiza 11, misr 1 and misr 2 gave the highest values of loss % of the thousand kernel weight (gm.) (73.44, 69.35, 18.20 and 15.61%) respectively. followed by sakha 94.1(11.94%) and giza 171 (7.53%).

Table (5): Effect of yellow rust infection on grain yield 1000 kernel wheat of six wheat cultivars under field conditions at Sakha Agriculture Research Station in 2018/2019 growing season.

Cultivar	Rust induce		Mean grain 1000kernel weight(gm.)		
	a FRS.	b AUDPC.	Infected	Protected	Losses (%)
Sids 12	**83,33**	**925,00**	**12.66**	**47.66**	**73,44**
Gemmeiza 11	**76,67**	**865,00**	**15.03**	**49,03**	**69.35**
Misr 1	**56,67**	**534,00**	**39,55**	**48.35**	**18.20**
Misr 2	**43,33**	**400.00**	**38,70**	**45.86**	**15,61**
Sakha 94	**4,33**	**208,00**	**40.20**	**45.65**	**11,94**
Giza 171	**2,67**	**60,00**	**45.30**	**48.99**	**7,53**

a- (FRS). Final rust Severity. **b- (AUDPC). Area under disease progress curve**

Yellow rust, is considered as the most destructive disease of wheat. The losses may reach 100% on susceptible wheat cultivars when conditions favorable for disease (Singh *et al*., 2002). Stem rust can cause great damage to susceptible wheat crops over a broad number of geographical regions worldwide. A healthy crop before harvest can be destroyed by stem rust fungu , if sufficient inoculums arrives from infected fields . The nutrient flow in the plant is interrupted at a severe infection on the stems leading to shriveling of spikes and grain. Besides that, infected stems are weakened and therefore, prone to loding , leading to farther loss of grain (Roelfs *et al*., 1992 and Leonard and Szabo, 2005).

The effect of yellow rust infection on 1000 kernel weight of wheat genotypes i.e. Sids 12, Gemmeiza 11, Misr 1, Misr 2, Sakha 94 and Giza 171 were estimated under field conditions at Sakha Agricultures Research Stations for two successive growing seasons (2017/18-2018/19). Result obtained revealed that of 1000 kernel – weight was affected by yellow rust infection, and the differences between protected and infected wheat genotypes due to the difference in the level of disease severity of yellow rust. It was noticed that over the growing seasons, the tested wheat cultivars showed different disease severity, however the wheat cultivars which showed high yellow rust disease severity exhibited maximum values of area under diseases progress curve (AUDPC) and 1000 kernel weight, while the wheat cultivars which showed low disease severity exhibited minimum values of area under diseases progress curve (AUDPC).and 1000 kernel weight. However, the tested wheat cultivars

showed highly significant difference in Thousand kernel weight of the different cultivars was clearly affected by yellow rust infection particularly at high disease incidence (infected plants). Generally, 1000 kernel weight was decreased (loss increased) as the severity of stem rust increased. However. the percentages of loss in 1000 kernel weight differed significantly among the tested cultivars. The wheat cultivars sakha 94 and giza 171 revealed the lowest level of 1000 kernel weight loss. Whereas, the wheat cultivars sids 12, gemmeiza 11, misr 1 and misr 2 showed the highest loss in 1000 kernel weight in two growing seasons. Moreover the reduction in 1000 kernel weight was parallel to stem rust severity in all seasons. The among of the protected plants of the tested wheat cultivars was chemically controlled expected, this is because of this character is quantitatively and genetically controlled. On the other hand it is affected not only by the disease infection but also by further factors (Mousa, 2001). Boulot (2007) & Boulot *et al*. (2014) reported that the Egyptian wheat cultivars sids 1 exhibited the lowest of loss (%) in both grain weight and 1000 kernel weight in comparison with sids 9 and sakha 69 which they exhibited the highest loss (%) at the three locations of the study. Abdel-Malik (2011) reported that yield loss was correlated strongly with area under diseases progress curve (AUDPC). Which means that high level of partial resistance is needed to prevent significant yield loss. Also, Abdel Badeea (2011 & 2015) reported that infection with stem rust can severely reduce grain yield on susceptible cultivars and correlation was found between yield losses with disease severity and area under disease progress curve AUDPC). Therefore growing slow rusting cultivars will reduced the loss in grain yield. (Singh *et al*., 2013 and El-Sayed *et al.* , 2011).

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