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Contents

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Effect of ethyl acetate on vitality and virulence of entomopathogenic nematode species

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Abstract:

Entomopathogenic nematodes (EPNs) are the most important agents used in classical and augmentative biological control. The aim of this research work is to study, the effect of different concentrations of ethyl acetate (EA) on survival and virulence of two Egyptian isolates of the EPNs, Heterorhabditis indica Poinar, Karunakar and David (Rhabditida: Heterorhabditidae) and Steinernema carpocapsae (Weiser) (Rhabditida: Steinernematidae) in laboratory assays. EA showed a nematicidal effect against both species at a concentration higher than 0.1%. Infective juveniles (IJs) of H. indica were much more sensitive to the lethal effect of EA than S. carpocapsae. The medium lethal concentration (LC50) of EA for H. indica treated for 24 hrs. was nearly four times less than that of S. carpocapsae. In contrary, EA at low concentrations increased EPNs virulence by enhancing the ability of IJs to penetrated and kill the host and by increasing the proportion of both actively moving IJs in H. Indica and jumper "sprinter" IJs in S. carpocapsae. It is suggested that the lethal effect of EA on Entomopathogenic nematode (EPN) could be related to the damage of the nematode sensory apparatus due to its neurotropic effects. The activation effect of lower concentration on EPNs could be due to their stimulation to nervous receptors in the amphidial channel and channel un-blockage which increase the sensorimotor reactivity of nematode. The overall results suggested that EA at very low concentration is a promising candidate for EPNs activation prior field application.

Introduction

The entomopathogenic nematodes (EPNs) that belong to the families; Steinernematidae and Heterorhabditidae, have been used for biological control of many agricultural insect pests as a safe alternative to chemical insecticides. The third stage infective juveniles (IJs) of these nematodes can penetrate and kill their hosts within 24-48

hours in the laboratory (Poinar, 1986). However, in the field, they expose to various environmental extremes such as low humidity, solar radiation, plant- and agro-chemicals, which may limit their survival and field efficacy both in the soil and on plant surface (Ishibashi and Takii, 1993). Accordingly, finding environmentally safe activators that increase nematode efficacy and do not adversely affect nematodes vitality could be of great importance. Of the explored activators, juice from kale and aloe plant can stimulate nematode activity (Ishibashi, 1987), the insecticide carbamate oxamyl stimulated the locomotor movement of the IJs (Gaugler and Campbell, 1991), the pyrimidine fungicide nuarimol showed a beneficial effect on nematode vitality and movement (Gordon *et al.,* 1996) and the chlorine-based bleach sodium hypochlorite has been mentioned to activated the nematodes (Dempsey and Griffin, 2003). However, most of the studied chemical activators either environmentally unsafe, human-toxic pesticides or proved to be ineffective in the field (Ishibashi and Takii, 1993). Abd Elrahman and Abd Elrahman (2005) showed that treatment of *Heterorhabditis indica* Poinar, Karunakar and David (Rhabditida: Heterorhabditidae) IJs with a very low concentration of ethyl acetate stimulate nematode activity without any adverse effect in nematode survival. In contrary, Monzer and Al-Elimi (2002) and Monzer and Abd Elrahman (2003) mentioned that ethyl acetate has toxic effect against *H. indica*. However, the above-mentioned research did not provide detailed data on the effect of various EA concentrations and treatment periods on nematode survival and virulence as well as its effect on other nematode species.

Ethyl acetate (EA) is a colourless liquid with a characteristic fruity smell and is naturally present in plants such as *Anthemis nobilis* (*Roman chamomile*), *Rubus* species, several fruits (apple, banana and nectarines), cereal crops, radishes, palm tissues and during fermentation of plant materials (Monzer and Abd Elrahman, 2003 and Khan *et al.,* 2017). It is considered as a relatively safe product because of low toxicity to humans, animals, and the environment, thus it exempted from the requirement for tolerance when used in accordance with good agricultural practices as inert ingredients in pesticides (OECD, 2002). It also evaluated by the joint WHO/FAO experts committee on food additive (JECFA) and approved by FDA for a direct food additive (IPCS, 2002). The above-mentioned properties make EA an important candidate as a practical entom entomopathogenic nematode (EPN) activator. Hence, the exact effects that may EA have on the EPNs - a thorough examination.

Accordingly, the objective of this study was to examine in the laboratory the effects of various EA concentrations and exposure periods on the survival and virulence of *H. indica* and and *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae) that represent the two different genera of EPN with different foraging behaviour, as an attempt to find an effective EPN activator.

Materials and Methods

1.Nematode source:

Two Egyptian isolates of EPNs were tested in this study; *H. indica* (EGAZ2) and *S. carpocapsae* (EGAZ9). These two EPN species were identified using both morphometric analysis and molecular techniques (Azazy *et al.,* 2018). The nematodes were reared in late instar greater wax moths; *Galleria mellonella* L. (Lepidoptera Pyralidae), by the method of Dutky *et al.* (1964) and IJs were harvested with modified white traps (White, 1927). A suspension of 2000 IJ/ml was prepared and kept at 25 ± 1ºC for at least 24 h but for less than one week prior to testing. The concentration of IJs in the stock suspension was determined by counting aliquot samples and adjusting to the required concentrations by adding water.

2.Treatment of nematodes:

Seven concentrations of 0.0, 0.01, 0.1, 0.25, 0.5, 1.0 and 2.0% (v/v) of EA were prepared by adding 0.0, 0.01, 0.1, 0.25, 0.5, 1.0 or 2.0 ml of HPLC grade EA (Sigma Chemical Co., St. Louis, MO, USA) to 250 ml flasks each contained 100 ml from the stock nematode suspension and kept in the dark at 25 ± 1ºC.

3. Effect of ethyl acetate on nematode survival:

After 24 and 48 hrs. of nematode incubation in various concentrations of EA, the numbers of IJs in one ml of the suspension were counted under a stereomicroscope microscope and grouped into two categories: active and alive (sinusoidal undulation, "J"-shaped postured or inactive "S" posture; quiescent-looking till slight touch), or dead IJs (complete straight posture not responding to mechanical stimulation up to self-disintegrating). Sample extraction from each flask was repeated 3 times and survival percentages were calculated.

4.Effect of ethyl acetate on nematode virulence:

The ability of control and EA treated IJs to penetrate and cause host mortality was compared using both filter paper and sand row assays.

4.1. Filter paper assay:

Filter paper assays were conducted according to the standard filter paper method detailed by Monzer and Abd Elrahman (2003) with minor modifications as follows. Nematodes survived treatment with 0.0, 0.1 and 0.1 EA for 24 and 48 hrs. were concentrated and rinsed several time with distilled water and suspended in distilled water at a concentration of 100 IJs/ml. Eppendorf tubes (1.5 cm3) with several small holes made in their lids were lined with double layer filter paper (Whatman No. 1) and 200 µl (20 IJ) of nematode suspensions were transferred with a micropipette to each tube. Afterward, a single *G. mellonella* larva was placed directly inside each Eppendorf tube, which was then closed and kept at 25°C, in the dark. After 24 h, larvae were washed twice with distilled water to remove adhering IJ, dried and transferred to Petri dishes each lined with a piece of moistened filter paper. Over the following 5 days, the number of dead larvae was recorded before their dissection under stereomicroscope microscope to determine the number of nematodes in each. The penetration rate was determined by calculating the percentage of penetrated nematode in each cadaver relative to the total applied nematode (20 IJs).

4.2. Sand row assay:

The ability of EPNs to disperse and locate their host through the soil was tested using a sand assay as described by Abd Elrahman and Abd Elrahman (2005) and Azazy *et al.* (2014). A plastic tube (25 cm in length and 5 cm in diameters) was cut longitudinally into two symmetrical halves. The two ends of the half tube (25 cm in length and 2.5 cm in height) were sealed with wire mesh screens and were filled to height 2.0 cm with sterilized, wet sand (10% moisture with the particle size of 0.05-0.1 mm in diameter). Four full grown larvae of *G. mellonella* were used as bait providing host cues to attract the foraging IJs. Larvae were kept inside a wire screen cage (1mm pore size) filled with moist sand and placed at a trap zone located near one end of each container as shown in Figure (1). The prepared containers were incubated at 25±1ºC in the dark, for 24h to allow equilibration of any diffuses from the insects through the sand before applying the nematodes. Six thousand IJs in 3 ml distilled water were inoculated in sand of the inoculation zone opposing to the trap of each container. Containers were enclosed in plastic bags to minimize water evaporation and kept horizontally at 25 ± 1ºC in dark. After 24 hrs. of incubation, and in each container was divided into five equal sections (5 cm length for each) as illustrated in Figure (1) and every sand soil of each section (after excluding the first nematode inculcation section) was transferred to a separate petri dish, and the number of nematodes in each section was determined with a live-bait method modified from Fan and Hominick

(1991). Briefly, *G. mellomella* larvae were transferred to each dish. Larvae in the trap section were also transferred to a separate dish. After 3 days incubation at 25±1ºC, larvae were removed, washed in tap water, dried on an absorbent paper and transferred to Petri dishes lined with a piece of moistened filter paper. After 2 days the number of adult nematodes in the body cavity was determined by dissecting the Galleria larvae under a stereomicroscope. An index was calculated to determine the average net distance [N (D)] travelled per individual IJ according to the following equation:

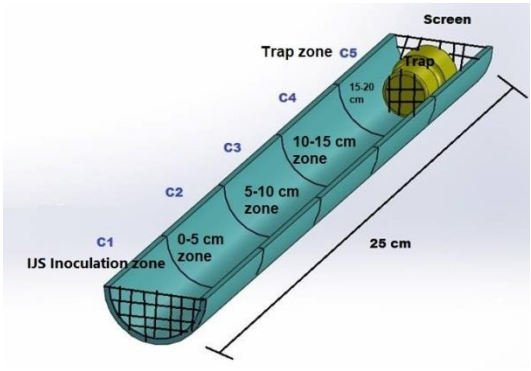
$$N(D) = (2.5a + 7.5b + 12.5c + 17.5T)/N$$

The values a, b, and c represent the number of nematodes recovered in a given section, T represents the number of nematodes recovered in larvae enclosed in trap, the constants (i.e., 2.5, 7.5, etc.) indicate the distance from the IJ inoculation zone to the midpoint of the section, and N is the total number of nematodes recovered outside the inoculation zone. This provides a weighted average of the distance travelled by nematodes within the column in 24 hrs., with greater values indicating increasing proximity to the hosts.

Nematode dispersal was quantified by:

a) Total number of IJ migrated outside the inoculation zone.
b) Percentage of IJs recovered in each section outside the inoculation zone relative to the total number of migrated IJs.
c) Total percentage of migrated IJs relative to total inoculated IJs (6000 IJs)
d) The average net distance N (D) travelled by each IJ outside the site of application.

Figure (1): Schematic of the sand assay (Modified from Azazy *et al.*, 2014)



5. Data analyses:

The entire assays were repeated using three generations of both *H. indica* and *S. carpocapsae* the results of the three experiments were combined for statistical analysis. Most of the results were expressed in percentage, although actual numbers were used for statistical tests. Probit analysis was done to calculate the Median Lethal Concentration (LC50) values and slope of EA, using LdP-Line® software (Bakr, 2007). Significantly different means were identified by analysis of variance (Tukey's honestly significant difference) at $P<0.05$ using CoStat® software (Costat, 2007). Results were recorded as the mean ± standard deviation (SD).

Results and Discussions

1. Effect of ethyl acetate on nematode survival:

Survival of IJs of *H. indica* and *S. carpocapsae* incubated in seven EA concentrations were recorded for 24 and 48 hrs. (Table, 1). There was no significant effect of EA at concentrations of 0.01 and 0.1% on the survival of IJs incubated for 24 and 48 hrs. of both nematode species (survival rate ranging from 100% to 95.1 ± 3.9%). However, significant differences were observed in the survival of IJs incubated in EA concentration higher than 0.1% ($p < 0.05$). Incubation of *H. indica* IJs in 0.25, 0.5, or 1.0% EA concentration for 24 hrs. sharply decreased nematode survival rate to 28.9 ± 2.5, 1.4 ± 2.5 or 1.2 ± 0.4, respectively. IJs of *S. carpocapsae* were more tolerant to the lethal effect of EA with a survival rate of 86.7 ± 1.5, 72.8 ± 3.7 or 38.9 ± 10, among IJs incubated in 0.25, 0.5, or 1.0% EA concentration, respectively, for 24 hrs. The difference in survival rate between *H. indica* and *S. carpocapsae* incubated in 0.25, 0.5, or

1.0% EA concentration for 24 hrs. was significant (P<0.01). Almost all IJs from both species that survived incubation in 0.5 and 1.0% EA concentration were not actively moving (i.e., motionless with straight or quiescent-looking postured till slight touch) after 24 hrs. of incubation. At EA concentration of 0.25 for 48 hrs., 0.0 and 30.2 ± 3.2% survival rate were recorded for *H. indica* and *S. carpocapsae*, respectively. IJs of both nematode species did not survive 0.5, 1.0% or 2.0% EA for 48 hrs. or 2% EA for 24 hrs. (Table, 1). Generally, EA was more toxic to *H. indica* than *S. carpocapsae* IJs as reflected by its calculated LC50 (Table, 1). The LC50 of EA for *H. indica* was 0.16 %, which is significantly lower than that for *S. carpocapsae* (0.78%).

Table (1): Mean survival rate (% ± SD) of *Heterorhabditis indica* and Steinernema *carpocapsae* treated with different concentrations of ethyl acetate for 24 and 48 hrs.

EA Concentration (%)	*H. indica*		*S. carpocapsae*	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
0 (control)	97.0 ± 4.2 a	95.2 ± 3.9 a	99.6 ± 0.4 a	99.2 ± 0.4 a
0.01	97.9 ± 1.5 a	95.1 ± 3.5 a	100 a	99.4 ± 0.3 a
0.1	95.3 ± 2.6 a	96.5 ± 0.4 a	99.9 ± 0.2 a	98.9 ± 0.7 a
0.25	28.9 ± 2.5 b	0.0 b	86.7 ± 1.5 b	30.2 ± 3.2 b
0.5	1.4 ± 2.5 c	0.0 b	72.8 ± 3.7 c	0.0 c
1.0	1.2 ± 0.4 c	0.0 b	38.9 ± 10 d	0.0 c
2.0	0.0 c	0.0 b	0.0 e	0.0 c
LC50	0.16e		0.78f	
Slope	2.37 ± 0.21		2.5 ± 0.2	

Means within the same column followed by the same letters do not differ significantly (p >0.05), SD = standard deviation.

2.Effect of EA on nematode virulence:

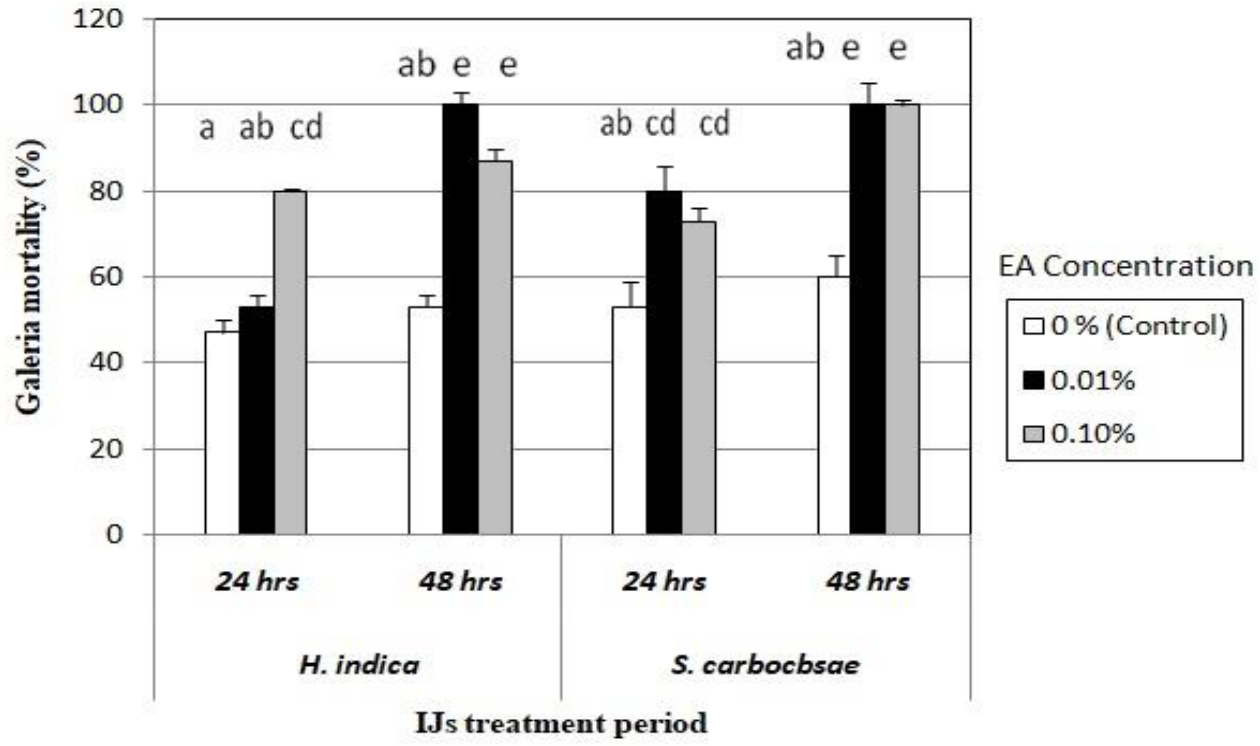
Results of nematode penetration rate in filter paper assays (Table, 2) indicated that treatment of IJs with EA generally increased nematode penetration rate. However, EA at a concentration equal to 0.01% for 48 hrs. increased nematode penetration rate significantly than untreated IJs for both nematode species. At such concentration of EA, the penetration rate of IJs reached 58.3 ± 12.6% and 61.7 ± 11.3% among *H. indica* and *S. carpocapsae*, respectively compared with 16.7 ± 7.6% and 30 ± 5.0 for untreated IJs, respectively. Figure (2) illustrated the effect of IJs treatments with EA on their ability to kill *G. mellonella* larvae in filter paper assays. Treatment of *H. indica* IJs with EA for 24 hrs. increased their ability to kill *G. mellonella* larvae than untreated control IJs, reached 80 ± 1.0 % among larvae exposed to IJs that previously treated with EA concentration of 0.1% for 24 hrs. The difference in mortality percentage among larvae exposed to EA-treated and control IJs was significant, P>0.01 at EA concentration of 0.1% and was not significant at EA concentration of 0.01%, p>0.01), However, IJs of *H. indica* that previously treated with 0.01 or 0.1% EA for 48 hrs. caused significantly higher mortality percentage among *G. mellonella* larvae compared with untreated IJs (100 and 87 ± 3.0% vs. 53 ± 3.0 %, respectively). On the other hand, treatment of *S. carpocapsae* IJs with either 0.01 or 0.1% concentration of EA significantly increase their ability to kill *G. mellonella* (P<0.01) than untreated control IJs.

Table (2): Filter paper assay: the average percentage (Mean ± SD) of nematodes counted in infected cadavers of *Galleria mellonella* larvae following exposure IJs of *Heterorhabditis indica* and Steinernema *carpocapsae* treated with 0.0, 0.01and 0.1% ethyl acetate concentration for 24 and 48 hrs.

Nematode species	EA concentrations	Exposure time	
		24 hrs.	48 hrs.
H. indica	0 (control)	11.7 ± 7.6 a	16.7 ± 7.6 acd
	0.01	13.3 ± 7.6 ab	58.3 ± 12.6 e
	0.1	26.7 ± 2.9 cd	28.3 ± 2.9 cd
S. carpocapsae	0 (control)	38.3 ± 5.8 c	30 ± 5.0 bcd
	0.01	56.7 ± 7.6 e	61.7 ± 11.3 e
	0.1	33.3 ± 2.9 c	36.7 ± 5.8 e

Means sharing the same letter are not significantly different (P>0.01), SD = standard deviation

Figure (2): Filter paper assay: the percentage mortality (Mean ± SD) of *Galleria mellonella* larvae following exposure to IJs of *Heterorhabditis indica* or *Steinernema carpocapsae* that previously treated with 0.0, 0.01and 0.1% ethyl acetate for 24 and 48 hrs. (Columns sharing the same over headed letter(s) do not differ significantly (p>0.05).



Results of virulence of *H. indica* against *G. mellonella* larvae in the sand assay were tabulated in Table (3). More than half of the dispersed IJs reached the trap zone of the container (64.9 ± 3.91% and 52.2 ±7.8% for control and treated IJs, respectively). No significant differences (p>0.05) were observed in the percentage of IJ recovered from 0-5, 10-15 cm zone, or trap zone between EA treated and control IJs. The total number of dispersed IJs was significantly (P<0.05) higher in EA-treated IJs relative to control (74.0 ± 3.0 versus 59 ± 5.8% IJs, respectively).

Table (3): Effect of treatment with 0.0 and 0.01% EA for 48 hrs. on the percentage of *Heterorhabditis indica* IJs dispersed after 24 hrs. of inoculation in the sand assay.

Distance (Cm)	%dispersed IJs relative to total dispersed IJs	
	0.0 (Control)	**0.01 %**
0-5	13.6 ± 5.44a	11.3 ± 6.9a
5-10	12.4 ± 0.01a	30.2 ± 5.5b
10-15	9.0 ± 7.0a	6.3 ± 2.8a
15-20 (Trap)	64.9 ± 3.91c	52.2 ±7.8c
	Total number of dispersed IJs	
0-20	59 ± 5.8 a	74.0 ± 3.0b
	Total dispersed IJs relative to inoculated IJs	
0-20	0.98 ± 0.04a	1.2 ± 0.05b

Means sharing the same letter within each section of the table are not significantly different (P>0.01), SD = standard deviation

On the other hand, most of *S. carpocapsae* IJs were recovered from the first two zones of the column for both control and EA-treated IJs of *S. Carpocapsae* (Table, 4). Table (4) also showed that percentage of IJs recovered from the trap zone was significantly higher in EA-treated *S. carpocapsae* IJs than control (41.6 ± 5.9% vs. 21.2 ± 7.0%, respectively), while, No significant difference was observed in total no of dispersed IJs between control and EA treated *S. carpocapsae*. Only 0.62 ± 0.03% of the inoculated IJ dispersed outside the inoculation zone for *S. carpocapsae* nematode, while the significantly higher proportion of *H. indica* IJ ($p<0.01$) were found outside the inoculation zone after 24 hrs. of inoculation (0.98 ± 0.04% of total inoculated IJs).

Table (4): Effect of treatment with 0.0 and 0.01% ethyl acetate for 48 hrs. on the percentage of Steinernema *carpocapsae* IJs dispersed after 24 hrs. of inoculation in the sand assay.

Distance (Cm)	%dispersed IJs relative to total dispersed IJs	
	0.0 (Control)	**0.01 %**
0-5	38.1 ± 8.6a	19.8 ± 1.7b
5-10	36.3 ± 13.6a	34.7 ± 10.4a
10-15	4.4 ± 1.5c	3.9 ± 1.7c
15-20 (Trap)	21.2 ± 7.0b	41.6 ± 5.9a
Total number of dispersed IJs		
0-20	37.7 ± 2.1a	33.7 ± 4.9a
Total dispersed IJs relative to inoculated IJs		
0-20	0.62 ± 0.03e	0.56 ± 0.08e

Means sharing the same letter within each section of the table are not significantly different (P>0.01), SD = standard deviation

The calculated N(D) (Table, 5) indicated that there was no significant difference in the average net distance travelled by EA-treated and untreated IJs of *H. indica*, while it was significantly higher in EA-treated IJs of *S. carpocapsae* than control ($P<0.05$). On the other hand, N (D) was significantly higher in *H. indica* than *S. Carpocapsae* IJs for both control and EA-treated IJs.

Table (5): Effect of treatment with 0.01% ethyl acetate for 48 hrs. on the average net distance N (D) travelled by dispersed IJs of *Heterorhabditis indica* and Steinernema *carpocapsae* in the sand assay.

Nematode species	ND (cm)	
	0.0 (Control)	**0.01 %**
H. indica	13.7 ± 0.8a	12.5 ± 0.6a
S. carpocapsae	7.90 ± 0.78b	11. 0 ± 0.30c

Means sharing the same letter are not significantly different (P>0.01), SD = standard deviation.

Our laboratory studies were conducted by directly immersing the nematodes in series concentrations of EA for 24 and 48 hrs. Results showed that survival of IJs was generally unaffected by EA concentration up to 0.1%, then, significantly decreased with EA concentration for both nematode species and exposure periods. Furthermore, no IJs survived treatment with 0.5 and 1.0% EA for 48 hrs. or 2% EA for 24 hrs., indicating that EA has an obvious nematicidal effect. IJs of *H. indica*, on the other hand, proved to be much more sensitive to the lethal effect of EA, as the calculated EA LC50 for *H. indica* was nearly four times less than that of *S. carpocapsae*. This finding indicates species-specific differences in the response among nematodes. Campbell and Gaugler (1992) mentioned that heterorhabditids tends to be less tolerant of environmental stress than steinernematids. Popiel and Vasquez (1991) observed that the survival rate of *H. bacteriophora*, exposed to 22% glycerol for 24 hours, was three-fold lower than that of *S. carpocapsae* exposed to the same glycerol concentration. Also, Negrisoli *et al.* (2008) reported that the insecticide thiamethoxam and the fungicide cyproconazole were toxic to *H. bacteriophora*, but did not cause any toxic effect on *S. carpocapsae*. Our results, in general, are consistent with the observations of the effect of EA on nematodes reported by previous studies (Monzer and AL-Elimi, 2002 and Monzer and Abd Elrahman, 2003). As treatment with EA concentrations of 0.01% and 0.1% for 24 and 48 hrs. did not show any significant effect on IJs from both *H. indica* and *S. Carpocapsae* survival relative to control, they selected for studying their effect on nematode virulence.

Virulence of EPNs is the ability of IJs to search, recognize, penetrate and kill insect hosts (Glazer, 1992). It was evaluated in this study using both filter paper and sand assays. The filter paper assay put the IJs in close proximity to the host, thereby assuring their contact with the host and measured their ability to penetrate and kill the host. In the sand assay, there is no host contact, and host finding by IJ is required, thus measured the nematode's ability to detect, disperse, reach and penetrate the target host (Campbell and Gaugler, 1992 and Ricci *et al.*, 1996). Results of filter paper assay indicated that EA-treated nematode were significantly more efficient in penetrating *G. mellonella* larvae than control, especially in IJs of *H. indica* and *S. carpocapsae* treated with 0.01% EA for 48 hrs. Mating is essential for further reproduction inside host in *S. carpocapsae*, thus penetration with a high number of individuals increases the probability of mating and further reproduction, and consequently, increases nematode efficiency (Ricci *et al.*, 1996). However, a single juvenile of *H. indica* can potentially reproduce and few IJs will be sufficient to establish the second generation, thus penetration with high number may not imply that *H. indica* nematode has lower efficiency in killing the host. Accordingly, percentage mortality of *G. mellonella* larvae following exposure to EA treated IJs of both nematode species for 24 and 48 hrs. were calculated. Again, results of this study indicated that treatment of IJs with EA at 0.01% concentration for 48 significantly increases their efficiency in killing their contact host for both nematode species, and suggest that EA treatment enhanced the nematode's host- penetration and killing abilities.

Due to the above-discussed results of filter paper assay, IJs treated with 0.0% (control) and 0.01% EA for 48 hrs. from both species were selected to further explore their ability to detect, disperse, and penetrate the target host in the sand assay. Results of virulence in sand assay revealed that very small proportion (less than 1%) of inoculated untreated IJs from both species dispersed laterally throughout the sand toward the host within 24 hrs. Corroborating our findings, Lacey *et al.* (2001) and Manimaran et al. (2012) reported that only around 0.1% of both S. *carpocapsae* and *H. indica* IJs dispersed 8 cm after 24 hrs. of inoculation in sand column assay in the presence of a host. The sand assay also indicated that a total number of dispersed IJs related to *H. indica* was significantly higher than that of S. *carpocapsae*. In addition, the highest proportion of dispersed *S. carpocapsae* IJs was recovered from the first (0-5) zone adjacent to inoculation zone, while the highest proportion of dispersed *H. indica* IJs was recovered from the trap zone at the far end of the sand row. This reflects the difference in foraging behaviour between the two nematode species in the soil. According to foraging behaviour, EPN has been classified into cruisers (active searchers) and ambushers (sit and wait foragers) (Bal *et al.*, 2015). The dispersed IJs of *H. indica*, which is cruiser forager species, moved actively toward *G. mellonella* cue thus higher proportions of them reached the trap zone after 24 hrs. of inoculation. On the other hand, S. *carpocapsae* is ambush forager and do not disperse very well in sand as most of IJs prefers to wait for the host, although a small number of IJs disperse slowly by waving (Campbell and Kaya, 2000 and Lacey *et al.*, 2001). However, the results of this study showed that a significant proportion of dispersed *S. carpocapsae* IJs reached the trap zone after 24 hrs. of inoculation despite their ambush foraging nature. This could be attributed to jumping or "sprinter" behaviour. Bal *et al.* (2014) and Labaude and Griffin (2018) mentioned that even so *S. carpocapsae* is ambush foraging, it possesses a small group of sprinters that able to fast disperse on the soil toward the host cue by jumping movement.

The main result of this study is that treatment of *H. indica* IJ with 0.01% EA for 48 hrs. increased slightly but significant number of dispersing IJs than untreated control although it did not affect the average net distance N(D) travelled by dispersed IJs. On the other hand, treatment of *S. carpocapsae* with EA under the same concentrations did not affect a number of dispersing IJs but significantly increased the percentage of IJs that detect and reach *G. mellonella* larvae enclosed in the trap zone than untreated control. The activation effect of EA on the ability of *S. carpocapsae* IJs to disperse was reflected by the average net distance N(D) they travelled which was significantly longer in EA treated IJs than control. It could be concluded that treatment with EA increased proportion of actively moving IJ relative to total *H. indica* IJs that was injected in the inoculation zone, while increase proportion of "sprinters" in *S. carpocapsae* relative to the total dispersing IJs, but not total injected in the inoculation zone.

It is not yet understood the exact mechanism by which EA act on EPNs. Monzer and Abd Elrahman (2003) related the lethal effect of EA on EPNs to the damage of the sensory apparatus of the IJs due to its neurotropic effects. EA is not likely to penetrate inside nematode body as it adsorbs by the glycoprotein surface of the IJ duple sheath coat (Djian *et al.*, 1991 and Glazer, 2002). In addition, natural openings of the IJs, such as the mouth and anus, are closed while living outside hosts (Endo and Nickle, 1994). The only vital organ in contact with the external environment and that could expose to EA would have been the nervous receptors in the amphidial channel opening near the head of IJ to detect aqueous chemo-attractants and repellents (Ashton *et al.*,1999). Compounds such as carbamates and organophosphates act through their neurotropic effects on the

nervous system throught amphidial channel (Hara and Kaya, 1982). However, neurotropic effects of EA do not explain the detected activation effect of EA low concentrayions on IJ active movement in *H. indica* and jumbling movement in case of S. *carpocapsae*. Bowen and Balster (1997) reported increase sensorimotor reactivity among mice inhaled low concentration of EA. Accordingly, the increase in nematode virulence by low concentrations of EA could be due to its stimulation to nervous receptors in the amphidial channel, which increases sensorimotor reactivity and response of nematode to external stimuli such as host cue, higher concentrations damage of the sensory apparatus as postulated by Monzer and Abd Elrahman (2003). Alternatively, we hypothesized that an increase in nematode virulence due to EA treatment could be related to amphidial channel un-blockage. IJs naturally expose inside decomposed *G. mellonella* or during the extraction process, to microscopic fat micro-droplets and other organic debris, various micro-organisms and/or fungal adhesion that could infiltrate inside and block the amphidial channel in a significant number of IJs. Nematodes with blocked amphidial channel will be unable to detect chemical host cue that triggering their actively moving response in *H. indica* or sprint in case of *S. carpocapsae*. EA as an effective organic solvent; capable of dissolving many polar and non-polar organic compound, may act through dissolving many amphidial channel blocker. Removal of such blocker by EA could enhance the response of IJs to host cue and increase their virulence.

Based on the present study we conclude that EA at low concentration (0.01-0.1%) increases EPNs virulence, while at higher concentrations acts as a nematicide. Pending further research on the effect of EA on other nematode species and its exact mode of action on EPNs, EA appears as a promising candidate for nematode activation prior field application. However, the biological significance of the above discussed results in the field is yet to be determined since laboratory bioassays are generally thought to provide better results than field tests (Ishibashi and Takii, 1993).

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Synergistic effect of allelopathic plant extract on fluroxypyr efficiently to suppress weeds in corn

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Abstract:

Successful weed control is important to maximize crop yield. Use herbicides to control weeds has several undesirable effects on the environment leading to the search for other alternatives, of which allelopathy has great potential. The aim of the study is to evaluate the effect of adding the methanolic crude extracts of *Eucalyptus citriodora* and *Cucurbita pepo* leaves on Fluroxypyr herbicidal activity against corn weeds, *Corchorus olitorius* L., *Amaranthus retroflexus* L., *Portulaca oleracea* L. and *Echinochloa colonum* L. So, a field study carried out at the experimental farm, Faculty of Agric., Tanta Univ, during 2008 and 2009 summer seasons. With *E. colonum* weed species, the hoeing (H) was the most effective treatments in reducing the weed biomass density, followed by 0.75 fluroxypyr +0.25 *E. citirodora* H. mixture (F+E.c3:1) and fluroxypyr (F) treatments. In the other weed species, (F) were the most effective treatments, followed by F+E.c3:1mixture and H treatments. The control treatment had the highest weed density. There are no significant differences between the fluroxypyr and F+E.c3:1mixture treatments or between the two *Zea mays* L. (Family: Poaceae) hybrids treatments. The hybrid TWC 321 had the lowest weed plant density. It is concluded that the effective weed control through the use of a reduced rate of herbicide (75% of the recommended rate) mixed with the *E. citirodora* plant extract. But, maximum values of the benefit-cost ratio (BCR) had recorded in fluroxypyr (F) and F+E.c3:1 treatment.

Introduction

Corn is the third essential cereal crops in Egypt, while it positions third of the most growing crops in the world. It has a significant economic importance worldwide as human food, animal feed and as a crude material for an increasing range and variety of food and nonfood industry (Paliwal, 2000). Egypt has 703,921 hectares of corn, which produces 5.69 million tons/Annam, with a gap between production and consumption estimated at 7.8 million tons in 2014 (Abd ElFatah *et al*., 2015). This gap offset by imports, which places a burden on the country's budget. Because of the water

resources insufficiency, cannot increase corn yield by increasing the cultivated area. The other way to increase corn productivity is by increasing unit area production (Zohry *et al*., 2016).

Weeds are undesirable plants, which compete with crops for light, soil, water and nutrients (Rajcan, and Swanton, 2001). The corn yield reduction due to weed contest reached 66-90 % (Abouziena *et al*., 2007 and Dalley *et al*., 2006). There are many difficulties that correspond to the dependence on hand-hoeing in corn, which is the lack of sufficient labor and lack of workable field conditions at critical stages of the crop-weed competition. In such a circumstance utilization of herbicides become necessary (Singh *et al*., 2009). Herbicides are effective in controlling weeds, but when used, it can lead to: disturb the ecosystem by increasing soil and water contamination (Ahmad *et al*., 2000) also; they may increase the herbicide-resistant weeds (Narwal *et al*., 2005) and they are hazardous to humans and animals (Einhellig, 2002).

Rice (1984) defined allelopathy as the effects of one plant on another plant via the release of chemicals into the environments; these effective compounds called allelochemicals (Whittaker and Feeney, 1971). Therefore, incorporating allelopathy in weed control may reduce the herbicides uses, environment pollution and diminish herbicide toxicity hazards (Chon *et al*., 2002).

Recent research identified a various species that possess chemicals capable of reducing the weeds development that associated with corn without causing significant damage to corn, including *Eucalyptus citriodora* L. (Family: Myrtaceae) and *Cucurbita pepo* L. (Family: Cucurbitaceae) (Abdallah and Amine, 2016), *Tithonia diversifolia* (Hemsl.) (Family: Asteraceae) (Oyerinde *et al*., 2009), *Sorghum halepense* L. (Poaceae) and *Cyperus rotundus* L. (Cyperaceae) (Soufan and Almouemar, 2009). Otherwise, weed control in corn can achieve with a reduced rate of the herbicides, without a yield loss (Kir and Doğan, 2009 and Pannacci and Covarelli, 2009). So, the main goal of this study is to achieve an effective weed control through the using a reduced rate of recommended herbicide Fluroxypyr tank mixed with the methanolic crude extracts of *E. citriodora* and *C. pepo* leaves.

Materials and Methods

1. Collection of plant materials:

In the flowering development stage, squash (*C. pepo*) and camphor (*E. citriodora*) leaves gathered from the Experimental Farm of Faculty of Agric., Tanta Univ., during 2007, at El-Gharbia Governorate.

2. Preparation of methanolic crude extract *Cucurbita pepo* and *Eucalyptus citriodora*:

Selected plants leaves were washed with tap water, air-dried for 15 days at room temperature (25± 2C^{o}). Dried leaves, milled to a fine powder, soaked in methanolic alcohol (400 g /1500 ml methanol). At room temperature, the solution stirred well at a rate of 100: 120 RPM by a shaker water bath, filtered through Whitman filter paper, evaporated to dryness. A 20% concentration prepared.

3. Field experiments:

To assess the allelopathic effect of plant extracts, 7 preparations of 0, 66, 75 and 100% of fluroxypyr herbicide + either of plant extract of squash or camphor examined against corn weeds (Table, 1). A field experiment conducted at the Experimental Farm, Faculty of Agric., Tanta Univ, during 2008 and 2009 corn growing seasons. The grains of corn cultivars (TWC 321 and TWC 351) seeded during the first week of June in both seasons. Corn cultivars seeds got from the Agricultural Research Center, Cairo, Egypt. Three seeds planted per hill, germinated seeds thinned to one seedling/hill after Two weeks after sowing. Corn plants received regular agricultural practices. The experiment laid out in a complete randomized block design (RCBD) with 3 replicates. Table (1) shows the different spray treatments used. The size of the

experimental unit was 42 m^2. Soil texture was as clay soil (pH=7.82, organic matter = 0.91 and E. C= 2.6) without notable changes in the texture. Fluroxypyr herbicide, plant extracts, and the plant extract/fluroxypyr mixtures diluted with water and sprayed using a knapsack sprayer (Model CP3) fitted with one nozzle (2 ml / 1.5 liters). The spray has done once 15 days after sowing. Field samples and other observations logged, either with corn plants or weed population.

Table (1): Different treatments used in the field experiments.

No.	Weed control treatments	Code	Rate / Hectare
1	Control	C	----
2	Fluroxypyr (20 %)	F	480 ml/ Hectare
3	Plant extract of squash (20 %)	C.p	480 ml/ Hectare
4	Plant extract of camphor (20 %)	E.c	480 ml/ Hectare
5	Fluroxypyr + camphor leaf extract mixture (0.66: 0.33v/v)	F+E.c 2:1	320 ml + 160 ml = 480 ml/ Hectare
6	Fluroxypyr + *camphor* leaf extract mixture (0.75: 0.25 v/v)	F+E.c 3:1	360 ml + 120 ml = 480 ml/ Hectare
7	Fluroxypyr + *squash* leaf extract mixture (0.66: 0.33 v/v)	F+C.p 2:1	320 ml + 160 ml = 480 ml/ Hectare
8	Fluroxypyr + *squash* leaf extract mixture (0.75: 0.25 v/v)	F+C.p 3:1	360 ml + 120 ml = 480 ml/ Hectare
9	Hoeing (Hand weeded)	H	Twice (after 15, 45 day)

4. Weed development:

After 90 days of planting, weed samples collected within the two experimental sessions by harvesting the grown weeds from one square meter of each plot. Weeds identified and classified, dried at 105º C for 24 hours. The percent reduction in dry weight (% R) calculated according to the following formula: -

Dry weight reduction (% R)

$$= [(A - B) / A] \times 100$$

Whereas:

A = Dry matter weight of weed plants taken from the control/plot.

B = Dry matter weight of weed plants taken from treatment/plot.

5. Yield and its components:

At harvest stage (120 days from sowing), three inner rows chosen from each plot. Random samples (ten guarded plants) taken from each plot to estimate: number of rows/ears, number of grains/rows, 100 grains weight (g), ear weight (g), grain yield/plant (g) and total grain yield by kg/hectare.

6. Economic analysis:

Agriculture is an economic process. Therefore, the cost of production elements had calculated for the various weed control treatments, which represented in the (Land preparation and cultivation, seeds, mineral fertilizers, herbicides, pesticide spraying, hoeing, other pest control, the hiring charges of human labor, irrigation, harvesting and land rent). Gross revenue has calculated by multiplying the total yield in kg/ha and corn market price/kg. Net return (NR) calculated as the difference between the gross revenue and the total cost., the Benefit-cost ratio (BCR) calculated according to Li *et al*. (2005): BCR= NR/total Costs

7. Statistical analysis:

Data subjected to the proper statistical analysis as the technique of analysis of variance (ANOVA) of a complete randomized block design as described by Gomez and Gomez (1984). Means compared using the L.S.D. test as outlined by Waller and Duncan (1969). The computation was done using computer software MstateC version 3.4.

Results and Discussion

1. Weed biomass density:

Effect of fluroxypyr herbicide treatment on weed development compared with plant extracts or plant extracts/ fluroxypyr mixtures treatments studied after 90 days of corn planted (Figures, 1and 2).

Regarding season 2008, in all weed species, the weed control had the highest weed density. Also, in all weed species, except *Echinochloa colonum* L., the fluroxypyr herbicide treatments were the lowest weed density followed by 0.75 fluroxypyr +0.25 *E. citirodora* H. mixture and hoeing treatments. While with *E. colonum* weed species, the hoeing treatments were the most effective treatments for reducing weed biomass density followed by 0.75 fluroxypyr +0.25 *E. citirodora* H. mixture and the fluroxypyr herbicide treatments. There are no significant differences between the F herbicide treatments and F+E.c3:1in all treatments. The H treatments were the most effective treatments for reducing the *E. colonum* biomass density.

Regarding season 2009, the same trend had shown. Whilst, the highest weed density recorded in C treatments. The most effective treatments for reducing biomass density were the F herbicide treatments followed by F+E.c3:1mixture with *C. olitorius, A. retroflexus, P. oleracea* L. species. The H treatments were the most effective treatments in case of *E. colonum* species. There are no significant differences between the herbicide and F+E.c3:1 mixture treatment. The weed biomass increasing in all treatment with time, there is no significant difference between the two seasons.

Regarding *E. colonum* weed species, in all season, the H treatments were the most effective treatments in decline the weed biomass density, followed by F+E.c3:1 and F treatments. While in the other weed species, the lowest biomass density recorded in herbicide treatments, followed by F+E.c3:1mixture and H treatments. The C treatment had the highest weed density. There are no significant differences between the F and F+E.c3:1mixture treatments. Whilst there are significant differences between the two *Zea mays* hybrids treatments in weed biomass density. The hybrid TWC 321 had the lowest weed plant density.

Regarding *E. colonum* weed species, the most effective treatments for depressing the weed density were H following by F+E.c3:1mixture and F treatments. With respect too there weed species, the herbicide F treatments were the most effective treatments for dipping the weed biomass, followed by F+E.c3:1mixture and H treatments. The *C. pepo* plant extract and their mixtures treatments were the lowest effective one.

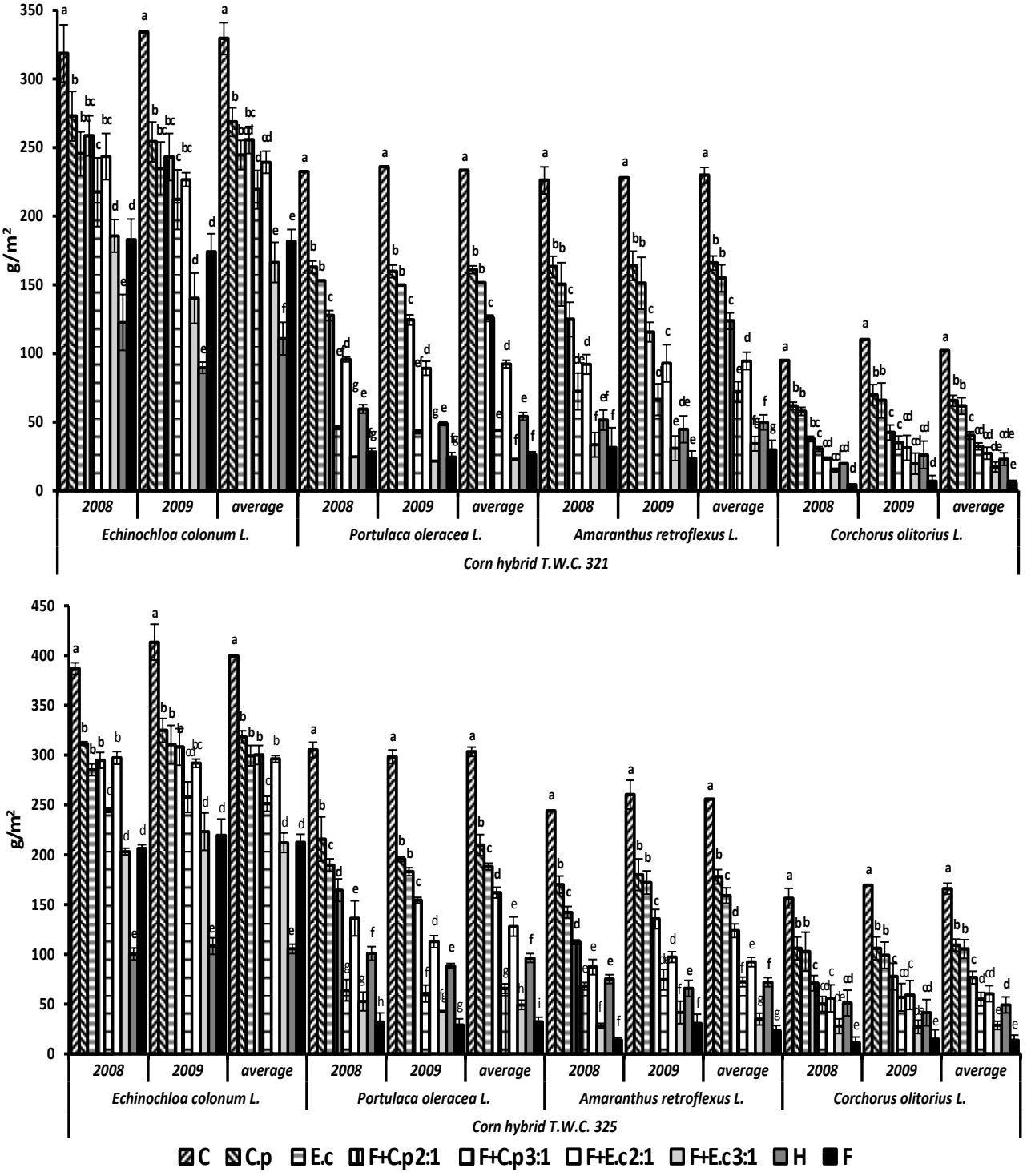

Figure (1): Effect of certain weed control treatments on weed biomass density.

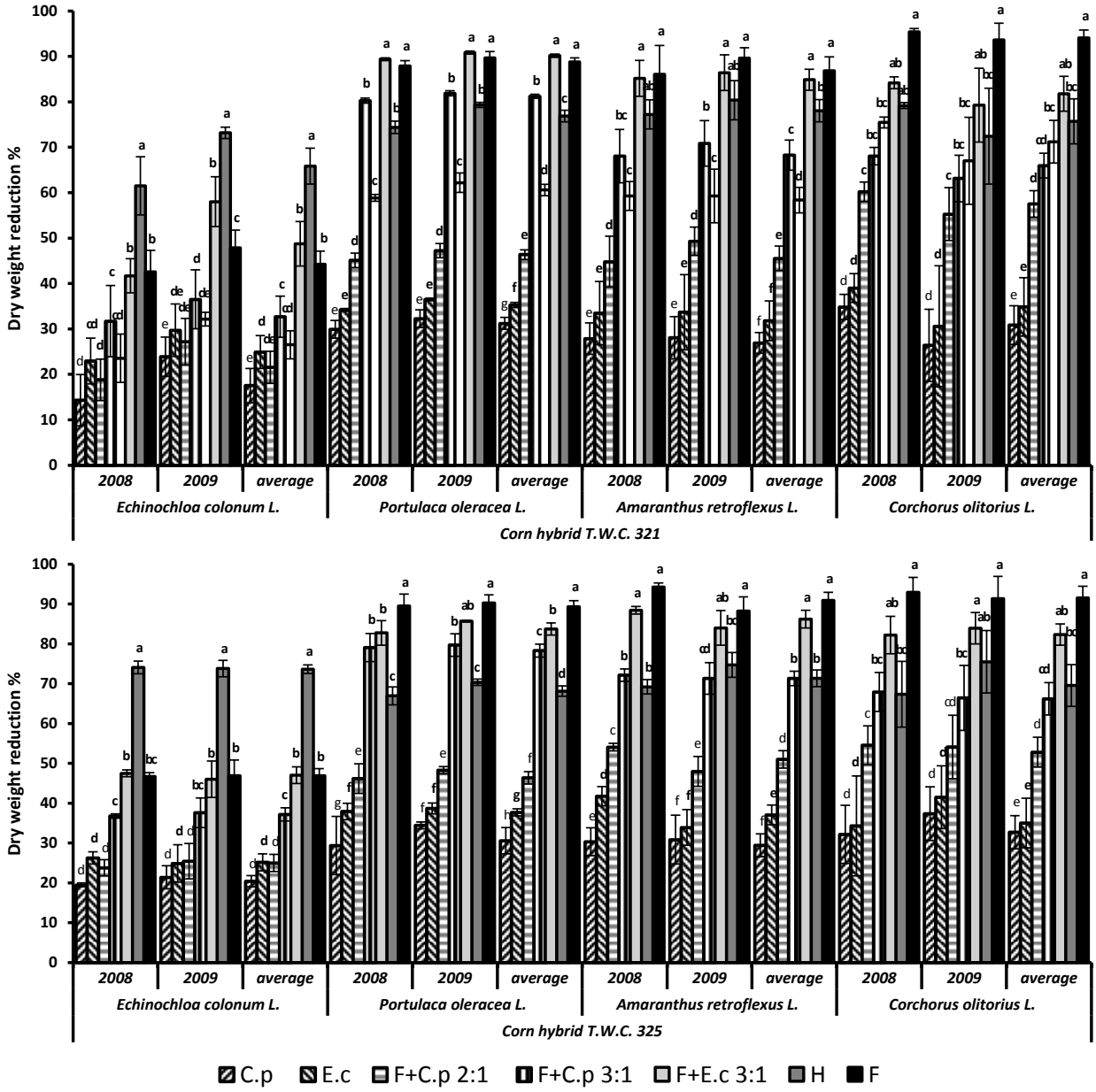

Figure (2): Dry weight reduction of certain herbs as affected by studied weed control treatments.

From the previous data, we can conclude that fluroxypyr herbicide treatments had the maximum efficacy against all weed's species except E. colonum species. The hoeing treatments were the most effective treatments for reducing the E. colonum biomass density. These outcomes concur with those of Abouziena et al. (2007), who stated that herbicide treatments had a selective activity in reducing the weed biomass while hoeing removes all weeds types. Similar funding show in other herbicides, (Guar et al., 1991) reported that apply atrazine 0.5 kg/ha had controlled the broad-leaved weeds, with no effect on the grass weeds. Pandey et al. (200l) found that Atrazine was more efficient against Ageratum conyzoides and less efficient against E. colonum and Brachiaria ramosa. Also, data revealed that; cultivars significantly influenced suppressing weed growth. Where the hybrid TWC 321 had the lowest weed plant density. This may be because of that TWC 321 hybrid was taller

than TWC351(Amine 2013), also, maybe because of the differential rooting patterns, higher leaf area index, more light interception, vegetative growth habit and allelochemicals (Abouziena et al., 2008; Dhima et al., 2008 and Seavers and Wright, 1999). Similar findings on the effect of corn cultivars on weeds recorded by Begna et al. (2001) and Gurney et al. (2002). Abouziena et al. (2013) found that SC 164 had lower weed dry weight than that of SC 166 cv. In contrast, Oliveira et al. (2011) reported that no differences found between dry matters of weeds shoot that occurred in plots of the three cultivars tested.

2. Effect of certain weed control treatments on corn yield and yield component:

The effect of fluroxypyr herbicides, plant extract, and plant extracts/fluroxypyr mixtures on the corn yield and yield component studied (Figures, 3 and 4).

Data revealed that in season 2008: corn hybrid 321 treated with 0.66 fluroxypyr +0.33 *E. citirodora* (F+E.C2:1) mixture had the highest values of the raw No./ear. Whilst the control treatment had the lowest one. There are no significant differences among other treatments. While, with corn hybrid-351, there are no significant differences between treatments. There are significant differences between the two-corn hybrid. However, the 351-hybrid had the highest values in this respect.

Corn hybrid-321 control weeded and *C. pepo* plant extract treatments had the lowest values of grain No./raw. Whilst the F treatments were the highest one. Regarding corn hybrid-351, the highest grain No./raw values recorded in hoeing, F+E.c3:1, F+E.c2:1treatments. *C. pepo* plant extract and control treatments had the lowest values in this respect.

Regarding corn hybrid 321, the highest values of 100-grain weight recorded in F+E.c3:1treatments followed by hoeing and F+E.c2:1treatments. The weeded control treatment had the lowest values in this respect. Regarding corn hybrid-351, the control treatments had the lowest values in 100-grain weight. Whilst there are no significant differences among the other treatments.

In corn hybrid-321, ear weight/plant recorded the highest values with F herbicide, F+E.c3:1, F+E.c2:1and H treatments. The control-weeded treatments had the lowest values in this respect. Whilst, in the case of hybrid-351, F+E.c3:1, F+E.c2:1, H (twice) and *E. citirodora* plant extract treatments had the highest values of the ear weight/plant. Whilst control treatments had the lowest one.

Regarding corn hybrid-321, F treatments had the highest values of grain yield/plant (204.8g/plant) followed by hoeing and F+E.c3:1treatments (180.2, 189.0 g/plant respectively). Whilst, control treatments had the lowest values in this respect (61.7g/plant). With respect to corn hybrid-351, F+E.c3:1treatments overcome the other treatments in grain yield/plant (170.0 g/plant). Followed by the F, hoeing, and F+E.c2:1treatments (168.6, 167.7 and 166.9 g/plant respectively).

In season 2009, In the case of corn hybrid-321**,** H treatments had the highest values of rows No./ear. Whilst, the control treatments had the lowest values in this respect. There are no significant differences among the other treatments.

On the other hand, with respect to corn hybrid-351, control treatment had the lowest values of ear rows No./ear. There are no significant differences among the other treatments.

Corn 321-hybrid treated with F, H, and F+E.c3:1 had the highest values of the grain No./plant. No significant differences found among the other treatments.

With respect to hybrid-351, the highest values of grain No./ear recorded in F+E.c3:1treatments while *C. pepo* plant extract treatments had the lowest values in this respect.

Corn hybrid-321 control treatments had the lowest values of 100-grain weight. There are no significant differences among the other treatments. The same trend had

shown in corn hybrid-351. With respect to corn hybrid-321, F treatments followed by hoeing and F+E.c3:1treatments had the highest values of ear weight/plant. The control treatments had the lowest values in this manner. With respect to corn hybrid-351, the F followed by F+E.c3:1 treatment had the highest values of the ear weight/plant. The control-weeded treatment had the lowest value in this respect.

Corn hybrid-321 treated with F+E.c3:1had defeat other treatments in grain yield/plant, followed by F, H and F+E.c2:1treatments (184.2, 184, 171.6 and 141.5 g/plant respectively.). In the case of *corn* hybrid-351, F treatment had the highest value of grain yield/plant (171.1 g/plant) followed by F+E.c3:1and F+E.c2:1treatments (165.1, 162.6 g/plant respectively). The weeded control had the lowest value in this respect (76.7 g/plant). With respect to the two corn hybrids, the control treatments had the lowest values of raw No./ear. There are no significant differences among the other treatments.

Grain No./raw had recorded the highest *values* in *corn* hybrid-321 treated with F and hoeing treatments followed by F+E.c3:1treatment. While in the case of *corn* hybrid-351 the highest values of grain No./raw had recorded in, F+E.c3:1treatment followed by H and F treatments. The control-weeded treatments had the lowest values of grain No./raw in the two hybrids.

Corn hybrid-321 treated with F+E.c3:1had the highest value of 100-grain weight followed by H and F treatments. Control-weeded treatment had the lowest value in this respect. With respect to corn hybrid-351, control-weeded treatment had the lowest value of 100-grain weight. There are no significant differences among other treatments.

Ear weight/plant had recorded the highest values in F, F+E.c3:1and H corn hybrid-321 treated treatments. However, control treatment had the lowest value in this respect. The same trend had shown in the case of corn hybrid-351. F, F+E.c3:1, and hoeing treatments had the highest values of ear weight/plant. Whilst the control-weeded treatment had the lowest one.

With respect to corn hybrid-321, F, and F+E.c3:1overcome the other treatments in grain yield/plant (194.4 and 186.6 g/plant respectively). Control treatment had the lowest value in this respect (61.02 g/plant). With respect to corn hybrid-351, F, F+E.c3:1, F+E.c2:1and H treatments had the highest values of grain yield/plant (169.8, 167.6, 164.8 and 157.8 g/plant respectively). The control treatment had the lowest value (57.5 g/plant).

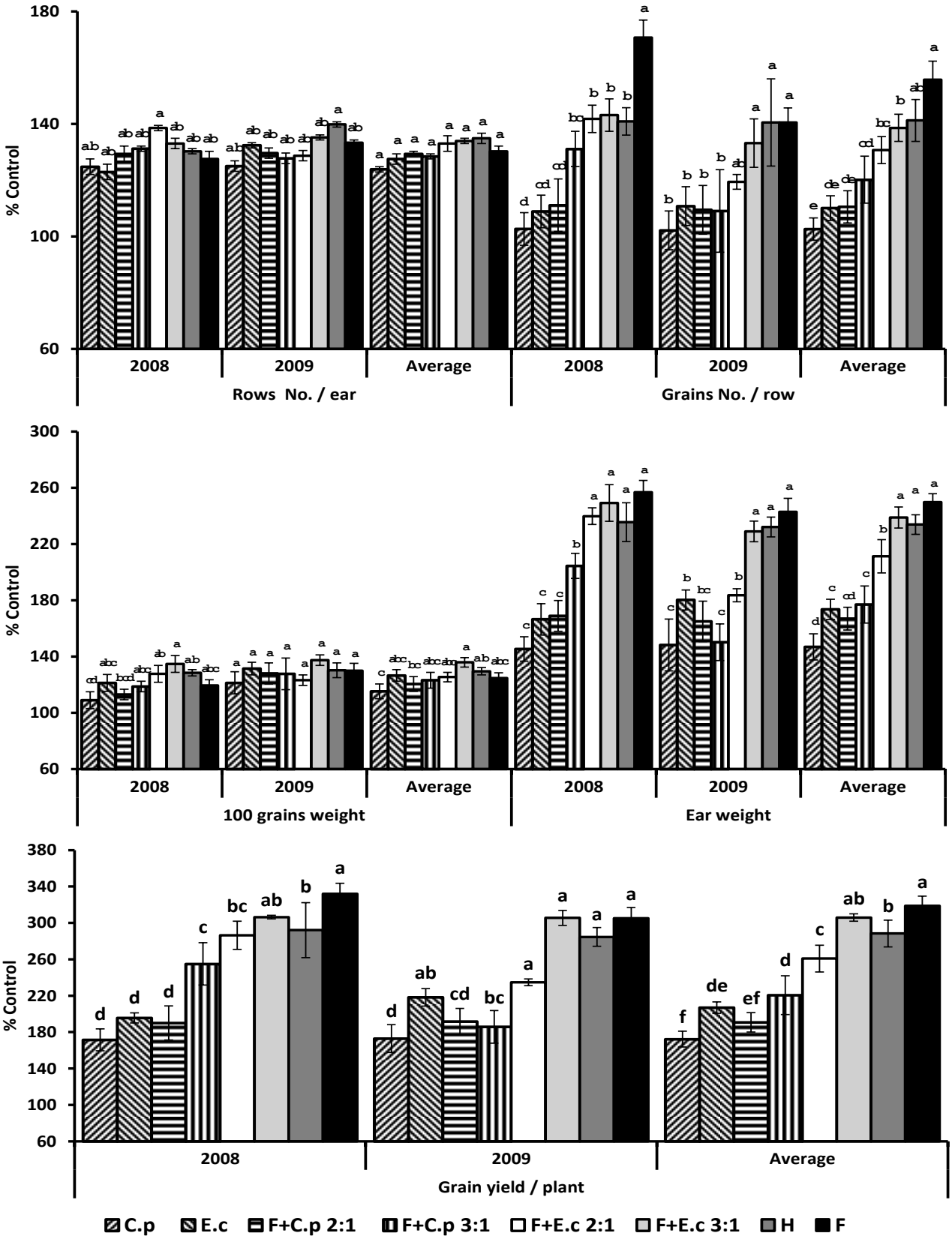

Figure (3): Effect of certain weed control treatments on corn yield and yield component of corn hybrid T.W.C 321.

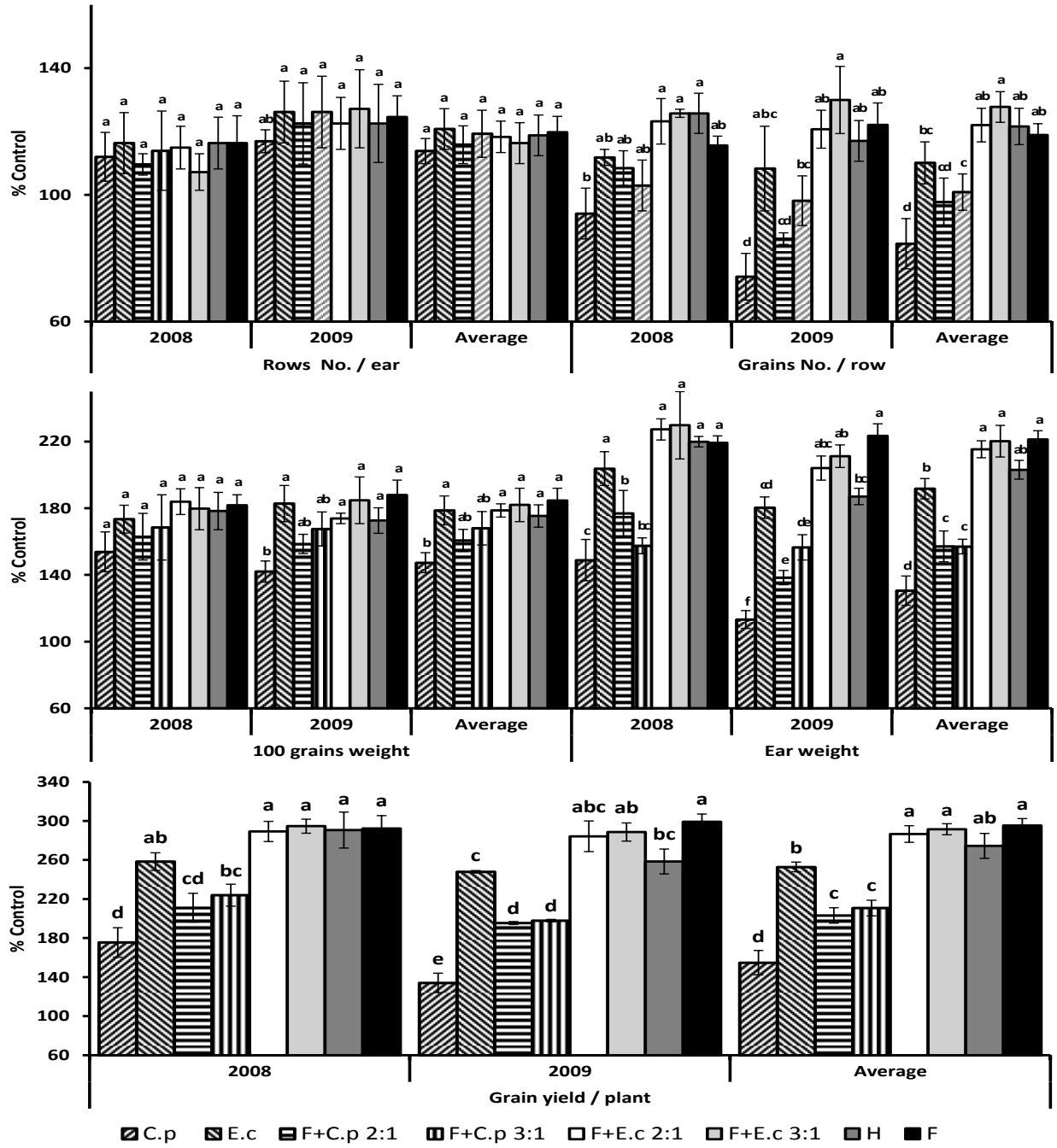

Figure (4): Effect of certain weed control treatments on corn yield and yield component of corn hybrid T.W.C. 325.

There is a significant difference between the two-corn cultivar in all yield and yield component characteristics. Significant differences in grain yield and yield components between corn cultivars reported by Ahmed and El-Housini, 2012; Abdel-Wahed et al., 2006; Maswada and El Gamal, 2013; El-Gizawy and Salem, 2010; Ibeawuchi et al., 2008; Mehasen and Al-Fageh, 2004; Salah et al., 2011 and Sedhom et al., 2012. These differences may be because of the genetical differences among cultivars and different genotypes regarding dry matter partitioning (carbon equivalent, yield energy/plant and per feddan for kernels and straw yields, shoot biomass and harvest index coefficient energy) (Salah et al., 2011.(

The control treatments had the lowest value in all parameters of yield and yield component., fluroxypyr herbicide treatments had the highest values in this respect. A similar finding observed by Mohammadi et al. (2012) who reported that full season weedy condition diminished 100-seed weight, seedling vigor index and seed protein content of the produced seeds. Also, Abouziena et al. (2013) reported that the control treatment had the minimum harvest index, with a significant reduction in seed protein and total soluble carbohydrates content. The significant diminish got in yield and yield parameters for un-weeded corn crop reflect the reduced effect of weed competition (Akobundu, 1992). While several researchers showed that fluroxypyr had a height efficacy in controlling weeds in corn (Abouziena et al., 2007 and Yehia et al. 1992). Ahmed et al. (2008) showed that Fluroxypyr provided the best treatment for controlling broad-leaved weeds.

Regarding corn hybrid-351, the fluroxypyr herbicide treatments had followed by the 0.75 fluroxypyr +0.25E. citirodora (F+E.C3:1) treatments and 0.66 fluroxypyr+0.33E. citirodora (F+E.C2:1) treatments with no significant difference among them, that in all yield and yield component criteria. While hoeing treatments influenced it. Regarding corn hybrid-321, the fluroxypyr herbicide treatments followed by hoeing treatments on grain no./raw, raw no./ear parameters. While fluroxypyr herbicide treatments followed by 0.75fluroxypyr +0.25E. citirodora (F+E.C3:1) treatments and hoeing in 100-grain weight and ear weight/plant, with no significant difference among them.

Regarding the two corn hybrids, regardless of control treatment, there are no significant differences among the other treatments in the values of raw No./ear. This agrees with, Abouziena et al. (2013) who report that no critical varied between the two cultivars tested in raw No./ear, kernels No. /row, ear grain weight, and biological yield criteria.

Using a reduced rate of herbicide (75% of recommended rate) mixed with the E. citirodora plant extract had secure the same effect in reducing biomass of weed. In addition, this mixture was more effective in controlling the narrow-leaved weeds E. colonum. This may due to that E. citirodora plant extract influenced seed germination of E. colonum (AbdAllah and Amin, 2016). Anyway, many researchers found that using a low rate of herbicide gave a sufficing weed control in corn crop (Abouziena et al., 2013; El-Metwally et al., 2002 and Parwada and Mudimu, 2011.(

Use of allelopathic plant extracts with reduced rates of herbicides to control weeds in arable crops has become turned into an entrenched fact (Jabran et al., 2008). The effect of combined Allelopathic plant extracts and herbicides application helps to reduce the amount of herbicide used (Cheema et al., 2005 and Razzaq et al., 2012.(

For instance, reduced rates of herbicides like glyphosate, bromoxynil, butachlor, ethoxysulfuron ethyl, iodosulfuron, isoproturon, MCPA, mesopleuron, metribuzin, peninsula, fenoxaprop, fenoxaprop-p-ethyl, pretilachlor when tank mixed with allopathic water extracts of crops (sorghum, sunflower, brassica, rice) proffer booming weed control in cotton, brassica, wheat and rice (Cheema et al., 2010; Elahi et al., 2011; Iqbal et al., 2009; Mahmood et al., 2009; Razzaq et al., 2010 and 2012; Rehman et al., 2010 and Wazir et al., 2011). Likewise, Khaliq et al. (2012) assess the economic effect of reduced rates (a quarter and a half of the label dose) of a post-emergence bispyribac sodium herbicide applied alone or in a blend with Eucalyptus camaldulensis Dehnh., Mangifera indica L., and Morus alba L. water extracts in direct seeded rice (Oryza sativa L.) fields. They found that tank mixing of E. camaldulensis water extracts with reduced herbicide dose were more effective in suppression the weed density and dry weight than those recorded for the same herbicide dose used alone. Shahid et al.

(2007) tested the herbicidal potential of aqueous extracts of sorghum (Sorghum bicolor), sunflower (Helianthus annuus), johnsongrass (Sorghum helepense), neem (Azadirachta indica), eucalyptus (Eucalyptus camaldulensis) and acacia (Acacia nilotica) alone and in incorporation with herbicides against weeds of wheat. They found that blend of Sunflower extracts with Carfentrazone–ethyl ester (half of the label dose) exhibited almost similar weeds control and gain more wheat grain yield.

3. Benefit-cost ratio (BCR) and net return (NR):

The benefit-cost ratio (BCR) and net return (NR) affected by weed control treatments (Table, 2). Regarding corn hybrid-321, Benefit cost ratio (BCR) had recorded the maximum values in fluroxypyr (F), 0.75 fluroxypyr +0.25 *E. citirodora* (F+E.c3:1) and hoeing(H) corn hybrid-321 treated treatments. While control(c) treatment had the lowest value in this respect (2.49, 2.37, 2.07 and 0.15 respectively). The similar trend had shown with corn hybrid-351 F, F+E.c3:1and F+E.c2:1treatments had the highest values of BCR (1.90, 1.88, and 184 respectively), followed by the H treatment (1.62). Whilst the C treatment had the lowest one (0.03).

When taking a net return (NR) into consideration, the herbicide treatments had the highest values of NR (2773.08 and 2120.14 $/ha for corn for hybrid-321 and 351 respectively). Followed by the 0.75 fluroxypyr +0.25 *E. citirodora* (F+E.c3:1) treatments (2624.12 and 2083.94 $/ha for corn hybrid-321 and 351 respectively).; hoeing treatments had a moderate NR (2371.01 and 1857.58 $/ha for corn hybrid-321 and 351 respectively), because of the high costs of the hoeing process compared to other treatments.

Table (2): Inputs and outputs items of maize crop as affected by weed control treatments (means over 2008 and 2009).

Economical items	Characters	Unit	Weed control treatments								
			C	F	C.P	F+C.P 2:1	F+C.P 3:1	E.C	F+E.C 2:1	F+E.C 3:1	H
List of inputs	Land preparation and cultivation	$ ha^{-1}	52	52	52	52	52	52	52	52	52
	Seed price		86.6	86.6	86.6	86.6	86.6	86.6	86.6	86.6	86.6
	Mineral fertilizers		129.9	129.9	129.9	129.9	129.9	129.9	129.9	129.9	129.9
	Herbicide price		-	32.5	4.3	23.1	25.4	4.3	23.1	25.4	-
	Spray cost		-	21.7	21.7	21.7	21.7	21.7	21.7	21.7	-
	Hoeing cost		-	-	-	-	-	-	-	-	86.6
	Another pest control		43.3	43.3	43.3	43.3	43.3	43.3	43.3	43.3	43.3
	Labor costs		43.3	43.3	43.3	43.3	43.3	43.3	43.3	43.3	43.3
	irrigation		121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2	121.2
	Harvesting		64.9	64.9	64.9	64.9	64.9	64.9	64.9	64.9	64.9
	Land rent		519.5	519.5	519.5	519.5	519.5	519.5	519.5	519.5	519.5
Total cost ha^{-1} season^{-1}			1060.6	1060.6	1114.7	1086.6	1105.3	1107.7	1086.6	1105.3	1107.7
TWC 321 hybrid											
List of outputs	Grain yield	Kg ha^{-1}	3196.3	10182.3	5503.1	6097.1	7050.5	6607.9	8333.8	9773.8	9214.3
	Farm gate price (locally price)	$ Kg^{-1}	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Gross revenue		$ ha^{-1}	1220.40	1220.4	3887.8	2101.2	2328	2692	2523	3182	3731.8
Net return (NR)		$ ha^{-1}	159.8	159.8	2773.1	1014.6	1222.7	1584.3	1436.4	2076.7	2624.1
Benefit-cost ratio (BCR)			0.15	2.49	0.93	1.11	1.43	1.32	1.88	2.37	2.07
TWC 351 hybrid											
List of outputs	Grain yield	Kg ha^{-1}	3009.8	8895.9	4662.4	6118.6	6347.0	7617.8	8629.8	8777	8263.1
	Farm gate price (locally price)	$ Kg^{-1}	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Gross revenue		$ ha^{-1}	1094.5	3234.9	1695.4	2225	2308.0	2770.1	3138.1	3191.6	3004.8
Net return (NR)		$ ha^{-1}	33.9	2120.1	608.9	1119.6	1200.3	1683.5	2032.8	2083.9	1857.6
Benefit-cost ratio (BCR)			0.03	1.90	0.56	1.01	1.08	1.55	1.84	1.88	1.62

Notes: (C), Control; (F), Fluroxypyr ; (C.p), C. pepo plant extract; (F+C.p2:1), Fluroxypyr+C. pepo(0.66 :0.33); (F+C.p3:1), Fluroxypyr+C.pepo(0.75 :0.25); (E.C), E.citirodora plant extract; (F+E.c2:1), Fluroxypyr +E.citirodora(0.66 :0.33); (F+E.c3:1), Fluroxypyr+E.citirodora (0.75 :0.25); (H); Hoeing; (Exchange rate: EGP (LE) ≈ 0.18 US$; rate in 2009.)

It is conclded that the effective weed control through the use of a reduced rate of herbicide (75% of the recommended rate) mixed with the *E. citirodora* plant extract. But, maximum values of benefit-cost ratio (BCR) had recorded in fluroxypyr (F) and 0.75 fluroxypyr +0.25E. This means that the use of herbicide fluroxypyr was more effective compared with the reduced dose of herbicides/plant extract mixture. This may be because of the low price of the herbicide and low cost of herbicide spraying process, in that time. But it compensates desired to benefit lowering environmental pollution and reduce toxicity to non-target organisms. Also, the tank mixed technique has difficulties, which represented in the difficult to implement by the simple farmer. Therefore, we hope to study how to convert plant extracts into formulation ready-to-use.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Effects of *Ocimum sanctum* extract against biochemical aspects of *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae

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Abstract:

The methylene chloride leaves extract of *Ocimum sanctum* (L.) was studied against the 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) strain. The LC50 value of the *O. sanctum* extract was lower against the 2nd instar larvae than the 4th instar and the time effect on the toxicity of the extract, where the LC50 after the 48 hrs was lower than after 24 hrs. against the two ages of the larvae. The biochemical aspects of *S. littoralis* larvae were detected using the LC50 of *O. sanctum* extract against the 2nd and 4th instar larvae. The *O. sanctum* extract exhibited variations of activities for each enzyme. It increased the activity of AST (Aspartate Transaminase) and beta esterases, on the contrary they decreased the activity of ALT (Alanine Transaminase), alpha esterases, Acetylcholinesterase (AchE), alkaline phosphatases, total protein and total lipids in both 2nd and 4th instar larvae, while *O. sanctum* extract decreased the activity of acid phosphatase only in the 2nd instar larvae and elevated it in the 4th instar larvae. Conversely, the carbohydrates increased in the 2nd and decreased in the 4th instar larvae. The results of the research show that the overall effects of *O. sanctum* extract on some biochemical components in *S. littoralis* larvae can facilitate the development of selective natural product as insecticides that can be employed in integrated pest management strategies.

Introduction

Ocimum sanctum (L.) (OS) is an herb belonging to the family Lamiaceae, known for its medicinal value in various traditional medicines in India and other Asian nations, particularly Ayurveda and Unani type of medicine (Satyavati,1987). The important bioactive constituents of *O. sanctum* are ursolic acid,-a triterpenoid and rosmarinic acid a phenylpropanoid. It contains volatile oil comprising mainly of eugenol and β-caryophyllene with minor terpenes like bornyl acetate, β-elemene, methyl eugenol, neral, β-pinene etc (Rastogi and Mehrsotra, 1998). Gupta *et al.* (2007) isolated three new compounds, ocimumoside A, (2) Ocimumoside B, and ocimarin, from an extract of the leaves of holy basil (*Ocimum sanctum*), together

with other known substances. apigenin, apigenin-7-O-beta- d-glucopyranoside, apigenin-7-O-beta- d-glucuronic acid, apigenin-7-O-beta- d-glucuronic acid 6"-methyl ester, luteolin-7- O-beta- d-glucuronic acid 6"-methyl ester, luteolin-7-O-beta-dglucopyranoside, luteolin-5-O-beta-d-glucopyranoside, 4-allyl-1-O-beta-dglucopyronosyl-2-ydroxybenzene and two known cerebrosides. Singh *et al.* (1991) found that the ethanol extract of OS leaves prevent the reduction in adrenergic neurotransmitters in brain of rats. Sukari *et al*. (1992) recorded a high toxicity of *O. sanctum* against *Tilapia mossambica*. While Chitwood (2002) mentioned the leaf extracts of lantana (*Lantana camara*), *Citrus* oil, tulsi (*Ocimum basilicum, O. sanctum*) and vetiver (*Vetiverazi zanoides*) are useful in controlling leaf miners in potato, beans, brinjal, tomato and chilies.

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered as the major pest in a wide range of cultivation including cotton, corn, soybeans, peanuts, and vegetables. This pest is not only widely spread in Egypt but also in other Middle East countries in addition to temperate zones in Asia and Africa (Salama *et al*., 1990). Over the last few decades, the intensive use of broad-spectrum insecticides against the Egyptian cotton leafworm, *S. littoralis* has led to the development of resistance to many registered pesticides making their control even more difficult (Miles and Lysandrou, 2002). The extensive use of these synthetic pesticides has given rise to problems such as residuals toxicity (pollution), pesticide resistance and harmful effects on beneficial insects, such as natural enemies, honey bees and beneficial birds. For the above mentioned reasons, the general trends in last three decade were the substitution of synthetic pesticides by natural products (Aydin and Gurkan, 2006).The aim of this research work is to study effects of *O. sanctum* extract against biochemical aspects of the Egyptian cotton leafworm, *S. littoralis* larvae.

Materials and Methods

1. Plant materials:

The plant was collected from Khulias, Jeddah, Kingdom of Saudi Arabia, in February 2017.

2. Preparation of the extract:

A weight of 50 g fresh leaves of the plant was grounded and then macerated in 200 ml of methylene chloride solution and left 7 days, then filtered through Whatman No. 40 filter paper. The solvents were removed under reduced pressure using a rotary evaporator to obtain 1.61 g extract for *O. sanctum*.

3. Test insect:

The 2nd and 4th instar larvae of *S.littoralis* strain, used in this study, was obtained from the Faculty of Agriculture, Cairo University and was reared in the laboratory of the Pest Physiology Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, for several generations away from any insecticidal contamination, under constant laboratory conditions as described by El-Defrawi *et al*. (1964).

4. Toxicity assay:

The concentrations 20, 10, 5 % (extract / diet) from *O. sanctum extract* were prepared. Ten larvae for each 2nd and 4th instar of *S. littoralis* were transferred individually to the surface of each treated diet kept in glass jars, four replicates for each concentration. Ten larvae were allowed to feed on untreated diet as a control treatment for each the 2nd and the 4th larvae. The glass jars kept under the previous controlled conditions and inspected daily. Mortality percentages were recorded after 24 and 48 hrs. the obtained data subjected to Ldp line analysis (**Bakr, 2007**) and the toxicity then estimated. In this paper, try to study the biochemical changes. So, samples for analysis been taken 48hrs. post treatment with LC50 of tested *O. sanctum* extract and before the onset of the mortality.

5. Preparation of insects for biochemical studies:

The preparation of samples involved the use of the 2nd and 4th instar larvae of *S. littoralis* after 48hrs. of all treatments at LC50 level and control. The larvae were homogenized in distilled water (50 mg /1 ml) using a Teflon homogenizer surrounded with jacket of crushed ice for three minutes. Homogenates were centrifuged at 8000 r.p.m. for 15 min. at 2℃ in a refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, were used directly for the biochemical analysis (Amin, 1998).

6. Biochemical measurements:

- Transaminases; (ALT) alanine aminotransferase activity (GPT) and (AST) aspartate aminotransferase activities (GOT) were determined according to Reitman and Frankle (1957)
- Phosphatases were demonstrated according to Powell and Smith (1954)
- α- and β-esterases were detected according to Van Asperen (1962)
- AchE (acetyl cholinesterase) activity was measured according to Simpson *et al.* (1964)
- Total lipids Total lipids were estimated by the method of Knight *et al.* (1972)
- Total carbohydrates were determined according to Dubois *et al.* (1956)
- Total proteins were determined according to Bradford (1976)

7. Statistical analysis:

Significant differences were calculated by ANOVA and Duncan's multiple range tests. Differences among treatments were determined by Tukey's multiple range test ($P < 0.05$) CoStat - Statistics Software (CoStat, 2007).

Results and Discussion

Table (1) Showed that the LC50 of the *O. sanctum* extract to the 2nd instar larvae of *S. littoralis* is 47.62% after 24 hrs. and 0.99% after 48 hrs., where in Table (2) found the LC50 of the *O. sanctum* extract to the 4th instar larvae of S. *littoralis* is 140.06% after 24 hrs. and 27.02% after 48 hrs. The results showed, also, that the LC50 value of the *O. sanctum* extract was lower against the 2nd instar larvae of *S. littoralis* than against the 4th instar larvae and the time effect on the toxicity of the extract where the lC50 after the 48 hrs was lower than after 24hrs against the two ages of the larvae of *S. littoralis*. Anees (2008) found that, the LC50 values of *O. sanctum* leaf extract against the larvae of *Ae. aegypti* was 425.94, and against the larvae of *Cx. quinquefasciatus* was 592.60 ppm. Average of 60% mortality was recorded for *Periplenta americana* due to the toxicity of leaf extract of *O. sanctum* (Hazarikaa and Boruah, 2014). Yousef et al. (2016) mentioned that the LC50 values for *C. procera* extract was (0.14g /100 g diet) and recorded (0.0032 g/100 g diet) for *O. sanctum* against the cotton pink bollworm, *Pectinophora gossypiella (*Saunds.*)* they elucidate the high toxic effect of *O. sanctum* may be due to the presence of high amount of lead in the extract.

In Tables (3 and 4) *O. sanctum* extract caused decreased to the total protein non-significantly in the 2nd and significantly in the 4th instar larvae of *S. littoralis* compared with the control, the effect of *O. sanctum* is comparable to the effect of the novel insecticide pyridalyl was obtained by Dahi *et al.* (2011), they observed a conspicuous depletion in total protein content in both 4th and 6th treated larval instar with LC50 of the novel insecticide pyridalyl. Similar results were obtained by (Sokar, 1995) and (Assar *et al.*, 2016) for the total protein of the same species treated with teflubenzuron and hexaflumuron. Wilkinson (1976) stated that protein help to synthesize microsomal detoxifying enzymes which assist in the detoxificaition of toxicants that enter into the insect body. Proteins are the most important components of biochemical of insect that bind the

Table (1): Mortality percentage of the 2nd instar larvae of *Spodoptera littoralis* treated with *Ocimum sanctum* extract.

Conc.	Corrected Mortality % ± SD	
	After 24 hrs.	After 48 hrs.
20%	38.46±0.00 b	92.30±2.5 a
10%	41.02±2.5 ab	74.35±2.8 b
5%	48.71±4.08 a	79.48±4.0 b
control	0±0 c	0±0 c
Lc50	47.6284	0.9945
Slope	0.3447+/-0.2141	0.9609+/-0.2397
F value	62	176.44
LSD	0.8320	0.9434
P	.0000***	.0000***

Means in the same column with the same letter(s) are not significantly different. (P<0.05)
*SD = standard deviation LSD : least significant difference. ***: highly significant*

Table (2): Mortality percentage of the 4th instar larvae of *Spodoptera littoralis* treated with *Ocimum sanctum* extract.

Conc.	Corrected Mortality %± SD	
	After 24 hrs	After 48 hrs
20%	20±4.08 a	42.50± 2.50 a
10%	7.5±2.50 b	22.50±4.78 b
5%	7.5±4.78 b	12.50±2.88 b
control	0.00±0.00 b	0.00±0.00 c
Lc50	140.0685	27.0286
Slope	1.0747+/-0.6386	1.6206+/-0.5363
F value	6	33.333
LSD	1.04302	0.9434
P	.0097**	.0000***

Means in the same column with the same letter(s) are not significantly different. (P<0.05)
****: highly significant SD = standard deviation LSD: least significant difference*

foreign compounds. In general, the problem of protein synthesis is intimately related to metabolism of nucleic acids.

Total carbohydrates show a non-significantly increase 12.26% higher in the 2nd instar larvae of *S. littoralis* was treated with LC50 of *O. sanctum* extract than in the control. These observations agreed with Assar *et al.* (2016), indicated that all tested insecticides (emamectin, spinetoram, hexaflumuron and teflubenzuron) led to increase in total carbohydrates compared with control. In contrast, total carbohydrates in the 4th instar larvae was non-significantly decreased (-3.69%) than in the control. That agree with Osman and Abou-Zeid (2015), they noticed decrease of total carbohydrate when they used the plant extract of *Capsicum annuum L*, and Organophosphorous insecticide Profenofos (selecron) and the mixture of them for controlling 4th instar larvae of cotton leaf worm under the semi field circumstances. The disturbance in carbohydrate content can be understood in the light of the ability of the organism to modify the synthesis of certain metabolite and disrupt the functionality of the organism (Rodriguez-Ortega *et al.*, 2003). The obtained results in this work recorded non-significantly decrease in total lipids values in the 2nd instar larvae and significantly in the 4th

instar larvae, which were treated with LC50 of *O. sanctum* extract.

The same results were obtained by Assar *et al.* (2016) were stated the reduction in total lipids with hexaflumuron and teflubenzuron as IGR, s against 4th instar larvae of *S. littoralis*. Similar reduction in total lipids was recorded by (El-Sheikh *et al.*, 2013). Different results were obtained by (Abdel-Mageed *et al.*, 2018), in they work recorded significantly increase in total lipids values 6.63, 5.74, 6.09 for flufenoxuron, chlorfluazuron, triflumuron respectively, while it was 4.33 for control. And elucidate the exceptional cases of increasing lipid content in *S. littoralis* treated with Chitin synthesis inhibitors (CSIs) may indicate its pronounced interference with not only the synthesis of lipids but also their mobilization as promoted to convert into other metabolites or fatty acids.

Table (3): Effect of LC.50 of *Ocimum sanctum* extract on some biomolecules of treated 2nd instar *Spodoptera littoralis*.

Biomolecules	Mean of the enzyme activity ± SD		Change%	F value	LSD	P
	Treatment	Control				
Total carbohydrate *(mg/g.b.wt)*	16.2±0.43 a	14.43±0.66 a	12.26	4.8937	2.21729	0.0914 ns
Total lipids *(mg/g.b.wt)*	6.4±0.26 a	6.96±0.24 a	-8.0	2.5130	0.9924	0.01881 ns
Total protein *(mg/g.b.wt)*	33.53±1.35 a	38.1±1.47 a	-11.99	5.2078	5.5559	0.0846 ns

Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05), SD = standard deviation LSD: least significant difference.

Table (4): Effect of LC.50 of *Ocimum sanctum* extract on some biomolecules of treated 4th instar *Spodoptera littoralis*.

Biomolecules	**Mean of the enzyme activity ± SD**		**Change%**	**F value**	**LSD**	**P**
	Treatment	**Control**				
Total carbohydrate *(mg/g.b.wt)*	**10.43±0.12 a**	**10. 83±0.27 a**	**-3.69**	**1.8**	**0.8277**	**0.2508 ns**
Total lipids *(mg/g.b.wt)*	**3.8±0.115 b**	**6.10±0.118 a**	**-37.70**	**194.0191**	**0.45911**	**0.0002 *****
Total protein*(mg/g.b.wt)*	**43±1.60 b**	**57.76±1.47 a**	**-25.55**	**45.7670**	**6.06031**	**0.0025 ****

*Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05), ***: highly significant SD = standard deviation LSD: least significant difference.*

In Tables (5 and 6) *O. sanctum* extract caused (ALT) alanine aminotransferase activity (GPT) decreased significantly (-38.38) in the 2nd instar *S. littoralis* larvae than in the control, where significant elevation to (AST) aspartate aminotransferase activities (GOT) (71.34) in comparing to the control. Also caused significant increased to AST (138.20) and significant decreased to ALT (-45.08) of the 4th instar *S. littoralis* larvae than in control. Declined level of (ALT) in *S. littoralis* larvae by *O. sanctum* extract, in the present study, in agreement with decreased activity in *S. Littoralis* by several insect growth regulators (IGRs) and CSIs, for example, hexaflumuron (Sokar, 1995), teflubenzuron, flufenoxuron and pyriproxyfen (El-Kordy *et al.*, 1995), and flufenoxuron, Chlorfluazuron and Triflumuron (Abdel-Mageed *et al.*, 2018). Azmi *et al.* (1998) dissected the inhibited activity of (GPT) in haemolymph of *S. littoralis* larvae by CSIs can be understood since pyruvate is the precursors of Krebs cycle compounds, related to the mitochondrial oxidation phenomenon and ATP products. Anyhow, diverse effects of the tested CSIs on GPT activity in larvae could be due to their effects on the synthesis or functional levels of this enzyme directly or indirectly by varying the cell cytomorphology (Nath,

2000), or the neurosecretory hormonal pattern (Abdel-Mageed *et al.*, 2018).

On the other hand, increased the activity of AST in *S. littoralis* larvae by *O. sanctum* extract, in agreement with Sokar (1995), El-Kordy *et al.* (1995) and Abdel-Mageed *et al.* (2018) treated *S. littoralis* with several IGRs or insecticides, e.g. hexaflumuron, flufenoxuron, pyriproxyfen or teflubenzuron, Chlorfluazuron and Triflumuron.

Table (5): Effect of LC 50 of *Ocimum sanctum* extract on enzymes activity of 2nd instar larvae of *Spodoptera littoralis*.

Enzymes	Mean of the enzyme activity ± SD		Change%	F value	LSD	P
	Treatment	control				
AST *(U/g.b.wt)*	**8.91±0.14 a**	**5.20± 0.10 b**	**71.34**	**444.64**	**0.48**	**.0000 *****
ALT *(U/g.b.wt)*	**2.36±0.14 b**	**3.83±0.17 a**	**-38.38**	**41.1914**	**0.634478**	**.0030 ****
Alkaline phosphatase *(mU/g.b.wt)*	**2988.33±57.32 b**	**6054±101.23 a**	**-50.64**	**694.348**	**323.0169**	**0.0000 *****
Acid phosphatase *(mU/g.b.wt)*	**329±8.02 b**	**433.33±10.13 a**	**-24.07**	**65.1389**	**35.89151**	**0.0013 ****
∞-esterase *(ugα-naphthol/min/g.b.wt)*	**704.66±9.70 a**	**746±25.73 a**	**-5.54**	**0.14739**	**57.85561**	**0.7206 ns**
β-esterase *(ugβ-naphthol/min/g.b.wt*	**1425±19.31 a**	**1206.66±12.57 b**	**18.15**	**89.7541**	**63.9855**	**.0007 *****
AchE *(ugAchBr/min/g.b.wt)*	**193.33±8.81 b**	**533.66± 14.49 a**	**-63.77**	**402.331**	**47.10874**	**0.0000 *****

Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05),
****: highly significant*
SD = standard deviation
LSD: least significant difference.

Table (6): Effect of LC50 concentrations of *Ocimum sanctum* extract on enzymes activity of 4th instar larvae of *Spodoptera littoralis*.

Enzymes	Mean of the enzyme activity ± SD		Change%	F value	LSD	P
	Treatment	control				
AST *(U/g.b.wt)*	**10.4±0.152 a**	**4.366±0.185 b**	**138.20**	**630.01923**	**0.667374**	**.0000 *****
ALT *(U/g.b.wt)*	**1.72±0.06 b**	**3.19±0.15 a**	**-45.08**	**76.5673**	**0.46642**	**0.0009 *****
Alkaline phosphatase *(mU/g.b.wt)*	**470.33±10.17 b**	**1063.33±31.79 a**	**-55.76**	**315.506**	**92.6915**	**0.0001 *****
Acid phosphatase *(mU/g.b.wt)*	**232.33±7.310 a**	**217±6.92 a**	**7.06**	**2.3176**	**27.9642**	**0.2026 ns**
∞-esterase *(ugα-naphthol/min/g.b.wt)*	**339±7.37 b**	**425.33±7.31 a**	**-20.29**	**69.1556**	**28.8239**	**0.0011 *****
β-esterase *(ugβ-naphthol/min/g.b.wt*	**1098.33±7.83 a**	**1032.33±17.07 b**	**6.39**	**12.343**	**52.1564**	**0.0246 ***
AchE *(ugAchBr/min/g.b.wt)*	**744±8.32 b**	**824±12.12 a**	**-9.70**	**29.58397**	**40.83671**	**0.0055 ****

Means in the same row with the same letter(s) are not significantly (ns.) different. (P<0.05),
****: highly significant SD = standard deviation LSD: least significant difference*

The obtained data show that significantly low activity of alkaline phosphatase (ALK-P) was noticed in 2nd and 4th instar larvae of *S. littoralis* was treated with *O. sanctum* extract (-50.64 and -55.76%, respectively) lower than in control. At the same respect, *O. sanctum* extract caused significant decrease in acid

phosphatase (AC-P) activity -24.07% in the 2nd instar larvae compared to the control. These results are in agreement with those obtained on *S. littoralis* by (El-Barky *et al.*, 2008 and El-Sheikh, 2012) using spinetoram with significant decrease in both acid and alkaline phosphatases. On the contrast, *O. sanctum* extract caused increased in (AC-P) activity 7. 06 % in the 4th instar larvae compared to control. Some increase in the activity of acid phosphatase in the same insect recorded by Sokar (1995) using hexaflumuron. Sridhara and Bhat (1963) stated that the increase or decrease of both phosphatase enzymes development is reflected in increase or decrease in acid-soluble phosphorus content.

O. sanctum extract caused decreased to the alpha esterase activity non-significantly, in the 2nd instar larvae of *S. littoralis* recorded -5.54% followed by 4th instar larvae which have -20.29% significantly lower than the control level. Assar *et al.* (2016) concluded that both alpha and beta esterases in *S. littoralis* was highly inhibited with hexaflumuron., On the other hand, *O. sanctum* extract increased significantly the activity of beta esterase enzymes in the 2nd and 4th instar larvae to 18.15 and 6.39%, respectively, higher than in the control. Bakr *et al.* (2013) noticed that IGR's may be cause different levels of significant changes in alpha and beta esterases on *S. littoralis*.

The most common resistance mechanisms in insects are modified levels or activities of esterase detoxification enzymes. These esterases comprise six families of proteins belonging to the a/ß hydrolase fold superfamily (Oakeshott *et al.,* 1993 and Cygler *et al.,* 1993). Numerous studies have demonstrated that esterases play an important role in participate to insecticide detoxifications in insect and other arthrsopod species (Mouches *et al.,* 1986).

Acetylcholinesterase (AChE) significantly decreased in the 2nd and 4th instar larvae of *S. littoralis, which were treated* with LC50 of *O. sanctum* extract recorded -63.77 and -9.70%, respectively, lower than in control. AChE is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. In insects, AChE is the only cholinesterase (Salgado, 1998). It is one of the most known defensive esterases as it is the major target for organophosphate and carbamate insecticides. This property led to the development of inhibitors of this enzyme as insecticides, they covalently bind to the active site. (Aldridge, 1950) and cause the death of the insect. (Fournier *et al.,* 1992).

The effect of *O. sanctum* extract on the enzymes; esterses , AChE, AST, ALT and phosphatases, may be useful in the management of insect populations where insecticide resistance has developed as a result of altered enzyme activities. It could be concluded that *O. sanctum* extract effects on the cotton leaf-worm are significant and could be added to its toxic effects, so it is suggested that *O. sanctum* is potentially potent substitution to chemical pesticides for control of *S. littoralis.*

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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The effect of *Bacillus* species on the response of common bean to *Tetranychus urticae* (Acari: Tetranychidae) infestation

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Abstract:

The alternative of chemical pesticides and bio-pesticides are now widely used to preserve the environment and prevent the spread of pests. In this study, two bacterial isolates well known for their ability to work as bio-control agent as well as plant growth promoting bacteria were tested as bio-pesticides against *Tetranychus urticae* (Koch.) (Acari: Tetranychidae). The foliar spray of common bean plants with the bacterial strains, phylogenetically relevant to *Lysinibacillus sphaericus* and *Bacillus amyloliquefaciens*, led to a significant decrease in *T. urticae* population by 37% after 3 days of treatment, a result that was supported by GC-MS analysis of the metabolites of both bacteria which indicated the presence of different phthalate derivatives as major constituents. On the other hand, the bacterial treatment led to a significant increase in total soluble carbohydrates, proteins and chlorophylls compared with the control indicating the ability of these isolates to alleviate the mites affect as a result of their plant growth promoting activity. Our results also, indicated the possibility of using these bacterial isolates as potential bio-control agents for *T. urticae*.

Introduction

The intensive use of pesticides helped the farmers for some extent to control; however, long-term damage has become more of its benefits as the use of pesticides affect human health (Senthil-Nathan, 2015) as well as the biodiversity in the environment (Pavela, 2015). In order to achieve the goals of sustainable agriculture, It became necessary to use biopesticides (Senthil-Nathan, 2015) that is developed from naturally occurring source and does not leave residues after use. Biopesticides could be microbial pesticide that contain microbe of specific action against the desired pest without negative effect on the treated crop. Microbial pesticides gains an increasing interest in order to achieve Integrated Crop Management (ICM) in an environmentally friendly way (Copping and Menn, 2000).

Mites such as *Tetranychus urticae* (Koch.) (Acari: Tetranychidae) are among the pests threating crop production (20-45%

of the yield might be lost depending on season as growth, chlorophyll contents and fruit size as well as quality are greatly affected in case of severe mites infection (Rhodes *et al.*, 2006 and Premalatha *et al.*, 2018). *T. urticae* has a high rate of fecundity and a short developmental time that can be as brief as one week at high temperatures of about 32°C. The long term intensive use of acaricides leads to the dominance of resistant population that could not be affected by chemical pesticides and that would be reflected on plant yield (Fraulo and Liburd, 2007).

Plant-Incorporated protectants biopesticides has been reported very recently (Pavela, 2015 and Premalatha *et al.*, 2018). Some fungal species, such as *Hirsutella*, have been used as a bio-control agent against mites (Burges, 2012), however, little is known about the use of bacterial pesticides against mites. The use of bacteria in sustainable agriculture is of particular special status for sustainable agriculture because some of these bacteria could have a duel role as a plant growth promoting bacteria beside its ability to control pests (Compant *et al.*, 2005).

Directly, the use of such plant growth promoting bacteria might provide plants with fixed nitrogen as the biological nitrogen fixation is not exclusive to rhizobia. Additionally, they might have the ability to make phosphate available to plants via phosphate solubilization. Siderophores as iron chelating agents has also been recognized as one of the direct benefits attained by these bacteria by which soil unavailable iron becomes available to the host plant (Pérez-Montaño *et al.*, 2014). Host plant growth and development is also regulated by phytohormones produced by endophytes such as auxins (Das *et al.*, 2013) and GA3 (Vessey, 2003) despite its scarcity. Indirectly, ACC (1-aminocyclo-propane-1-carboxylate) deaminase activity provided by the plant growth promoting bacteria decreases the level of elevated ethylene delaying senescence and restoring proper plant growth. In addition, such bacteria would raise the level of plant induced resistance to diseases and insect infections (Ramamoorthy *et al.*, 2001 and Li *et al.*, 2015). In this study, the effect of two types of local bacillus isolates has been evaluated as pesticides against *T. urticae* in a semi-field condition.

Materials and methods

1. **The bacterial isolates and *Tetranychus urticae* culture:**

Two bacterial isolates were used in this study. One of them has been isolated from the north coast of the Mediterranean Sea and molecularly identified as *Lysinibacillus* MFNC5 (accession no KT803879) with a close homology to *Lysinibacillus sphaericus* (Mowafy *et al.*, 2016). The second one was isolated from the nodules of common bean plants and has been identified as *Bacillus MAP3* (accession no MG214652) with a close homology to *Bacillus amyloliquefaciens*. Both strains were cultivated in 250 ml LB media and incubated at 28^{o}C for 2 days. The cells were collected by centrifugation at 5000 rpm for 10 min at 4^{o}C and then re-suspended in sterilized distilled water in order to adjust the CFU concentration of the used isolate using spectrophotometer at 600 nm. The O.D. was confirmed after suspension to be 1 in order to fix the number of cells in treatments via foliar application for both bacillus isolates used in this study considering that O.D 1 = 8 x 10^{8} cells (Anith *et al.*, 2004). The culture of *T. urticae* was obtained from a laboratory pure colony that was maintained on common bean leaves incubated in petri-dishes at 25±2^{o}C.

2. **The gas chromatography mass spectrometry analysis of the bacterial secondary metabolites:**

The *LB media* of both bacterial strains were exhaustively extracted using 1 L of ethyl acetate (4 x 250 mL). The organic layers were combined together, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. A sample of each crude extract was analyzed using the gas

chromatography mass spectrometry (GC-MS) in order to identify volatile organic metabolites. The GC-MS analysis was conducted at the Central Laboratory of the Ministry of Agriculture, Al-Bhooth Street, Giza, using an Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric column PAS-5ms (30 m x 0.32 mm x0.25 um film thickness). The bacterial extracts were injected under the following conditions. Helium was used as carrier gas at approximately 1.0 ml /min, pulsed splitless mode. The solvent delay was 3 min, and the injection size was 1.0 µl. The mass spectrophotometric detector was operated in electron impact ionization mode an ionizing energy of 70 eV scanning from *m/z* 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained at 1250 V above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C/min and 10 min hold at 280°C the detector and injector temperature were set at 280 and 250°C, respectively. Wiley275 and NIST05 mass spectral databases were used in the identification of the separated peaks.

3. The semi-field experiment:

A homogeneous lot of apparent uniform common bean seeds were used in this experiment. Pure strains of seeds were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The experiment has been started in April 2017 in the experimental field of Faculty of Agriculture, Mansoura University. The used soil was a mixture of clay and sand (2:1 v/v). The pots used in this study were filled with 3 Kg of soil. Before cultivation, the soil was supplied with super phosphate fertilizer (1g/ each pot). Plants were inoculated with *T. urticae* obtained from large sensitive laboratory colony as mentioned previously. The required number of *T. urticae* was transferred from the colony to a 1.5 cm diameter common bean leaf disc that was then placed onto one leaf of the experimental plant.

The pots were divided into 4 groups including a control. The first and the second group were subjected for foliar spray by *Lysinibacillus* MFNC5 and *Bacillus MAP3* in which the O.D. of the bacterial suspension was kept 1 to justify the no of cells from each treatment. The third group was treated with Abmectin (Vertimec® 1.8% EC) with 40 cm^3/100 liter water. The last group was subjected to water foliar application to work as a negative control. The number of living mites was counted before treatment and (3, 7 & 14) days after treatment. The percentage of reduction was estimated according to the equation described before (Henderson and Tilton, 1955).

4. Estimation of photosynthetic pigments:

Chlorophyll a and chlorophyll b were determined at the flowering stages of plant growth using the spectrophotometric method as recommended by (Dye, 1962) for pigments as adopted by (Taylor and Achanzar, 1972)**.** A known fresh weight of plant leaves was cut and ground with 80 % acetone. After centrifugation, the supernatant absorbance was measured at 644 and 663 nm**.**

5. Estimation of total soluble carbohydrates:

A known volume of the dry leaf powdered tissue was submerged overnight in 10 ml 80 % (v/v) ethanol at 25°C with periodic shaking. After one day, the obtained ethanol mixture was filtered, made up to 20 ml and kept in the refrigerator (Vedder, 1915).Total soluble sugars (TSS) content was determined using the procedures described previously (Hansen and Møller, 1975). An aliquot of 0.1 ml of the alcoholic extract was added to 3 ml of freshly prepared anthrone and incubated in a boiling water bath for 10 min and the absorbance was obtained at 625 nm. The amounts of TSS in plant extracts were obtained using the standard curve of glucose.

6- Estimation of total proteins:

The method of protein extraction was adopted by (Scarponi and Perucci, 1986). A known weight of fresh plant tissue was cut into small pieces and homogenized in five volumes of chilled acetone using a homogenizer for one minute followed by sonication. The crude homogenate was filtered and the residue was used for determination of protein content after re-suspension in 50 mM tris-HCl buffer pH 9. Protein content was determined spectrophotometrically according to the method adopted by (Bradford, 1976). Bovine serum albumin was used as standard in this experiment. Data were analyzed by one way analysis of varience (ANOVA), and the means were separated using Duncan's Multiple Range Test (Snedecor, 1980).

Results and Discussion

1. The gas chromatography mass spectrometry analysis of the bacterial secondary metabolites:

The organic volatile constituents (Figure 1 and Table 1) of the ethyl acetate extract were detected using the GC-MS technique.

The GC-MS profile of Lysinibacillus MFNC5 indicates the presence of thirteen organic compounds belonging to several classes including 2,5-diketopiperazine alkaloids, long chain saturated fatty acids, phthalate esters in addition to other organic compounds. Furthermore, twenty two volatile compounds were elucidated from the GC-MS analysis of Bacillus amyloliquefaciens indicating the presence of 2,5-diketopiperazine alkaloids (2,5-DKPs), piperidone alkaloids, pyrimidine alkaloids, long chain saturated fatty acids and phthalate esters .

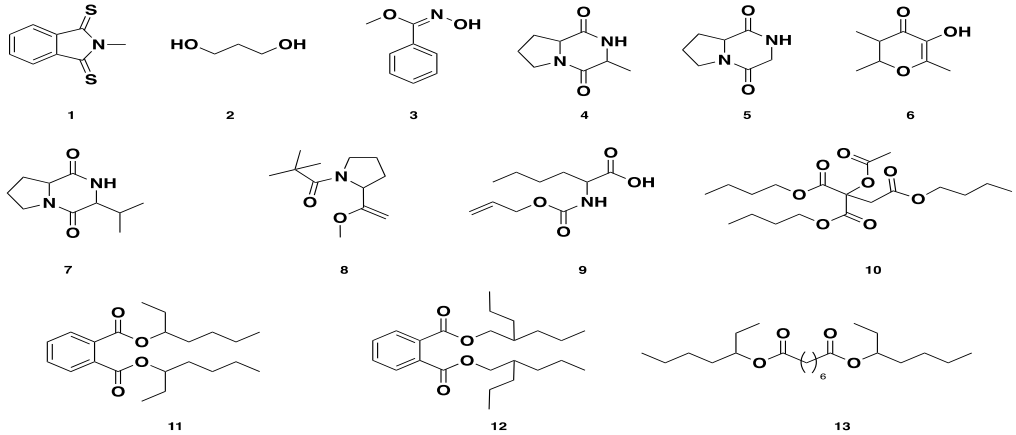

Figure (1): The structure of identified compounds 1-13.

Table (1) : Identified volatile compounds from *Lysinibacillus* MF5 via GC-MS analysis.

Peak	Compound	Formula	R. T	Peak area %	Quality
1	2-methylisoindoline- 1,3-dithione	$C_{10}H_9NS_2$	3.13	3.30	83
2	Propane 1,3- diol	$C_3H_8O_2$	3.91	7.59	72
3	Methyl (Z)-N-hydroxybenzamide	$C_8H_9NO_2$	5.08	0.80	83
4	3-methylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_8H_{12}N_2O_2$	25.58	0.30	52
5	Hexahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_7H_{10}N_2O_2$	25.93	1.05	95
6	3-Hydroxy-2,5,6-trimethyl-4-H-pyran-4-one	$C_8H_{10}O_3$	26.88	0.12	47
7	3-isopropylhexahydropyrrolol[1,2-a]pyrazine-1,4-dione	$C_{10}H_{16}N_2O_2$	27.40	0.41	64
8	1-(2-(1-ethoxyvinyl)pyrrolidin-1-yl)-2,2-dimethylpropan-1-one	$C_{13}H_{24}NO_2$	29.37	0.71	53
9	2(((allyloxy)carbonyl)amino) hexanoic acid	$C_{10}H_{18}NO_4$	33.82	3.20	47
10	Tributyl 2-acetoxypropane- 1,2,3-tricaboxylate	$C_{20}H_{34}O_8$	34.96	0.3	64
11	Di(octan-4-yl) phalate	$C_{22}H_{34}O_4$	38.70	0.22	91
12	Bis(2-propylpentyl)phthalate	$C_{24}H_{38}O_4$	39.66	69.16	90
13	Bis(2-ethylhexyl) decanedioate	$C_{26}H_{50}O_4$	43.12	0.21	84

2. Effect of the used bacterial isolates on *Tetranychus urticae* population and on the response of common bean to *Tetranychus urticae* infection:

The data represented in Figure (2) showed the foliar spray with both bacterial isolates led to a significant decrease in the number of *T. urticae* individuals/ plant leaf by almost 37% after 3 days of treatment, however, the reduction percent was 89% in case of Abamectin. The number of individuals after 7 days was still significantly lower than that of the control in response to bacterial treatments although the reduction percent was reduced to at least 15.25% after 7 days of treatment compared with 86% in case of Abamectin treatment. After 14 days, there was almost no significant difference between water treatment and bacterial treatments at the time which Abamectin treatment still able to significantly decrease individual populations (70%).

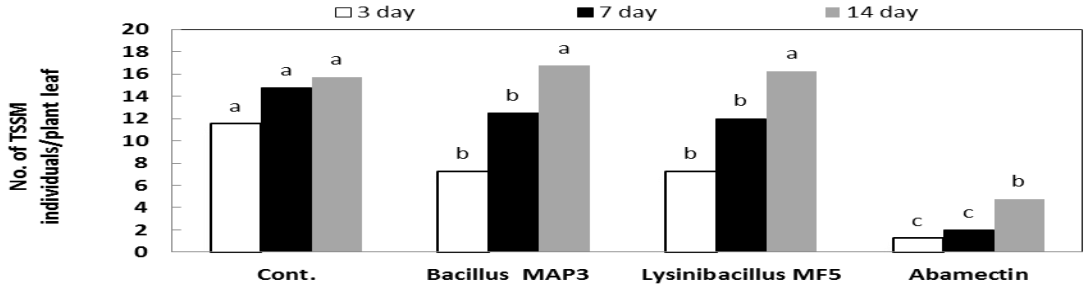

Figure (2): The effect of the foliar spray of both bacterial isolates in addition to the insecticide Abamectin to act as a positive control and water (Cont.) to represent negative control on the number of *Tetranychus urticae* individuals/ plant leaf.

The data represented in Figure (3) shows that the amounts of total carbohydrates and total proteins as a response to bacterial treatments were significantly more than that of the negative control. The same was observed in response to Abamectin treatment and there was no significant difference compared to bacterial treatments. The total chlorophyll was significantly higher in response to both bacterial treatments compared with water treatment and Abamectin.

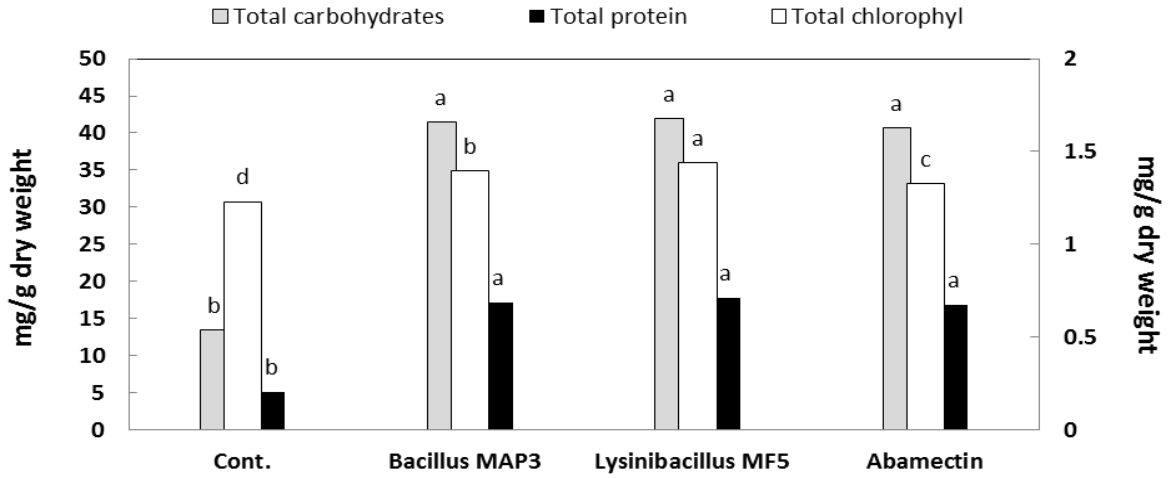

Figure (3): The effect of the foliar spray of both bacterial isolates in addition to the insecticide Abamectin to act as a positive control and water (Cont.) to represent negative control on plant total soluble carbohydrates, total protein and total chlorophylls. The values of total chlorophylls are represented according to the scale in the secondary axe.

In this study, the used bacterial isolates, with phylogenetic relevance to *Lysinibacillus sphaericus* and *Bacillus amyloliquefaciens*, were selected due to their significant effect on several pests. *Lysinibacillus sphaericus* has been regarded as a plant growth promoting bacteria as well as its ability to act as a bio-control agent against several phyto-pathogenic fungi (Naureen *et al.*, 2017). It also could be used as insect pathogen (Berry, 2012). The GC-MS analysis of *Lysinibacillus* MF5 isolate indicated the presence of divers volatile compounds. Among them, the bis (2-propylpentyl) phthalate (12), which represents the major component with 69.16% in addition to its derivative di (octan-4-yl) phthalate (11) that represents 0.22% of the total extract composition. Both compounds and their analogues were found to exhibit several biological activities including antibacterial and antilarval activities (Qi *et al.*, 2009). The observed decrease in *T. urticae* population as a result of *Lysinibacillus* spraying might be attributed to the production of the afromentioned compounds as well as the reported effect of the surface layer protein "S-layer" (Allievi *et al.*, 2014). The reported ability of *Lysinibacillus* to promote plant growth has been observed in the value of the detected metabolites, protein and carbohydrate, and chlorophylls treated with the bacterial isolate while infested with *T. urticae* in comparison with that of the untreated mite-infected plants.

Additionally, *Bacillus amyloliquefaciens* has been regarded as a bio-control agent against phytopathogenic fungi (Danielsson *et al.*, 2007) and bacteria (Wulff *et al.*, 2002). It also has been found to enhance and increase ornamental hosta resistance to insects (Li *et al.*, 2015). It also has been regarded as a plant growth promoting bacteria (Idris *et al.*, 2007 and Nautiyal *et al.*, 2013). The GC-MS analysis performed on our *Bacillus* MAP3 isolate showed the presence of two phthalates derivatives, bis (2- ethylhexyl) phthalate (BEHP) as the major chemical constituent with (81.25%) of the total ethyl acetate extract, along with another regioisomer, phthalic acid, di(oct-3-yl) ester with 0.12%. Both compounds are well-known to exhibit antibacterial and ant larval properties (Qi *et al.*, 2009). The observed decrease in *T. urticae* population might be directly attributed to the effect of the afro-mentioned metabolites of both bacterial strains that have been detected by GC-MS analysis particularly (2-propylpentyl) phthalate and di(octan-4-yl) phthalate for *Lysinibacillus* MF5 and bis(2-ethylhexyl) phthalate (BEHP) and phthalic acid that have been detected to *Bacillus* MAP3.

The activity against *T. urticae* might also be attributed to the surface layer protein "S-layer" as evidenced previously for *Lysinibacillus sphaericus* (Allievi *et al.*, 2014) or indirectly, it might be due to the increase in induced systemic resistance due to the volatile organic compounds produced by *Bacillus* (Farag *et al.*, 2013). The observed increase in carbohydrates, proteins and chlorophylls for plants treated with bacteria, might be attributed to the effect of the used bacterial isolate on plant growth and metabolism. These isolates, for their effect on plant, have been termed as plant growth promoting bacteria (PGPB). They promote plant growth either directly by phytohormones production such as IAA and GA3 (Egamberdieva and Lugtenberg, 2014), ACC deaminase activity that would decrease the level of ethylene in plant tissue to alleviate stress and delay senescence (Penrose and Glick, 2003), siderophore production to accumulate and make iron availability to plant (Bashan and De-Bashan, 2005) and phosphate solubilization to make phosphate in the available form to plant (Rodríguez *et al.*, 2006) or indirectly via pathogen control.

It is concluded that the data of this study are considered as a result that describing the use of bacterial pesticides in *T. urticae* management. Up to our knowledge, it might be the first work to describe such interaction although the results gave the

impression that the bacterial pesticide used in this study need to be formulated and developed to be more efficient against *T. urticae*.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Haemocytes and biochemical changes in *Locusta migratoria* (Orthoptera: Acrididae) after treated with some essential oils

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Abstract:

The migratory locust**,** *Locusta migratoria* L. (Orthoptera: Acrididae) is the most widespread species throughout different parts of the world. It feeds on grass and often causes serious damage to agricultural crops. The present study deals with the effect of essential oils of garlic, cumin and basil against the migratory locust, *L. migratoria* in terms of percent mortality. Total Haemocyte Count (THC) and Phenoloxidase (PO), Acid Phosphatase (ACP) and Alkaline Phosphatise (ALP) activity. The effect of different concentrations (0.1, 0.5 and 1%) of essential oils were evaluated on last nymphal instar of L. *migratoria*. The results clearly demonstrated that the tested oils (garlic, cumin and basil) had stomach toxicity through the nymphal feeding on treated diet. the mortality percentages were estimated that reached to 67.86, 48 and 37.93 % in one day old nymph treatment with basil, garlic and cumin oils respectively after ten days of treatment with concentration (1%), The LC_{50} of different oils varied from one to other, the best treatment among all the tested oils was Basil oil with LC_{50}(0.45%), it was more toxic as stomach poison than garlic oil with LC_{50} (1.31%), finally cumin oil with LC_{50} (3.07%). THC increased after 1 and 2 days while decreased after 3 and 4 days after treated with basil essential oil compared to control. Garlic and cumin essential oils showed decreased the (THC) after 2, 3 and 4 days in the same time occurred increase in THC after 1 day compared to control. The insect enzymes PO, ACP and ALP activities were affected fluctuated between increasing and decreasing 2, 4, 6 and 8 days after treatment with garlic, cumin and basil (at LC_{50} value). ACP activity and PO activity were dramatically increased at 2^{nd} and 4^{th}in all treatments and decreased at 6^{th} and 8^{th} days comparing with untreated nymphs. Also, highly significant decline in ALP were recorded in them by garlic and cumin during the study.

Introduction

The migratory locust, *Locusta migratoria* L. (Orthoptera: Acrididae) is greatly distributed in the old world (Uvarov, 1977). It feeds on grass and often raises dangerous damage to agricultural crops (Pener and Simpson, 2009). Population densities increases and nymphs start aggregating (Tanaka and Nishide, 2012). The

main outbreak area in Africa of the *L. migratoria* is existing on flood plain of Niger River in Mali. The huge plague (1928-1934) was started in this region and spread towards the south of the continent. Two other probable outbreak areas are detected north of the Equator: The Blue Nile region in Sudan and the Lake Chad basin. Grangerisation and population in these two areas are less favourable and have never induced a plague (Lecoq, 1991). Around the suitability of the Lake Chad basin area appeared some doubt about the full development of the gregarious phase (Davey and Johnston, 1956). However, sometimes local upsurges occur and are so dangerous that wide areas have to be sprayed for crop protection. The major classes of insecticides in the field of pest control in use today are organophosphates and carbamates (Ware, 1982 and Dorow, 1993). Because of the serious side effects on the environment and human health of these insecticides, alternative agents are being examined for the insect pest control (Franzen, 1993). Botanicals are a hopeful source of pest control compounds. Over 2000 species of plants are known today to possess some insecticidal activity (Jacobson, 1989). Botanicals considered friendly way to environment be used in pest management, but effort has not been made yet to use them as the possible alternatives for the management of pests (Khanikor and Bora, 2012). Essential oils are complex compounds, volatile, natural, distinguished by a strong odour and are formed as secondary metabolites by plants. In nature, essential oils play a necessary role in protection of the plants as antiviral, antifungal, antibacterial, insecticides, and also against herbivorous by reducing their appetite for such plants. (Bakkali *et al*., 2008). Essential oils which are extracted from aromatic plants are used to control insect pests. These essential oils are investigated and will be documented (Isman, 2006; Koul *et al*., 2008 and Rajendran and Sriranjini, 2008). Insect haemocytes are part of immune response in invertebrates in case presence of foreign material, toxin and microorganisms (Gupta, 1991 and Pathak, 1993) and the response is showed in terms of phagocytosis in case of small sized particle and in case of large sized material and pathogens is encapsulation and nodulation (Gillespie and Kanost, 1997). In addition, Acid Phosphatase (ACP) and Phenoloxidase (PO) are two important protein molecules involved in insect immunity (Pathak, 1993). Soltan (2014) showed decreased Total Haemocyte Count (THC) by Neem on the 5thnymphal instar of *Schistocerca gregarea*. PO is required in sequential conversion of dopa into melanin; it is existing in cuticle, haemolymph and oenocyte, and thus helps in the fight against nonself. If phenoloxidase plays paramount role in development of resistance against certain insecticides (Liu *et al*., 2009) or can work as indicator for immune competence in host-parasite model system, as a marker for evaluating toxic action may be used PO activity. Therefore, the possible effect of the plants in question were attempted to be evaluated through studies on effect of the plant's essential oil on percent mortality, THC and PO, ACP and ALP enzymes activity.

Materials and methods

Experimental Insect:

The migratory locust was used as an experimental insect in thiswork. These nymphs obtained from department ofLocust and grasshopper, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. Insects were reared in wooden formed cages measuring: 60 cm length x 60 cm Width x 70 cm height, with a small door in the front side to facilitate daily routine work. An electric lamp (100 watt) was adjusted to maintain a continuous photoperiod of 12 L: 12 D in each cage as well as in order to maintain an ambient temperature of 32±2 ºC. The insects werehandled andreared under the crowded conditions outlined by Hunter–Jones (1961). The dead locusts, faeces and food remains dailywere removed before introducing the freshly food. Berseem, *Alexandranium*

trifolium fresh leaves, in winter, and the leaves of leguminous plant *Sesbaniaaegyptiaca*, in summer, were used as a food for insects. On the other hand, during the experimental work the Berseemleaves only were introduced as food for insects.

1. Essential oils:

Three essential oils:

Garlic: *Allium sativum L.* (Family: Liliaceae)
Cumin: *Cuminumcyminum L.* (Family: Apiaceae)
Basil: (*Ocimumbasilicum L.*) (Family: Lamiaceae)
Were obtained from EL-Captain Company, elcaptain@elcaptain Co., Al-Obour city (Cairo), Egypt.

2. Nymphal treatments:

Leaves of Berseemwere dippedfor three minutes in three different concentrations of essential oils Garlic, Cumin and Basil (0.1, 0.5, and 1.0 %) andwere allowed to dry before offering to the newly moulted fifth nymphal instar of *L. migratoria*. A day after treatment, treated and untreated nymphs were provided with untreated fresh leaves. Three replicates (ten nymphs for replicate) were used for each concentration. After 24 hrs. from treatment all mortalitieswere recorded to treated and control insects, this data were summarized as estimates of the Median Lethal Concentration (MLC). LC_{25}, LC_{50} and LC_{90} values were calculated by using Lpd line software for calculating and drawing the mortality curve according to Finney Method (1971).

3. Total haemocyte count:

Ninety nymphs treated with our essential oils concentrations samples of the haemolymph were taken at different intervals of 1, 2, 3 and 4 days after treatment. After the flowing of haemolymph, it was quickly drawn up to the "0.5" mark in a Thoma white blood cells diluting pipette. The haemolymph was then diluted to the "II" mark with Tuerks solution (1.5% glacial acetic acid containing few drops of genetian violet) with shaking for 1 min. The diluted haemocytes were counted by a haemocytometer of improved nebauer chamber in four corners squares multiplied by 50 to give the number of cells per cm^3 (Wintrobe, 1974).

4. Sample collection and preparation:

One hundred and Fifty treated nymphs were divided into three replications. Nymphs were kept in cages (25 x 25 x 60cm) with a fluorescent lamp as a light source. The control insects were placed in other cages (Robert *et al.*, 2002). Samples of the haemolymph were taken at different intervals of 2, 4, 6 and 8 days after treatment. The haemolymph was collected through a fine puncture in the hind leg membrane and transferred into clean dry centrifuge tubes. A known volume of the collected haemolymph was centrifuged on 13000 rpm to 15 min. to remove blood cells and pigments. Then the supernatant collected for analyses (El Gawhary, 1997).

5. Phenoloxidase determination:

Determination of phenoloxidase activity was based on a method described by Ishaaya (1972) with some modification. The reaction mixture consisted of 200 µl enzyme solution, 2ml phosphate buffer (0.2 M, pH 7) and 0.5 µl 2 % Catechol. The reaction mixture was incubated at 25 ˚C. The activity was then recorded after 2 min from the beginning of the reaction at absorbency 470 nm.

6. Acid and alkaline phosphatises determinations:

ACP and ALP were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown colour is produced. The reaction mixture consists 1 ml citric buffer (pH 4.9), 1 ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml nymphal haemolymph. Mix gently and incubate for exactly 30 minutes at 37 ºC. At the end of incubation period, 0.8 ml of NaOH was added to stop the reaction. Then added 1.2 ml of $NaHCO_3$, followed by 1 ml of 4-

aminoantipyrine solution and 1 ml of potassium ferrricyanide. The produced color was measured, immediately, by spectrophotometer at 510 nm. The enzymatic activity is expresses as mg phenol released/ ml haemolymph. Phenol standard curve was prepared as a stock of phenol was prepared by dissolving 1 gm pure crystalline phenol in 1 literHCl. 10 ml of the stock solution (containing 10 mg) was diluted to 100 ml with distilled water. Aliquots of 0.05, 0.1, 0.2, 0.3 and 0.4 ml of the diluted phenol (equal to 5, 10, 20, 30, and 40 mg phenol) were pipetted into test tubes and the volume was completed to 1 ml with distilled water. 1.1 ml of buffer was added followed by 0.8 ml of NaOH, 1.2 ml of NaHCO3, 1ml of aminoantipyrine and 1ml of potassium ferrricyanide. Each tube was mixed well after each addition. The developedcolour was measured at 510 nm.

7. Statistical analysis:

The percentage of nymphal mortality was corrected according to Abbotts formula (Abbott, 1925)

Corrected % = {1 – (n in T after treatment/ n in Co after treatment)} *100

Where: n = Insect population, T = treated, Co = control.

LC_{25}, LC_{50}, LC_{90} values and slope of regression lines were calculated by using (Lpd line) software for calculating and drawing toxicity lines according to Finney Method (1971).

Other Data were analyzed by analysis of variance (ANOVA) means, within row, bearing different subscripts are significantly different ($P<0.05$) by SPSS Program software (SPSS,2009).

Results and Discussion

1. Effectiveness of basil, cumin and garlic on *Locusta migratoria* by feeding technique:

1.1. Effectiveness of Basil against 5th nymphal instar of *Locusta migratoria*:

Results in Table (1) showed effect three concentrations of basil essential oil (1.0, 0.5 and 0.1%) on the percentages mortality of 5th nymphal instar of *L. migratoria* from one to ten days. Data cleared that the percentages of mortality of the 5th nymphal instar were 33.33,41.38 and 67.86% after 10 days at concentration of 0.1, 0.5 and 1 %, respectively. Figure (1) and Table (2) appeared the lethal concentration values (LC_{25}, LC_{50} and LC_{75}) from basil essential oil were 0.06, 0.45 and 3.21 respectively.

Table (1): Mortality % of 5th nymphal instar of *Locusta migratoria* after treated with different concentrations of basil.

days	1% v/v					0.5%v/v					0.1% v/v				
	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	10	10	10	10	10	0	10	6.67	6.67	0	0	0	0	0
3	20	10	20	16.67	16.67	10	10	10	10	10	0	0	0	0	0
4	20	20	20	20	17.24	10	10	10	10	10	0	0	0	0	0
5	30	20	30	26.67	24.14	10	20	20	16.67	16.67	10	10	10	10	10
6	40	30	40	36.67	34.48	20	20	20	20	20	10	10	10	10	10
7	40	40	40	40	37.93	30	30	20	26.67	24.14	20	20	10	16.67	16.67
8	50	50	40	46.67	44.83	30	40	30	33.33	31.03	20	30	20	23.33	23.33
9	60	50	60	56.67	55.17	30	40	40	36.67	34.48	20	30	20	23.33	23.33
10	70	70	70	70	67.86	40	50	40	43.33	41.38	30	40	30	33.33	33.33

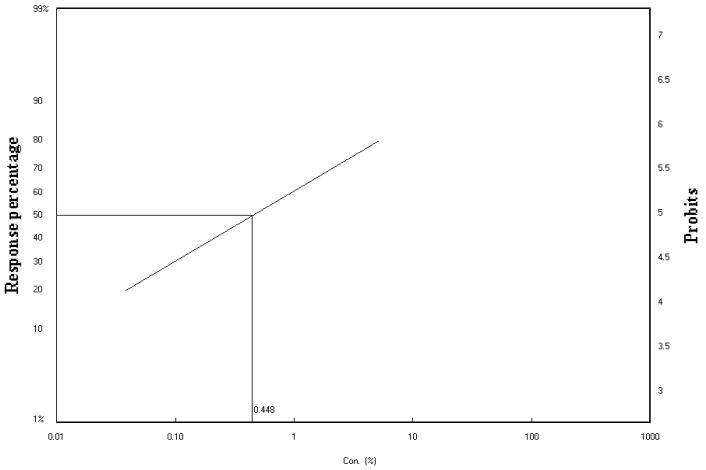

Figure (1): The Lethal Concentration (LC) values of basil by feeding technique on *Locusta migratoria.*

Table (2): The Lethal Concentration (LC) values of basil by feeding technique on *Locusta migratoria*.

LC	Con. %	Lower limit	Upper limit
25	0.06	0.0268	0.1371
50	0.45	0.2974	0.6632
75	3.21	1.3006	8.1479
90	18.92	4.0622	94.1268
95	54.69	7.928	412.313
99	400.36	27.5481	640.197

1.2. Effectiveness of cumin against 5th nymphal instar of *Locusta migratoria*:

Results in Table (3) cleared the percentages of mortality to 5th nymphal instar of *L. migratoria* after treated nymphs with three concentrations of cumin (1.0, 0.5 and 0.1%) from one to ten days were 37.93, 24.14 and 13.79% respectively, on other hand, data in Table (4) and Figure (2) showed the LC values (LC_{25}, LC_{50} and LC_{75}) of cumin against *L. migratoria* after ten days from treatment were 0.39, 3.07 and 23.75%.

Table (3): Mortality % of 5th nymphal instar of *Locusta migratoria* after treated with different concentrations of cumin.

Days	1% v/v					0.5%v/v					0.1% v/v				
	r1	r2	r3	mean	corrected	r1	r2	r3	mean	corrected	r1	r2	r3	mean	corrected
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	10	3.33	3.33	0	0	0	0	0	0	0	0	0	0
3	0	0	10	3.33	3.33	0	0	0	0	0	0	0	0	0	0
4	0	0	10	3.33	3.33	0	0	0	0	0	0	0	0	0	0
5	10	10	10	10	10	0	10	0	3.33	3.33	0	0	0	0	0
6	10	20	10	13.33	13.33	10	10	10	10	10	0	0	0	0	0
7	20	20	20	20	17.24	10	20	10	13.33	13.33	0	0	10	3.33	3.33
8	20	30	20	23.33	20.69	10	20	20	16.67	16.67	0	10	10	6.67	6.67
9	30	40	30	33.33	31.03	20	20	20	20	17.24	10	10	20	13.33	13.33
10	40	40	40	40	37.93	20	30	30	26.67	24.14	10	20	20	16.67	13.79

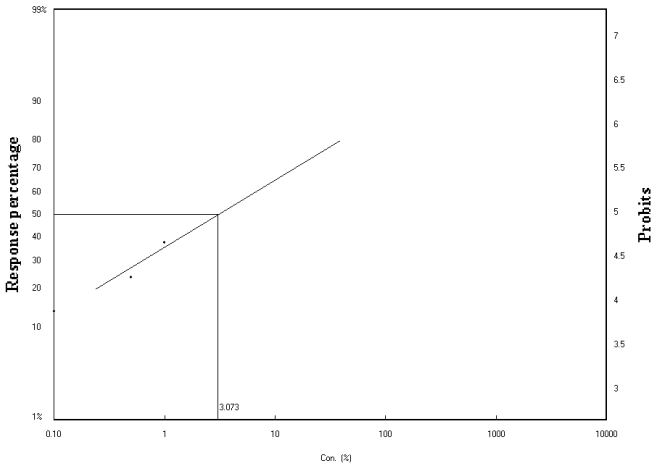

Figure (2): The Lethal Concentration (LC) values of cumin by feeding technique on *Locusta migratoria*.

Table (4): The Lethal Concentration (LC) values of cumin by feeding technique on *Locusta migratoria*.

LC	Con. %	Lower limit %	Upper limit %
25	0.39	0.22	0.67
50	3.07	1.41	29.54
75	23.75	5.68	2067.39
90	149.58	19.33	97619.22
95	449.96	40.04	984400.3
99	3550.37	156.30	75375153

1.3. Effectiveness of Garlic against 5th nymphal instar of *Locusta migratoria*:

Data presented in Table (5) showed the percent reduction of population of *L. migratoria* after ten days from treatment with three concentrations of garlic, this reduction was dose dependent. The percent reduction at 1 % was 48% but at 0.5% was 33.33% finally at 0.1% was 17.86%. Results in Table (6) and Figure (3) showed the LC values and the effect of three concentrations of garlic against *L. migratoria*, where LC_{25} , LC_{50} and LC_{75} were 0.21, 1.31 and 8.16, respectively.

Table (5): Mortality % of 5th nymphal instar of *Locusta migratoria* after treated with different concentrations of garlic.

days	1% v/v					0.5%v/v					0.1% v/v				
	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	0	10	6.67	6.67	0	0	10	3.33	3.33	0	0	0	0	0
3	20	10	10	13.33	10.34	10	10	10	10	6.9	0	0	0	0	0
4	20	10	20	16.67	13.79	10	10	10	10	6.9	0	0	0	0	0
5	20	20	20	20	14.29	10	20	20	16.67	13.79	0	10	0	3.33	3.33
6	30	20	30	26.67	21.43	20	20	20	20	14.29	10	10	10	10	6.9
7	40	30	30	33.33	25.93	30	20	20	23.33	17.86	10	10	10	10	6.9
8	40	40	30	36.67	26.92	30	30	30	30	25	20	10	10	13.33	10.34
9	50	40	40	43.33	34.62	30	30	30	30	25	20	10	20	16.67	13.79
10	60	60	50	56.67	48	50	40	30	40	33.33	30	20	20	23.33	17.86

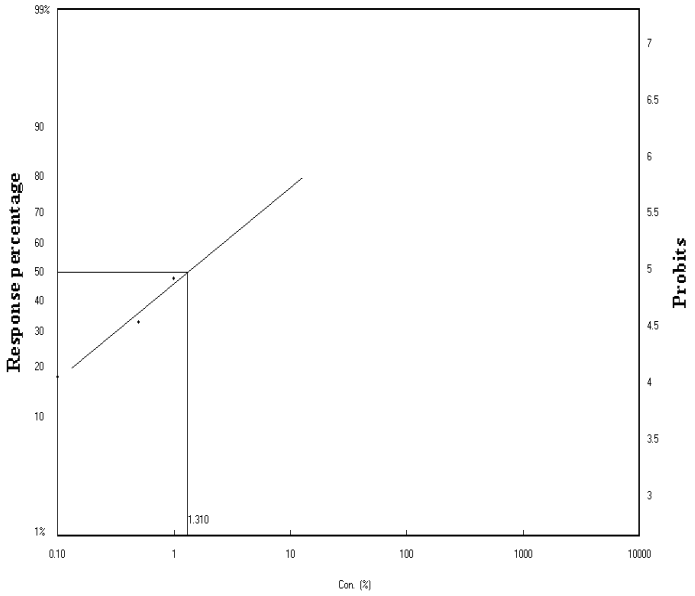

Figure (3):The Lethal Concentration (LC) values of garlic by feeding technique on *Locusta migratoria.*

Table (6): The Lethal Concentration (LC) values of Garlic by feeding technique on *Locusta migratoria*.

LC	Con. %	Lower limit %	Upper limit %
25	0.21	0.098	0.32
50	1.31	0.797	3.76
75	8.16	3.08	92.60
90	42.34	9.85	1748.95
95	113.39	19.63	10210.99
99	719.52	71.16	280839.4

Our results showed that out of the three essential oils of basil, garlic and cumin was the most toxic. The results reflected higher toxicity of basil essential oil against last nymphal instar at the dosages of 1.0, 0.5 and 0.1 % resulted in 67.86, 41.38 and 33.33% mortality of nymphs at 10 days of treatment while LC_{50} was 0.45%, and the order of toxicity of these three oils can be shown as cumin < garlic < basil. The study revealed that last instar nymphs of *L. migratoria* were susceptible to the action of essential oils basil, garlic, where were resistant and could survive after exposure to essential oil cumin treatments. Such resistance of last nymphal instar of *L. migratoria* to the essential oil under consideration may be attributed to age dependent changes in bio constituents. Insecticides upon entering insect body may be acted upon by detoxifying enzyme system for detoxification before they reach the site of action. Age dependent changes in enzyme activity and their subsequent correlation with insecticide toxicity have been reported for several insects with respect to cyto-chrome P450, glutathione S-transferase, malathioncarboxylesterase and microsomal oxidase enzyme (Yu, 1983; Gui *et al*., 2009; Lee *et al*., 1996 and Rajatileka *et al*., 2011). After application of essential oils, values of LC_{50} was considered for study in order to assess response of immune system of *L. migratoria* in terms of total haemocyte count and phenoloxidase, acid phosphatase and alkaline phosphatase enzymes activities.

2. Determination of total haemocyte count on *Locusta migratoria*:

After application of essential oils (Table,7), values of LC_{50} was considered for study in order to assess response of immune system of *L. migratoria* in terms of total haemocyte count and phenoloxidase, acid phosphatase and alkaline phosphatase enzymes activities. On application of essential oil of basil concentration of 0.45% on *L. migratoria* nymphs, THC significantly increased at 1st day (4600±50) which then came down to the level of control (2900±43.3, 2800±90.1 and 2100±25) at 2nd, 3rd and 4th days. On application of 1.31% garlic oil, THC increased significantly at 1st day (5400 ±300), but THC decreased significantly from 2nd day till 4 day (2800 ±25, 1800 ±132.3 and 1380 ±147.3). Application of 3.07% of essential oil of

cumin initially increased THC level at 1st and 2nd days (6800 ±180.3 and 4300 ±86.6) and then significantly decreased at 3rd and 4th days (1750 ±180.3 and 930 ±60.8).

THC is correlated with the rate of phenomena occurring during insect immune response such as phagocytosis, nodule formation, encapsulation, recognition of foreign bodies and wound healing and hence the total number of haemocytes reflect the involvement of immune system to deal with pathogens or chemical molecules (Kraaijeveld *et al*., 2001). In the present study, increase of THC at 1st day of treatment at essential oils, basil, cumin and garlic might reflect activation of immune response. Speedy haemocyte division to facing with the foreign particles may increase THC. The rise and down of THC level which take place on application of essential oil might indicate an active involvement of the defence system to outdo the toxic action in which the haemocytes in the circulation have been used up for the aim of defence. At concentration (LC_{50}) of three essential oils, although at 24 h post treatment THC was high in cases of all the oil, at 48 h in response to action of cumin THC increased. Post treatment of insecticides initial increase followed by reduction at 2nd - 3rd days were reported by several workers (Sharma *et al*., 2008 and El-Aziz and Awad, 2010). Furthermore, dependent THC on ecdyson titre (Ayyangar and Rao, 1990). Abamectin is an insecticide caused the secretion of antidiuretic hormone from neurosecretory cells of the thoracico-abdominal ganglionic mass that slowed the rate of excretion resulting in increase of blood volume and that way decrease of total haemocyte count (Suhail *et al*., 2007). Reduction of haemocytes may also be due to disaster happened in hematopoietic organs which are responsible for production of haemocytes (Tiwari et al., 2002). Also, application of insecticides occurred decrease in THC after 12 h and 1st day of treatment in other insects (Ayyangar and Rao, 1990 and El-Aziz and Awad, 2010).

Table (7): The effect of basil, garlic and cumin on total haemocyte count of the 5th instar nymphs *Locusta migratoria* (the number of cells per cm^3).

Treatment / Days	Control	Basil	Garlic	Cumin
1nd	44a ±86.6	46b ±50	54b ±300	68c ±180.3
2th	34a ± 173.2	29b ±43.3	28b ±25	43c ±86.6
3th	30.5a ± 100	28a ±90.1	18b ±132.3	17.5c ±180.3
4th	33a ± 50	21b ±25	13.8c ±147.3	9.3d ±60.8

3. Effect of essential oils, basil, garlic and cuminon activity of phenoloxidase (O.D. unit x 10^3/min./ml haemolymph) in 5th nymphal instar of *Locusta migratoria*:

The effect of essential oils, basil, garlic and cumin with their LC_{50} values in Table (8). The PO activity measured during four days after treatment with essential oils. In the second day the PO activity highly increased than the control and in the 4th day the activity was still high but lowers than the first day. The PO activity in treatment showed decreasethan control in the 6th and 8th day in all treatment. Phenoloxidase enzyme plays a paramount role in recognition exotic particles and therefore give immunity against external chemicals and different microorganisms in various arthropods (Shelby *et al*., 2000; Shiao *et al*., 2001; Yu *et al*., 2003; Ling *et al*., 2005 and Zhu *et al*., 2009). Black colour nodules formed in the haemolymph, due to melanin which produced by the enzyme PO. studies appeared that PO was existing in major quantities in the serum than in the haemocytes. (Gillespie *et al*., 2000) tyrosine turns into Dopa by PO enzyme which is used in synthesis of melanin for later utilize in immune response and wound healing (Huang *et al*., 2002). Increase of PO activity has long been correlated with increased resistance (Sugumaran, 2002 and Shelby and Popham,

2006) and decrease of PO activity is attributed to weakening of immune system (Hiromori and Nishigaki, 2001). Prevent phenoloxidase production by locust haemocytes may be as a result of destruction of the cells that produce prophenoloxidse (Said, 2014).

Table (8):Effect of essential oils on activity of phenoloxidase (O.D. unit x 10^3/min./ml haemolymph) in 5th nymphal instar of *Locusta migratoria*.

Days	Control	Basil Oil	Garlic Oil	cumin Oil
2days after treatment	3483.33±35.35a	4250.00±132.29c	4133.33±140.47c	3833.33±90.74bb
4days after treatment	3423.33±68.07a	3863.33±47.26c	3616.67±28.87ab	3663.33±40.41bc
6 days after treatment	3460.00±36.05a	2946.67±45.09bc	3040.00±45.83b	2760.00±52.91c
8 days after treatment	3505.00±5.00a	2906.67±15.27bc	3006.67±15.27b	2750.00±62.45c

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***.*

4-Effect of on activity of Acid andAlkaline phosphatise (IU /ml haemolymph) of the 5th instar nymphs *Locusta migratoria*:

Results presented in Table (9) show that, the ALP activity significant decreased between Garlic oil 13.71±.11, 13.13±0.26, 11.92±0.81 and 9.8633±.08 unit (U) after 2, 4, 6 and 8 days from treatment. Also, in cumin oil significant decreased 10.12±.07, 9.8±.1, 6.27±.15 and 5.11±.08 U after 2, 4, 6 and 8 days after treatment. The ALP activity increased 19.66±.12, 18.98±.1, and 18.02±.01 U by Basil oil after 2, 4 and 6 days while become non-significant 16.9833±.10693a after 8 days compared to control 16.83±.28, 16.82±.11, 16.77±.06 and 16.54±.06 U. Activity of acid phosphatase in haemolymph of *L. migratoria* during treatment with essential oils, basil, cumin and garlic was found in Table (10). Eight days after treatment there was significant differences in ACP activity between Experiment and control. Where, there was a large increase in ACP activityin treated insects on second day and fourth day than become decrease insix and eight day after treatment. Our study showed that the activity of ACP at 2nd and days 4th of treatment fifth nymphal instar of *L. migratoria* treated with 0.45%, 1.31% and 3.07% of Basil, Garlic and cumin essential oils was determined. The tested compounds significantly increased the activity of ACP as compared to the control. The activity of ACP decreased in treated nymphs after day 6 and day 8. All treatments caused increase in ACP activity where, cumin gave the highest increase in ACP activity followed by Garlic than Basil, where these values after 2 day were 13.03±.11, 13.47±.26 and 15.17±.15 IU/l respectively as compared with 10.55±.085 IU/l in the control. ALP and ACP have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Tsumuki and Kanehisa, 1984). Great interest in ALP and ACP during developmental studies and so, because of its association with histolysis. Whereas, ALP and ACP hydrolyzes a diverse of orthophosphorylation reactions (Hollander, 1971). The number of lysosomes increases as a result for Ecdysone (Radford and Misch, 1971). This indicates that the decreased activity of ALP and ACP in this study may be due to decreased number of lysosomes. Sridhara and Bhat (1963) showed that during development the decrease or increase of phosphatases enzyme reflected an increase or decrease in the acid-soluble phosphorus content.

Table (9): Alkine phosphatase activity (IU /ml haemolymph) of the 5th nymphal instar of *Locusta migratoria* after treated with essential oils.

Days	Control	Basil Oil	Garlic Oil	cumin Oil
2days after treatment	16.83±.28284a	19.6600±.12166b	13.7100±.11000b	10.1200±.07211c
4days after treatment	16.8233±.10786a	18.9833±.10408b	13.1300±.02646b	9.8000±.10000c
6days after treatment	16.7667±.05774a	18.0200±.01000b	11.9233±.08145c	6.2667±.15275d
8days after treatment	16.5367±.06351a	16.9833±.10693a	9.8633±.07506b	5.1133±.07767c

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***.*

Table (10): Acid phosphatase activity (IU /ml haemolymph) of the 5th nymphal instar of *Locusta migratoria* after treated with essential oils.

Days	Control	Basil Oil	Garlic Oil	Cumin Oil
2 days after treatment	10.55±.08485a	13.0267±.11150b	13.4700±.25632b	15.1667±.15275c
4 days after treatment	11.0833±.10214a	11.9333±.09452b	12.1000±.20000b	13.9333±.25166c
6 days after treatment	11.1267±.06429a	9.4800±.10817b	10.6100±.11533a	12.0000±36056b
8 days after treatment	10.9900±.19672a	8.6200±.08185b	8.6667±.45092b	9.1767±.13650b

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****

It is concluded that the best treatment among all the tested oils was basil oil then garlic oil finally cumin oil, also all essential oils used effect on immune response in *L. migratoria* nymphs.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Evaluation of natural products on some biological and biochemical parameters of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)

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Abstract:

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera.: Gelechiidae), is considered a major cotton pest. Experiments were conducted to study the effect of some natural products namely *Thuja orintalis*, bug oil, sesame oil and α-pinene on some biological and biochemical aspects of *P. gossypiella* under laboratory conditions of 27±1°C and 70±5% RH. Results revealed that LC_{50s} values were 4.88, 6.29, 3.46 and 1.43 ppm, when newly hatched larvae treated with *Thuja orintalis*, bug oil, sesame oil and α-pinene, respectively. Percentages of accumulative larval mortality were estimated by 61, 68, 66 and 58% for previously mentioned compounds, respectively, compared with 5% in the untreated check. Also, the obtained results showed a prolongation in larval and pupal duration for the tested compounds except bug oil as it recorded 11.8 days for larval duration compared to 14.3 days for the untreated check. In contrast, all treatments caused considerable reduction in both larval and pupal weight, in comparison to the untreated check. According to the adult stage, results indicated high reduction in total eggs laid estimated by 140.3, 111, 67.66 and 157 eggs/female for *Thuja orintalis*, bug oil, sesame oil and α-pinene respectively, compared to 253 for the untreated check. Percentage of hatchability was reduced to reach 72, 73.6, 62.3 and 81% for the same compounds respectively compared to 96% in untreated check.

Introduction

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera.: Gelechiidae) (PBW) is worldwide distributed and is considered one of the most danger pests attack cotton cultivating in some countries. In Egypt, a very high rate of infestation by this pest on cotton fields is recorded since it has been discovered by Willcocks (1916).

Using different pesticides for controlling this pest on cotton resulted many of side effects such as; air, water and soil pollution, in addition to residue toxicity on crops, moreover, pest resistant to various classes of pesticides. Besides, pesticides disturb the natural balance between pests and their natural enemies.

Natural plant extracts or oil extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non-target organisms (Sharma *et al.*, 2006). There are more than 2400 plant

species belonging to 189 plant families which are considered as rich sources of bioactive organic compounds (Rao *et al.*, 2005). In Egypt, several studies were done on plant or oils extracts to evaluate the effect on various economic pests such as *Pectinophora gossypiella*, (Reda *et al*., 2014), *Agrotis ipsilon*, (Abd El-Zaher, 2005), *Bemisia tabaci* and *Empoasca discipiens*, (Salem *et al.*, 2003) in addition *Sesamia cretica* and *Ostrinia nubilalis* (Yacoub, 2006). Gaaboub *et al.* (2012) mentioned that jojoba oil proved efficiency against the 2nd and 4th instars larvae of *S. littoralis* after 24 hrs. of treatment. Bug oil has a repellent effect on pests till 21 days; it kills all the mobile stages of the pests and acts on the respiratory system of the pest in addition to a repellent effect. Sesame is a widely grown food plant producing both eaten seeds and oils. Most of plant parts are used as pesticides. Sesame oil also can be used as an insecticide with suffocating and synergistic modes of action. Sesame oil was first reported as a synergist with the botanical insecticides pyrethrin on the common housefly, *Musca domestica* (Eagleson, 1940). Alpha pinene is highly repellant to insects and is widely found in the oils of many species of many coniferous trees. Pinene is found in many essential oils as a main component.

The aim of the present work was to study the evaluation of some natural products activity on some biological and biochemical aspects of *P. gossypiella*.

Materials and methods

1. Insect used:

***Pectinophora gossypiella*:** The newly hatched larvae of *P. gossypiella* PBW susceptible laboratory strain used in these experiments were reared in the laboratory on semi-artificial diet according to methods described by Rashad and Ammer (1985).

2. Materials used:

2.1. *Thuja orintalis* leaves extract

-Name: *Thuja orintalis.*

-Active Ingredient: *α*-pinene (49.3%), *β*-phellandrene (9.6%) and *α*-cedrol (8.2%) and others in small amounts.

2.2. Bug oil

- contact insecticide and acaridae (EC) compositions; vegetables oils

-Active Ingredient: Tagetes erecta oil 0.75%, Thymus vulgaris oil, 0.75%, Nonyl phenol, 6.0% and Rapeseed oil, 92.5%

2.3. Sesame oil

- Name: Sesame Oil

-Active Ingredient: linoleic acid (41% of total), oleic acid (39%), palmitic acid (8%), stearic acid (5%) and others in small amounts.

-Other Names: Sesame, Oil of Sesame, Ground Sesame Plant, Sesame Oil, Benne Oil, Benniseed, Gingilli Oil, Sextra, Teel Oil, Sesame indicum L.

2.4. α-pinene

-Chemical Names: ALPHA-PINENE; α-pinene; 2-Pinene; 80-56-8; .alpha.-Pinene; Acintene A. -Molecular Formula: $C_{10}H_{16}$

-Molecular Weight: 136.238 g/mole

3. Extraction method:

The experiments plant extract of *Thuja orientals* (Fam: Cuprssaceae) was prepared in petroleum ether as described by (Abd El-Zaher, 2005) and then the essential oil (α-pinene) was obtained using Gas Chromatography and then kept in refrigerator at 4ºC to be used in the present studies.

4. Laboratory experiments:

4.1.Toxicological studies:

Pilot experiment was conducted to evaluate the LC_{50} values of *Thuja orintalis*, seasam oil, Bug oil and Alfa-pinene against newly hatched larvae of *P. gossypiella*. Serial concentrations of 20, 10, 5, 2.5 and 1.25% for *Thuja orintalis*, 10, 5, 2.5, 1.23 and 0.61 % for each of bug oil and seasam oil and 3, 2, 1, 0.5 and 0.25 % for Alfa pinene were freshly prepared. Three replicates of 20 tubes, each tube (2 X 7.5 cm) containing 3 gm. of an artificial diet (described by Rashad and Ammar, 1985) for each concentration was used. Newly hatched larvae of the pink bollworm were placed on diet surface (a larva/ tub) treated with 1.0 ml of the tested preparation. An equal number of maintained larvae were used as untreated check and placed on the surface of the diet treated with

water only. After 24 hrs larval mortalities were recorded and corrected according to Abbott's (1935). LC_{50} and LC_{95} Values of the tested compounds were calculated according to, Finney (1971). According to (Sun, 1950) toxicity index can be calculated:

Sun's toxicity index = (LC_{50} or LC_{90} of the most toxic compound/ LC_{50} or LC_{90} of the tested compounds) x 100.

4.2. Biological studies:

For some biological studies, the $LC_{50,}$ values of the four tested compounds were used against the on newly hatched larvae of *P. gossypiella*. LC_{50} value of each compound was spread on the upper surface of the diet poured in the glass tubes, while the untreated check was applied with distilled water only. Three replicates of 50 tubes, each tube (2 X 7.5 cm) containing 3 gm. of diet were used for each compound in addition to other three replicates for the untreated check. Three replicates of newly hatched larvae of *P. gossypiella* (each of 50 larvae) for each treatment were transferred individually to the diet tubes by camel hair brush, capped by cotton wool, kept in incubator under the control conditions and inspected daily until pupation. Larval and pupal durations, weight and pupation percentage were estimated in addition to adult emergence percentage. Resulted adults from different treatment were sexed in 15 pairs (male X female) divided into three replicates; each replicate (5 malesX5 females) in cages, the top and bottom of each cage were covered with screening mesh kept in position by rubber bands for stimulating eggs laying response in the females. Cages for each treatment were examined daily to estimate the pre-oviposion, oviposition and post-oviposition periods, females and males longevity, number of eggs laid in addition to percent of hatchability.

Sterility and corrected sterility percentages can also be calculated according to Tapozada *et al.* (1966).

% Sterility observed = 100 – Egg hatchability percentage

$$\%\ control\ of\ Sterility = \frac{\%\ Sterility\ observed - chek}{100 - chek} \times 100$$

4.3. Biochemical studies:

Preparation of insects for analysis: The treated larval samples were homogenized in distilled water. The homogenates were centrifuged at 8000 r. p. min. at 5^0C in refrigerated centrifuge. The supernatants were kept in deep freezer at 20^0C till use for biochemical assays.

-Total protein content of whole homogenate was measured according the method described by Bradford (1976).

-Total lipids were determined by colorimetric method described by Knight *et al.* (1972).

-Total carbohydrates were estimated colorimetric method described by Singh (1977).

5. Statistical analysis:

The obtained data were statistically analyzed using F-test at 0.05 of probability according to Costat statistical program to calculate LC_{50} and LC_{95} values, the data were analyzed using probit (proban) analysis software according to Finny (1971). The toxicity index was also calculated according to Sun (1950).

Results and Discussion

1.Toxicity of *Thuja orintalis*, bug oil, sesame oil and α-pinene against newly hatched larvae of the pink bollworm, *Pectinophora gossypiella*:

Results presented in Table (1) showed that α-pinene had the most potent compound against neonate larvae of PBW (LC_{50}= 1.43%), followed by sesame oil (LC_{50}= 3.46%), *Thuja orintalis (*LC_{50}= 4.88%), and bug oil (LC_{50}= 6.29%). Similar trend was appeared where LC_{95}values were 8.84, 33.5, 14.08 and 52.76 %, respectively. It could be mentioned that newly hatched larvae of PBw were the most susceptible for α-pinene than the other different compounds. The toxicity of these natural oils against newly hatched larvae go in the same trend with obtained results of Viitanen *et al.* (2000) who recorded that the toxicity of tar oil against both eggs

and newly hatched larvae of pink bollworm is due to its contents of phenols, creosote and anthrathane. Also, Sharaby *et al.* (2012) who recorded the toxic effect of three different natural essential oils of medicinal plants, namely garlic (*Allium sativum),* mint (*Mintha pipereta)* and eucalyptus (*Eucalyptus globulus)* against the 1st nymphal instar of the grasshopper (*Heteracris littoralis)*. The toxicity index is used in our study as a simple method in comparing the degree of toxicity of tested compounds in comparison to the most toxic one included in the evaluation in the study. As shown in Table (1) α-pinene is the most toxic compound and accordingly the toxicity index values of *Thuja orintalis*, bug oil and Sesame oil compounds were 29.30, 22.74and 41.33 respectively as effective as α-pinene for treated larvae.

Table (1): Toxicity of some potential extracts against newly hatched larvae of the pink bollworm, *Pectinophora gossypiella*.

Compounds used	LC_{50} (%)	LC_{95} (%)	Slope	Toxicity index based on lc_{50}
Thuja orintalis	4.88	41.08	1.77± 0.15	29.30
Bug oil	6.29	52.76	1.78± 0.15	22.74
Sesame oil	63.4	33.5	1.66± 0.20	41.33
α-pinene	1.43	8.84	2.08± 0.23	100

2. Biological studies:

2.1. Effect of some potential extracts on larval, pupal and adult emergence percentages of *Pectinophora gossypiella* treated as newly hatched larvae:

2.1.1. Larval mortality and malformation:

Data presented in Table (2) indicated the percentage of larval mortality treated as newly hatched larvae by LC_{50} values of *Thuja orintalis,* bug oil, Sesame oil and α-pinene. According to the obtained results, bug oil was the most effective one compared with other treatments. The percent of accumulative larval mortality (up to 20 days) estimated by 61, 68, 66 and 58 for treatment with previously mentioned compounds respectively, compared with 5% in the untreated check. In addition, a percent of malformed larvae was appeared as a result of treatments with most efficient product namely *Thuja orintalis,* wher the rat*e* of malformed larvae was (9%) followed by Alfa pinene (7 %) compared with (3and 5%) for bug oil and Sesame oil respectively, and it was 1% in the untreated check. The larvae were very small dwarfed with dark thorax and compressed abdomen turned to darken just after death. The small size of treated larvae may be explained as a result of antifeedent and repellent effect of these oils which lead finally to larval mortality, as recorded by Dwivedi and Shekhawat (2004) they reported that leaf extracts of *T. orientalis* were used as repellent agent against *Chilo partellus.* In this field of study, (Abd El-Zaher, 2005) reported that treatment with *Thuja orintalis extract* leads to loss of the eating ability of treated *A. ipsilon* larvae. Larvicidal activities of *T. orientalis* oil against 4th-instar larvae of *Aedes aegypti* and *Culex pipiens* pallens have been observed by Ju-Hyun *et al.* (2005). Results also, are in the same direction with that recorded by (Yacoub, 2006) who revealed that the toxicity and antifeedent effect of jojoba oil as well as growth and development inhibitor. In addition, Shukla *et al.* (2005) evaluated the inhibitor activity of soybean trypsin and plant lectins against *Helicoverpa armigera* in order to identify toxin genes for deployment through transgenic plants. Jain and Sharma (2017) reported high larvicidal activity of *T. orientalis* leaf oil against 4th instar larvae of *Aedes aegypti* and *Culex pipiens* pallens and sufficient repellent action against *Chilo partellus.*

Table (2): Effect of some potential extracts on larval, pupal and adult emergence percentages of pink bollworm. *Pectinophora gossypiella* treated as newly hatched larvae.

Compounds used	Conc. %	larval mortality percentages at indicated days after treatment				Accumulated larval mortality percentage (up to 20 days)	% Malformed larvae	% pupation	% Adults emergence
		(1) day	(3) days	(3) days	(12) days				
Thuja orintalis	4.88	51	5	3	2	61	9	30	73.66
Bug oil	6.29	49	6	4	9	68	3	29	63..6
Sesame oil	3.46	55	1	3	7	66	5	29	78.0
α-pinene	1.43	45	3	6	4	58	7	35	67.3
Untreated check	-	1	1	1	3	6	1	93	96.6

2.1. 2. Pupation:

According to Table (2) treatments highly affected pupation percentage as it reduced to (29%) for both bug oil and Sesame and (30 and 35 %) for *Thuja orintalis* and α-pinene respectively, while it recorded (93%) for untreated check. Oposit results were optained by Gaaboub *et al*. (2012) they recorded an increase in pupation percentage reach to 81.2% for pupae resulted from the 4th instar larvae of *S. littoralis* treated by jojoba oil.

2.1.3. Adult emergence:

As shown in Table (2) treating the newly hatched larvae of the pink bollworm with *Thuja orintalis*, bug oil, Sesame oil and α-pinene decreased the percent of the adult emergence to 73.66, 63.6, 78.0 and 67.3 respectively, compared with 96.6 in the untreated check.

2.2. Effect of some potential extracts on immature duration of *Pectinophora gossypiella* treated as newly hatched larvae:

2.2.1. Larval duration:

Data in Table (3) illustrated obvious increase in larval duration for all treatments as it recorded 21.5 and 17.9 days when newly hatched of *p. gossypiella* treated with LC_{50} of *Thuja orintalis*, Sesame oil, respectively, while it decreased to13.6 and 11.8 day when treated with bug oil and α-pinene, respectively, compared to 14.3 days in the untreated check. In addition, the average larval weight decreased to 0.002, 0.029, 0.018 and 0.032 g/ larva for the previous compounds, respectively, while it was 0.039 g/ larva in the untreated check.

Table (3): Effect of some potential extracts on immature duration of *Pectinophora gossypiella* treated as newly hatched larvae.

Compounds used	Conc. %	Larval stage		Pupal stage		Total immature duration
		Duration (days)	Weight (g/larva)	Duration (days)	Weigh (g/pupa)	
Thuja orintalis	**4.88**	**21.5±0.3^{a}**	**0.002±0.01^{c}**	**10.96±0.4^{a}**	**0.019±0.01^{bc}**	**32.4±0.55^{a}**
Bug oil	**6.29**	**11.8±0.1^{d}**	**0.029±0.002^{ab}**	**9.1±0.64^{b}**	**0.026±0.01^{b}**	**21.13±0.46^{c}**
Sesame oil	**3.46**	**17.9±0.4^{b}**	**0.018±0.1^{b}**	**10.3±0.37^{ab}**	**0.013±0.001^{c}**	**28.2±0.87^{b}**
α-pinene	**1.43**	**13.6±0.3^{c}**	**0.032±0.01^{a}**	**9.6±0. 4^{b}**	**0.030±0.01^{ab}**	**22.2±0.9^{c}**
Untreated check	*	**14.3±0.1^{c}**	**0.039±0.01^{a}**	**8.0±0.57^{c}**	**0.039±0.02^{a}**	**22.3±1.52^{c}**
LSD		**1.364**	**0.012**	**1.022**	**0.011**	**2.175**

Results are in partially agreement with that of Amer *et al*. (2013) they indicated that larval duration of *P. gossypiella* decreased to 15 days when the newly hatched larvae treated with *A. annua* + *C. annuum* compared to 20 days in the untreated check and more

decreasing in larval duration was occurred due to treatment with *A. annua* or *C. annuum* (13 days). In addition, Abd El-Zaher (2017) showed that the mortality of the 4th instar larvae of *S. littoralis* was increased with increasing the plant oil concentration as well as the periods of exposure of Flax oil, *Linum usitatissimum*; Ginger oil, *Zingiber Officinale*; Garlic oil *Allium sativum* and Jojoba oil, *Simmondsia chinensis*.

2.2.2. Pupal duration:

The obtained data presented in Table (3) indicate an increase in the pupal duration to reach 10.69, 9.1, 10.3 and 9.6 days when 1st larval instar of PBW treated with *Thuja orintalis*, bug oil, sesame oil and α-pinene, respectively with more efficiency of *Thuja orintalis* treatment which was the most efficient in an opposite way by reducing pupal weight to 0.019 g/pupa compared to 0.0388 g/pupa in the untreated check.

2. 3. Latent effect of some potential extracts on *Pectinophora gossypiella* adults treated as newly hatched larvae:

2.3.1. Oviposition periods:

Data summarized in Table (4) clear that the tested compounds; *Thuja orintalis*, Sesame oil and Alfa pinene elongate the pre-oviposition period and shortened the oviposition period of emerged females from treated newly hatched larvae.

Table (4): Latent effect of some potential extracts on *Pectinophora gossypiella* adults treated as newly hatched larvae.

Compounds used	Conc. (%)	Oviposition times /female in days			Longevity times	
		Pre-ovipostion	Ovipostion	Post-ovipostion	female	male
Thuja orintalis	4.88	5.00±0.10^{a}	09.8±0.11^{d}	3.3±0.29^{b}	18.2±0.35^{b}	13.1±0.3^{d}
Bug oil	6.29	2.76±0.56^{c}	18.3±0.33^{a}	6.1± 0.49^{a}	27.16±1.1^{a}	16.3±4.7^{a}
Sesame oil	63.4	3.96±0.48^{b}	10.4±0.32^{d}	1.6±0.20^{d}	15.9±0.3^{c}	11.6±0.3^{e}
Alfa pinene	1.43	3.50±0.60b^{c}	11.8±0.70^{c}	2.6±0.20^{c}	17.9±1.2^{b}	14.3±1.1^{c}
Untreated check	*	2.90±0.3^{c}	14.0±0.7^{b}	2.7±0.1^{c}	19.0±1.5^{b}	15.2±0.9^{b}
LSD		1.024	1.571	0.211	1.589	0.531

Pre-oviposit period recorded 5.00, 3.96 and 3.50 days while, oviposit period recorded 9.8, 10.4 and 11.8 days for previously mentioned compounds, respectively, opposite to bug oil treatment that shortened the pre-oviposit period to 2.70 days and elongate oviposit period to 18.3 day compared with 2.90 and14.00 days in the untreated check, respectively.

Data shown in Table (4) clear that the tested *Thuja orintalis* and bug oil elongated the post-oviposition period of *P. gossypiella* emerged as females from 2.7 in the untreated check to reach 3.3 and 6.1 days respectively and vice versa Sesame oil and Alfa pinene treatments that caused a reduction in post-oviposition period to be 1.6 and 2.6 days, respectively.

2.3.2. Adult longevity:

Data presented in Table (4) show that the adult females and males' longevity were shortened as a result of treating newly hatched larvae with *Thuja orintalis*, Sesame oil and Alfa pinene. These periods were 18.2, 15.9 and 17.9 days for females and 13.1, 11.6 and 14.3 days, for males respectively. In contrast an elongation was observed in longevity of females and males in case of bug oil which recorded 27.16 and 16.3 for female and male respectively Compared to the untreated check.

2.3.3. Reproductive potential:

Data shown in Table (5) appeared a high reduction in the total numbers of eggs laid by females for all treatments. The mean value of egg's numbers was 140.3,111,67.7 and 157 eggs/female for *Thuja orintalis*, bug oil, sesame oil and α-pinene, respectively compared to 253 eggs for the untreated check with a percent of Sterility estimated by 28.0, 26.4, 37.7 and 19.0 for the tested compounds, respectively.

Table (5): Latent effect of some potential extracts on the reproductive potential of *Pectinophora gossypiella* adults treated as newly hatched larvae.

Comp. used	Conc. %	reproductive / female		% Hatchability	% Sterility observed	% Corrected sterility
		Total no. of eggs/♀	Mean no. of eggs/♀/day			
Thuja orintalis	4.88	140.3±8.1^{c}	14.29±1.1^{b}	72.0	28.0	44.6
Bug oil	6.29	111.0±6.4^{d}	6.06±0.2^{d}	73.6	26.4	56.13
Sesame oil	63.4	67.7±3.7^{e}	9.39±0.4^{c}	62.3	37.7	73.24
α-pinene	1.43	157.0±3.9^{b}	13.31±0.9^{b}	81.0	19.0	37.95
Untreated check	*	253.0±4.6^{a}	18.07±0.7^{a}	96.0	4.00	*
LSD	*	5.166	2.471	*	*	*

The results are in agreement with that of (Halawa *et al.*, 2007), they stated that jojoba has inhibitory effects on growth and development as well as oviposition process.The effect also extended to the of eggs hatchability percentage causing a percent of reduction which is much obvious in case of sesame oil (62.3%) followed by *Thuja orintalis* (72.0%) bug oil (73.6%) and α-pinene (81.0%) compared to (96.0%)for the untreated check. On other hand the percentage of sterility increased due the result of treatments to reach 28.0, 26.4, 37.7 and19.0% for *Thuja orintalis*, bug oil, sesame oil and α-pinene, respectively compared to 4.0% for the untreated one. Reduction or failure of egg hatchability may be due to the penetration of insecticide into the eggs and prevent hatchability by interfering with embryonic cuticle synthesis so the new hatch probably cannot use its muscles to free itself from egg chorine, (Sammour *et al.*, 2008). Results are agreed with that of Gaaboub *et al.* (2012) as they recorded a significant reduction in egg numbers deposited by each female resulted from the 4th larval instar treated with jojoba oil compared with the control. Also, Amer *et al.* (2013) observed that the sterility increased from 11% in untreated chek to 20.1, 25.6 and 35.1 % when *P. gossypiella* treated as newly hatched larvae with *A. annua*, *C. annuum* and *A. annua* + *C. annuum*, respectively.

3. Biochemical analysis:

Data shown in Table (6) summarized the effect of the tested compounds on total protein and total lipids in the treated larvae. All treatments reduced both total protein to be 9.7, 8.0, 4.3 and 11.2 mg/g.b.wt and total lipids to 7.3, 4.3, 3.3 and 9.0 mg/g.b.wt) for *Thuja orintalis*, bug oil, sesame oil and α-pinene, respectively compared to 14.3 and 11.3 mg/g.b.wt for the untreated check, respectively.

Table (6): Effect of some potential extracts on the total protein, carbohydrate and lipid contents of *Pectinophora gossypiella* treated larvae.

Treatment	**Total protein** (mg/g.b.wt)	**Total lipid** (mg/g.b.wt)	**Total carbohydrate** (mg/g.b.wt)
Thuja orintalis	9.7±0.4	7.3±0.2	18.3±0.3
Bug oil	±0.48.0	4.3±0.1	9.9±0.6
Sesame oil	4.3±0.1	3.3±0.3	11.6±0.5
α-pinene	11.2±0.6	9.0±0.2	21.7±0.4
untreated check	4.3±0.11	11.3±0.4	20.9±0.8

According to total carbohydrate, treatments have the same reduction pattern except for α-pinene treatment that increase the total carbohydrate to 21.7 mg/g.b.wt compared to 20.9 mg/g.b.wt for the untreated check. Resulted data revealed that α-pinene treatment is the most efficient compared to the other treatments, which can be explained the contributed with the high reproductive potential due to the higher increase in total protein, total lipids and total carbohydrate than other treatment. This explanation is confirmed by the data of many authors that larval haemolymph protein contributed in developing ova in Lepidoptera (kong and kim, 1988).in addition our results are partially in agreement with that of Amer *et al.*, (2013); they stated slightly increase in total carbohydrates of pink bollworm larvae which being 10.69 mg/g.b.wt as affected by formulated tar oil compared with the untreated check value that was 9.38 mg/g.b.wt. in contrast, total lipids didn't affect approximately , the value was 14.08 mg/g.b.wt compared with the untreated check which reached 14.29 mg/g.b.wt in treating larvae of the pink bollworm by formulated tar oil.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Susceptibility of three squash cultivars to the two spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae) infestation in relation to phyto-chemical components of the leaves

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Tetranychus urticae, squash cultivars, carbohydrate, chlorophyll, phenol, protein, phyto- chemical components and morphological study.

Abstract:

The field experiment was conducted to evaluate the relative susceptibility of three squash cultivars, *Cucurbita pepo* L. (Mabroka, Brencesa and Eskandarani) against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) during two successive years 2016 and 2017 in Balaktar and Om Saber villages, Behera Governorate. Also, it was carried out to study the effect of the morphological leaf characteristics and the chemical components (phenol, protein, chlorophyll and carbohydrate) of the three previously mentioned squash cultivars and clarified the resistance of the pest infestations. Obtained results showed that there was no significant difference in eggs or motile stages or the total numbers of spider mite infestation on the three cultivars *(*LSD; $P > 0.05$). However, Mabroka cultivar was the most resistant cultivar to the spider mite pest as its leaves harbored the lowest mean numbers *(*LSD; $P < 0.05$) and produced higher average of yield than that of Eskandarani cultivar in both villages, followed by Brencessa cultivar which occupied a moderate mean numbers of the spider mite in spite of produced higher total of yield production than that of Eskandarani cultivar. Which suffered the highest infestation of the spider mite population during both seasons, also its production was significantly the lowest average yield production ($P < 0.05$) in both locations. The morphological leaf characters, the number of in the lower surfaces were different in their shapes, length and number of setae for Mabroka, Brencesa and Eskandarani, which in 1 mm were 29, 39 and 61 setae, respectively, the results of chemical analysis of leaf component after pest infestation indicated positive relationships in case of total phenol (r = 1) for the three cultivars. While the amount of protein, chlorophyll and carbohydrates were significantly decreased and their (r) values were (-1) in the three cultivars. It could be classified to three degrees, the severe infestation as represented by (Eskandarani), the moderate infestation (Brencessa) and the last of low infestation (Mabroka). It is concluded that growing the Mabroka cultivar better than the other two tested cultivars.

Introduction

Squash, *Cucrbita pepo* L. (Family: Cucurbitaceae) has a high nutritional value essential for the body metabolism and vitality as they contain different vitamins, sugar, starch, fats, proteins and minerals such calcium magnesium, potassium, sodium, phosphorus and iron. The squash plants are attacked by the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) which infest leaves, stems, branches and causing various degrees of damage, they usually feed on the leaves injuring the epidermis resulting in blotching, stippling or bronzing and consequently reduce quantitatively and qualitatively the yield. (Faris *et al.,* 2004 and Abdallah *et al*., 2009). The infestation with the spider mites may be affected by plant leaf morphological structure and its chemical contents (El-Saiedy *et al.,* 2011). Leaf structure has been showed to be related to mite damage or to symptoms commonly associated with damage (Abou-Zaid, 2013). The concentration of organic compounds in the tissue of plant leaf i.e. proteins, carbohydrates, phenolic compounds can be influenced by mite feeding as the reduction of total protein was occurred, the injured leaves protein can be degenerated or its synthesis is prohibited. In addition, changes in the concentration of soluble sugars and amino acids not only create better physical conditions for feeding but also provide a higher nutritive value of the food for spider mites. (Tomczyk and Kropczynska, 1985) Many studies were done to clarify the relation between mite infestation and leaf structure. Some varieties of Pelargonium with thicker cuticle and epidermis were more resistant to spider mite. Besides that, the upper leaf surface was less preferred by mites, because its cuticle and epidermis were, thicker than that of the lower surface (Kou *et al.,* 1972 and Luczynski *et al.,* 1990). Kielkiewicz (1994) and Magali (1997) stated that, leaf trichomes contributed to the reduction of *Tetranychus cinnabrinus* (Boisdaval) (Acari: Tetranychidae) density at beginning of mite feeding and on tomato and bean varieties. Adults of *T. urticae* could feed through the spongy and part of the palisade parenchyma of the leaf, while immature *T. urticae* could feed only through the spongy parenchyma (Park and Lee, 2002). Also, the length, density and thickness of leaf trichomes may be considered as other factors affecting the host plant resistance to infestation by *T. urticae* (El-Saiedy, 2003). A negative relationship between the thickness of the upper epidermis and spongy tissue of leaf of the 7 cucumber varieties and the abundance of *T. urticae* movable stages. While, it was positive in case of palisade tissue (Hanafy, 2004). Moreover, *T. urticae* preferred the lower leaf surface of the plants due to the very thin cuticular layer (Abo-Bakr and Ali, 2005). Also, the more thickness of the cuticle of the epidermis especially that of the lower surface could be considered as a physical resistance factor (Azouz, 2005). Resistant varieties possess thicker on either upper or lower leaf surface than susceptible ones. The more thickness of the cuticle epidermis especially that the lower surface could be considered as a physical tolerance factor against mechanism of spider mite (El-Sanady *et al.,* 2008). The present study was carried out as a trial to throw light on the susceptibility of the three squash cultivars to spider mite's infestation in realtion to phyto-chemical components of the leaves

Materials and Methods

1. Field studies:

1.1. Population study of the *Tetranychus urticae*:

The experiment was conducted in Balakter and Om-Saber villages, El Beheira Governorate to evaluate the population and the susceptibility of three squash cultivars (Mabroka, Brencessa and Eskandarani) to the spider mite infestation, *T. urticae*. An area of 350 m^2 for each cultivar was divided into four equal plots each of 87.5 m^2 (one plot for cultivar sensitivity and three plots for measurement

of different methods of control). Seeds of the three cultivars were sown on 11th& 14th of March in 2016 and 2017 seasons, respectively. Each plot consists of 10 ridges, 70 cm apart, 12 m long and thinned to 2 plants in the raw to give a population of 2400 plants per 1/4 feddan. The experimental design was a randomized complete block with three replications. All plots received the recommended agricultural practices.

Samples of randomly chosen leaves from the squash plants were picked up weekly from each cultivar after three weeks of cultivation until the end of the season (30 leaves / cultivars, continued for 17 weeks) and each sample were kept in a tight closed plastic bag. After that they were transferred to the laboratory for examination using stereomicroscope. Eggs and movable stages (larva, nymphs and adults) of the two-spotted spider mite attacked the leaf surface were estimated by counting their numbers on each leaf for the tested squash cultivars during growing stages.

2.Laboratory studies

2.1. Effect of chemical components and leaf anatomy on the density of *Tetranychus urticae* on the three cultivars of squash:

2.1.1. Effect of *Tetranychus urticae* infestation on the phyto-chemical components: of leaf:

Chemical analysis of leaf samples was carried out at the beginning (seedling stage) and at the peak of spider infestation (fruiting stage). Samples of squash leaves of the three cultivars were transferred to the Faculty of Agriculture Research Park, Cairo University for chemical analysis. Some specific chemical constituents of squash leaves cultivars were determined as follow: Total phenol content was determined by Folin-Ciocateu method as modified by Singelton and Rossi (1965). Total carbohydrates were extracted from the plant leaves and prepared for assay according to Crompton and Birt (1967). Total protein was calorically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966). Total chlorophyll was determined calorimetrically according to Holden (1965).

2.1.2. The leaf anatomy (morphological leaf characteristics):

Leaf samples of three squash cultivars were collected and imaged the lower surface of leaves using the Analytical Scanning Microscopic Technique (SEM) at the National Research Center according to (Karnousky, 1965 and Fischer *et al*. 2012). Trichomes were counted by using Computer Eye.

3. Data analysis:

Analysis of variance was conducted by one way to determine the significance between means of the tested cultivars by using the portable statistical analysis SAS 9.3.1. program (SAS Institute, 2003). Whereas the means were compared through LSD tests, least significant differences at 0.05 level. Mean numbers of the two-spotted spider mite, *T. urticae* on each squash cultivar (Mabroka, Brencessa and Eskandarani) in each village were compared using Student's t-test. The simple Correlation coefficient was also used by using Pearson Simple Correlation Coefficient Calculator*

Results and Discussion

1.Population of the two-spotted spider mite, *Tetranychus urticae* on the three tested cultivars:

The population abundance of the two-spotted spider mite, *T. urticae* egg and motile stages (immature stages and adults) on the three squash cultivars (Mabroka, Brencessa and Eskandarani) were studied during 2016 and 2017 seasons in Balaktar and Om Saber villages, Behera Governorate. Figures (1 and 2) showed the relation between time (week) and the total average numbers of the spider mite (individual) for the three cultivars. In the first season, the spider mite started on the leaves of the three squash cultivars (Mabroka, Brencesa and Eskandarani) on the

3rd of April in smaller numbers and the mite population remained very lower for the first three weeks from the beginning of the experimental survey. Then the total number of spider mite populations increased gradually through the next four weeks on 22nd of May and reached approximately 38.68, 47.51 and 59.14 individual/leaf; respectively in Balakter village and 90.66, 159.26 and 177.44 individual/leaf; respectively in Om Saber village. After that the spider mite populations continued to increase rapidly in numbers for the rest of the season reaching at the end of the season 216.22, 568.28 and 751.22 individual/leaf in Balakter village and 330.46, 815.35 and 1176.28 individual/leaf; respectively in Om Saber village (Figure, 1). In the second season**,** likewise the total number of spider mite on the three cultivars (Mabroka, Brencesa and Eskandarani) revealed the same trend and started on 31st of March in a small number as well and the curves were approximately consent for the first three weeks from the beginning of the experiment. Then the populations numbers increased and reached approximately 43.71, 54.42 and 65.24 individual/leaf; respectively in Balakter village and 73.07, 129.41 and 158.52 individual/leaf in Om Saber village, through the next four weeks on 14 of May and then continued to increase rapidly in numbers and this increase continued till the end of the season at which the population reached 245.41, 597.1 and 768.25 individual/leaf in Balakter village, while the total number of spider mite populations in Om Saber village reached to 355.45, 832.21 and 1213.24 individual/leaf; respectively (Figure, 2).

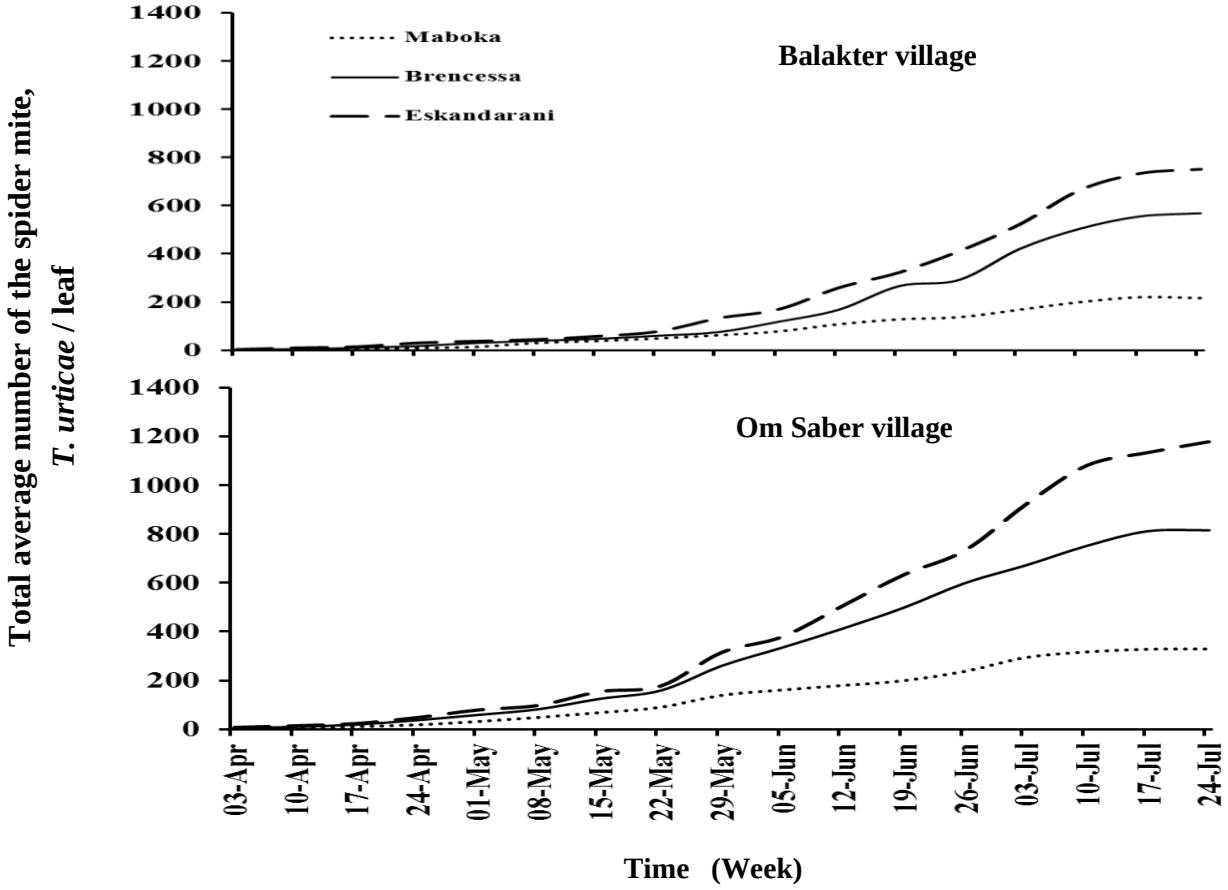


Figure (1): Weekly total average numbers of the spider mite, *Tetranychus urticae* /leaf on three squash cultivars; Mabroka, Brencessa and Eskandarani; under field conditions in Balakter and Om Saber villages, Behaira Governorate during season 2016.

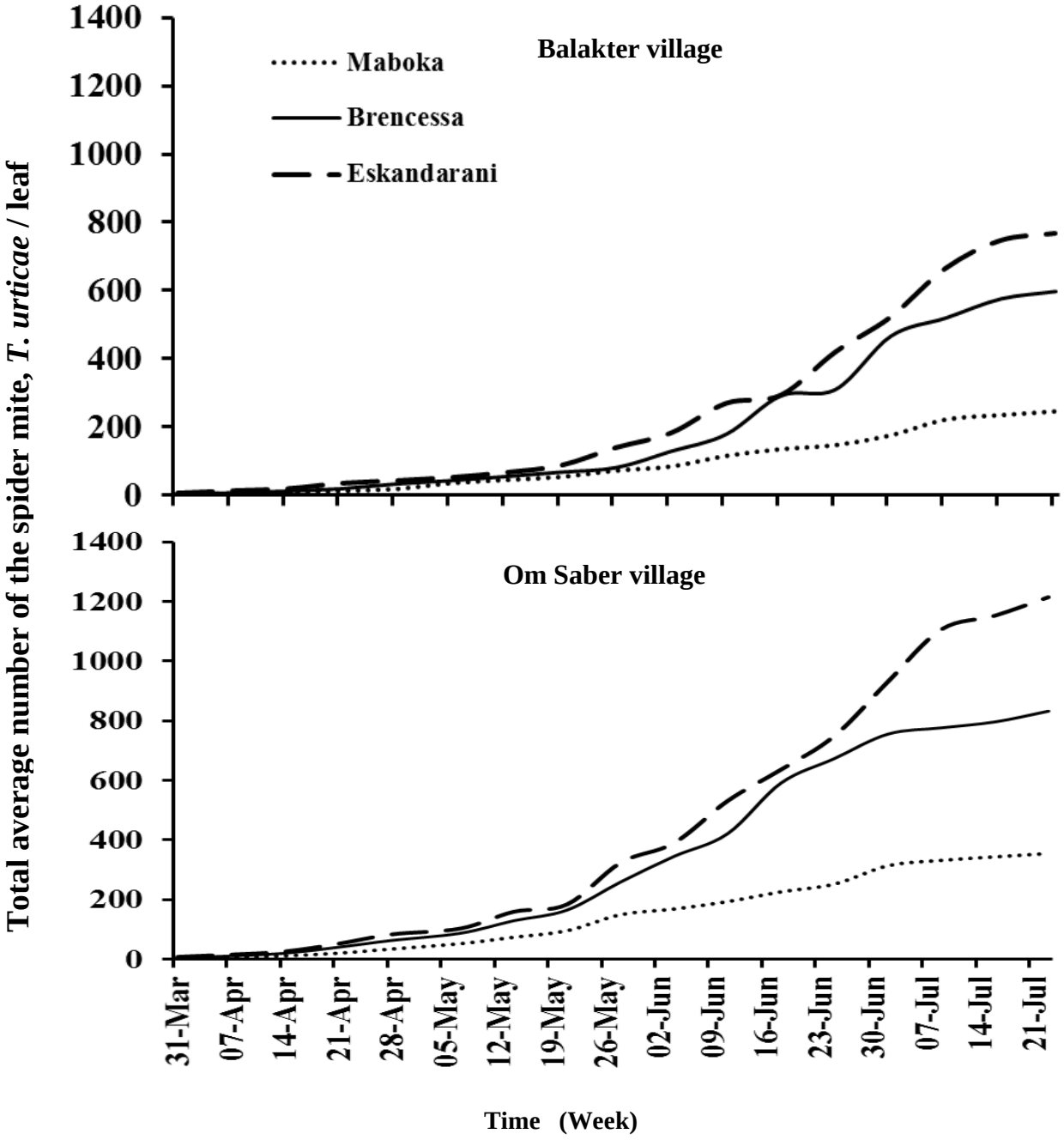


Figure (2) : Weekly total average numbers of the spider mite, *Tetranychus urticae* /leaf on three squash cultivars; Mabroka, Brencessa and Eskandarani; under field conditions in Balakter and Om Saber villages, Behaira Governorate during season 2017.

Table (1) described the average number for the egg and motile stages (immature stages and adults) as well as the total of spider mite, *T. urticae* ± standard error (SE), Maximum and on Minimum numbers on Mabroka, Brencessa and Eskandarani cultivars throughout 2016 and 2017 seasons in Balaktar and Om Saber villages.

Table (1): Total average numbers of the two-spotted spider mite, *Tetranychus urticae* /leaf of squash cultivars; Mabroka, Brencessa and Eskandarani under field conditions at Balaktar and Om Saber villages during both seasons.

Number of spider mite stages	Squash cultivars	2016					
		Balakter			Om Saber		
		Mean ± SE	Max.	Min.	Mean ± SE	Max.	Min.
Eggs	Mabroka	42.47 ± 9.07$_{aA}$	106.41	0.90	78.45 ± 16.95 $_{aB}$	190.14	0.40
	Brencessa	82.19 ± 20.60$_{abA}$	236.11	1.94	133.67 ± 26.45$_{abA}$	300.11	2.35
	Eskandarani	108.51 ± 27.41$_{bA}$	325.11	2.16	179.47 ± 39.17$_{bA}$	475.14	4.81
Motile stages	Mabroka	44.1 ± 9.97 $_{aA}$	113.55	0.14	66.82 ± 12.85 $_{aA}$	141.17	0.23
	Brencessa	105.04 ± 29.45$_{abA}$	332.17	0.98	199.18 ± 47.40$_{abA}$	515.24	1.88
	Eskandarani	141.85 ± 37.88$_{bA}$	427.11	1.75	260.54 ± 64.25$_{bA}$	701.14	3.11
Total	Mabroka	86.57 ± 19.00$_{aA}$	219.96	1.04	145.27 ± 29.69$_{aA}$	330.46	0.63
	Brencessa	187.22 ± 49.99$_{abA}$	568.28	2.92	332.85 ± 73.56$_{abA}$	815.35	4.23
	Eskandarani	250.36 ± 65.24$_{bA}$	751.22	3.91	440.01 ± 103.21$_{bA}$	1176.28	7.92
		2017					
Eggs	Mabroka	46.06 ± 9.91 $_{aA}$	122.15	1.11	81.8 ± 17.35 $_{aB}$	195.13	0.48
	Brencessa	86.00 ± 21.42$_{abA}$	248.90	1.82	136.97 ± 26.50$_{abA}$	300.10	2.60
	Eskandarani	111.42 ± 27.89$_{bA}$	330.14	2.20	184.07 ± 39.89$_{bA}$	481.13	4.32
Motile stages	Mabroka	47.83 ± 10.75$_{aA}$	123.26	0.18	72.59 ± 14.28$_{aA}$	160.32	0.14
	Brencessa	112.97 ± 31.09$_{abA}$	348.20	1.11	214.39 ± 51.22$_{abB}$	532.11	1.92
	Eskandarani	142.32 ± 37.83$_{bA}$	438.11	2.00	266.06 ± 65.68$_{bA}$	732.11	3.00
Total	Mabroka	93.88 ± 20.62$_{aA}$	245.41	1.29	154.39 ± 31.54$_{aA}$	355.45	0.62
	Brencessa	198.97 ± 52.45$_{abA}$	597.10	2.93	351.36 ± 77.32$_{abA}$	832.21	4.52
	Eskandarani	253.75 ± 65.6$_{8bA}$	768.25	4.20	450.13 ± 105.38$_{bA}$	1213.24	7.32

Means followed by different subscript small letters within columns for mite stages are significantly different from each other. Also, Means in row followed by different subscript capital letters within the row are significantly different from an another year (P < 0.05) LSD test

As shown in Table (1) no significant difference was found in the spider mite population among the three cultivar leaves (LSD; $P > 0.05$) during seasons 2016 and 2017 in both Balaktar and Om Saber villages. In the first season, there was significant difference in the number of spider mite eggs on Mabroka cultivar only between Balakter and Om Saber villages (LSD; $P < 0.05$). However, there were no significant differences between both villages in number of spider mite eggs or motile stages or total on the leaves of other cultivars (LSD; $P > 0.05$; Table, 1). There was no significant difference in eggs or motile stages or the total number of spider mites on the three cultivars (LSD; $P > 0.05$) (Table, 1). However, each stage of the spider mite on the three cultivars was ranked in ascending order on the leaves of Mabroka, Brencessa and Eskandarani cultivars during the two seasons in both villages. The total average number of the spider mite on Eskandarani cultivar was about folds that in Mabroka cultivar (250.36 and 86.57 individuals/ leaf, respectively in Balakter village; also, there were 440.01 and 145.27 individuals/ leaf, respectively in Om Saber village (Table, 1). While Brencesa cultivar had a moderate total average number of the spider mite 187.22 individuals/leaf among the tested cultivars in their infestation by *T. urticae*; in Balakter village and 332.85 individuals/leaf in Om Saber village (Table, 1). In the second season**,** similar results were

found in the infestation of the three cultivars under test with the spider mite, which were in the same direction as they were observed in both villages, except Brencessa cultivar which was a significant difference in the population number of motile stages on the leaves between both villages (LSD; $P < 0.05$) (Table, 1).

Moreover, the total average number of the spider mite on Eskandarani cultivar was about doubles that in Mabroka cultivar (253.75 and 93.88 individuals/leaf, respectively in Balakter village; also there were 450.13 and 154.39 individuals/ leaf, respectively in Om Saber village (Table, 1). While Brencesa cultivar had a moderate total average number of the spider mite 198.97 individuals/leaf among the tested cultivars in their infestation by *T. urticae*; in Balakter village and 351.36 individuals/leaf in Om Saber village (Table, 1). This cultivar was not significantly different from Mabroka or Eskandarani in both villages (LSD; $P > 0.05$).

The infestation rate by *T. urticae* in Om Saber village was higher than in Balakter and Om Saber village, with no significant difference between them (Figure, 3). Eskandarani was the most susceptible cultivar as its leaves harbored the highest total of spider mite population (250.36 and 440.01 individuals/leaf; in Balaktar and Om Saber villages, respectively during season 2016 and 253.75 and 450.13 individuals/leaf; in Balaktar and Om Saber villages, respectively during season 2017 (Table,1), followed not significantly by Brencessa cultivar (187.22 and 332.85 individuals/leaf; in Balaktar and Om Saber villages, respectively during season 2016 and 198.97 and 351.36 individuals/leaf; in Balaktar and Om Saber villages, respectively during season 2017, while the Mabroka cultivar was infested by the lowest total of spider mite population (86.57 and 145.27 individuals/leaf; in Balaktar and Om Saber villages, respectively during season 2016 and 93.88 and 154.39 individuals/leaf; in Balaktar and Om Saber villages, respectively during season 2017 (Table, 1 and Figure, 3), which is the most resistant one to the total of spider mite population. This result coincides with that obtained by Allam (2014) who studied the susceptibility of four squash varieties to spider mites and other different phytophagous pests' infestation and he concluded that the highest population of mite was recorded in variety of Eskandarani (baldy) and in contrary, Mabroka variety exhibited less population of the two-spotted spider mite.

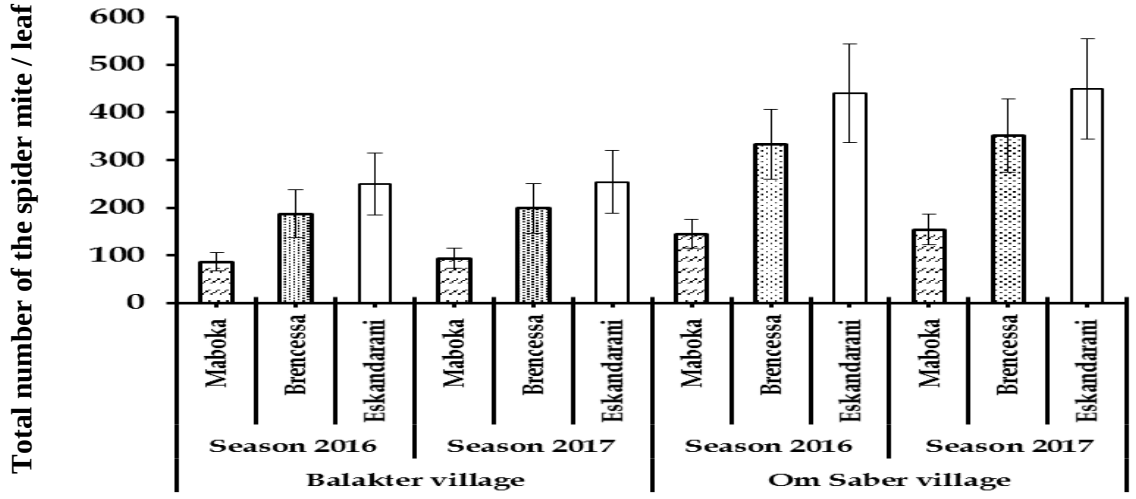


Figure (3): The total number of the two-spotted spider mite, *Tetranychus urticae* on the squash leaf of the three cultivars in Balakter and Om Saber villages in both seasons.

2. Measurements yielding of each squash cultivar:

Total of squash yields for each cultivar was collected and weighted to estimate the final yield per two karats. Table (2) described the average of the three squash yields in kg. /2 Karats, the mean of yield production, Maximum and on Minimum yield of Mabroka, Brencessa and Eskandarani cultivars in Balaktar and Om Saber villages. Data in that table showed the highest total of yield production were obtained of Brencessa cultivar recorded (835.76 and 707 Kg / 2 karats) in Balaktar and Om Saber villages, respectively, followed by Mabroka cultivar (664.6 and 585.4 Kg / 2 karats) then the lowest total of yield production were obtained for Eskandarani cultivar (446.86 and 362.5 Kg / 2 karats (Table, 2). There was significant difference among the three squash cultivars in total yield production/2 karats (LSD; $P < 0.05$) in both villages. Also, there were no significant difference between Brencessa and Mabroka cultivars in the mean of yields. However, there was a high significant difference between Mabroka and Eskandarani cultivar, which produced the lowest amount of yield production (LSD; $P < 0.05$) (Table, 2 and Figure, 4) in both locations.

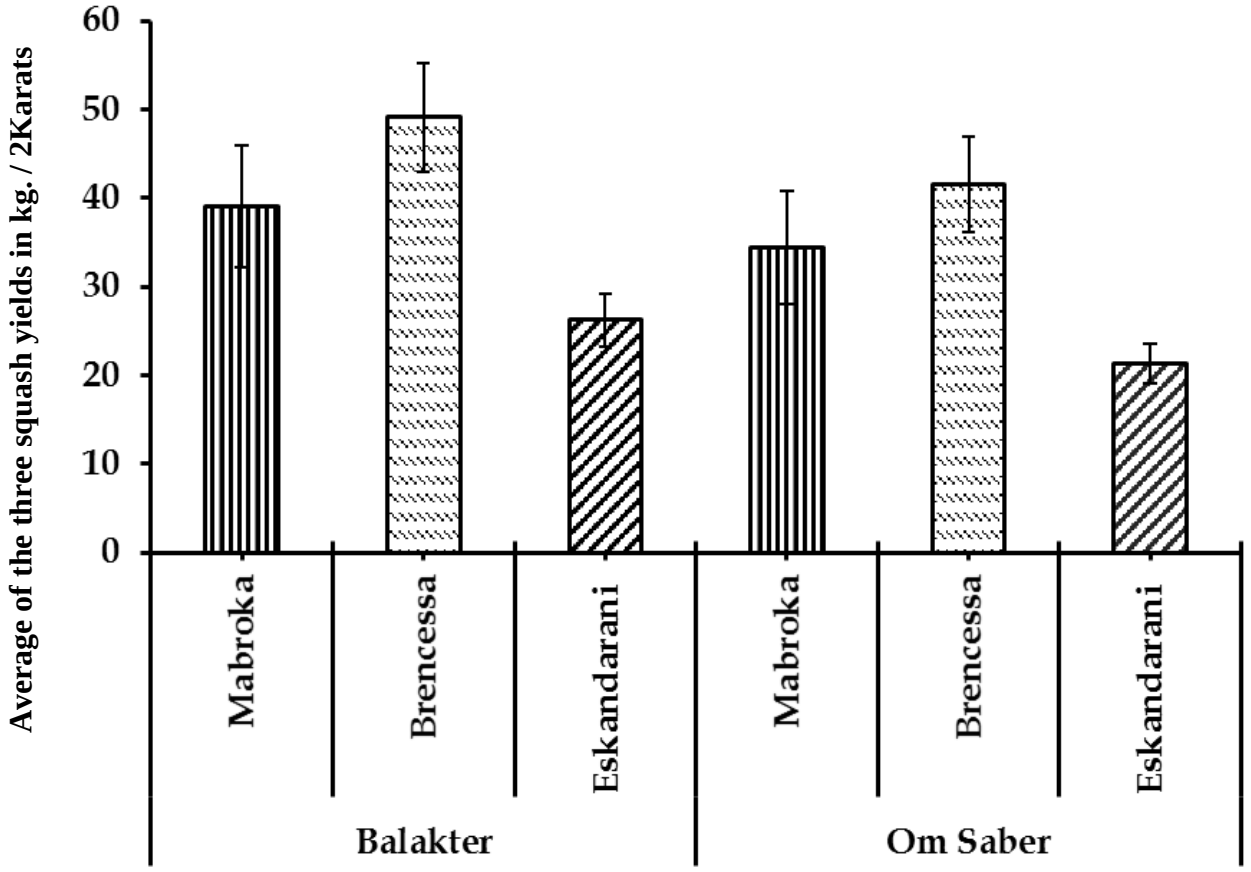


Figure (4) : Average of the three squash yields in kg. / 2Karats in both villages.

Table (2): Total of the three squash yields (Mabroka, Brencessa and Eskandarani) in kg. / 2 Karats in Balaktar and Om Saber villages.

Date	Balakter village			Om Saber village		
	Mabroka	Brencessa	Eskandarani	Mabroka	Brencessa	Eskandarani
25-May	8.8	42.3	14.5	5.7	38	10.4
29-May	13.2	20.6	20.8	9.5	17	16.7
02-Jun	20.3	24.94	9.4	17.6	20.3	7
06-Jun	70.9	16.54	13.8	65.4	12.7	10.8
10-Jun	43.4	40.8	20.5	39.3	35.4	17.4
14-Jun	96.7	70.9	35	83.7	55	30.2
18-Jun	50.6	90.3	21.5	47.4	73.8	17.5
22-Jun	20.4	76.94	40.2	19	67.3	33.6
26-Jun	32.3	81	61.4	28.3	79	41.5
30-Jun	30	51.54	31	27	46	27.3
04-Jul	14	71	27.96	10.8	53	23.4
08-Jul	18	41.3	30.9	13	37.3	25.2
12-Jul	10	44.8	21.5	7	39.7	18
16-Jul	26	87	29.6	20.2	73.5	24
20-Jul	50	21.8	30.8	43.5	18	26.5
24-Jul	90	30	22	82.7	24	19.3
28-Jul	70	24	16	65.3	17	13.7
Total	664.6	835.76	446.86	585.4	707	362.5
Mean	39.1_{ab}	49.2_{a}	26.3_{c}	34.4_{ab}	41.6_{a}	21.3_{c}
Max.	96.7	90.3	61.4	83.7	79	41.5
Min.	8.8	16.54	9.4	5.7	12.7	7.0

Means followed by different subscript letters within row are significantly different from each other (P < 0.05) LSD test.

3. The laboratory studies:

3.1.Effect of some chemical components of squash leaves and their relation to spider infestation:

The two-spotted spider mite, *T. urticae* infested the three tested cultivars of squash with different rates. Data in Table (3) showed the nutrient content of the tested squash cultivars in order to investigate the relationship between components and the mite pest infestation. The chemical analysis of leaf samples was carried out at the beginning and during the peak season of infestation.

Table (3): Some chemical components of squash leaves and their relation to spider infestation during season 2017.

Cultivars	Stage of plant	Mean No of spider mite	Total Phenol	Total protein	Total chlorophyll	Total carbohydrate
Mabroka	Seedling	4.56	12.05	23.96	122.54	14.68
	Fruiting	113.55	15.11	20.44	117.77	11.66
R			1	-0.99	-1	-0.99
Brencessa	Seedling	8.6	10.98	20.98	121.33	13.91
	Fruiting	321.11	12.82	18.95	114.65	10.23
R			1	-1	-1	-1
Eskandarani	Seedling	14.34	9.14	21.85	124.55	14.87
	Fruiting	427.11	11.58	18.23	113.22	10.85
R			1	-1	-1	-1

R: correlation coefficient

The three cultivars varied significantly in their infestations with the spider mite as the individuals were more abundant on plant leaves of Eskandarani followed by Brencessa while the lower abundant of the pest occurred in Mabroka.

Regarding Mabroka, it was noticed that at the beginning of infestation, when the population density of mites is minimum 4.56 individuals /leaf the value contents of protein, chlorophyll and carbohydrate were 23.96, 122.54 and 14.68 mg/100 gm, respectively; these values were decreased to 20.44, 117.77 and 11.66 mg /100 gm, respectively, when the population density of mites was in its peak of infestation recorded 113.55 individuals /leaf. While for total phenol throughout the beginning of infestation, recorded a low value 12.05 mg /100 gm, which increased to 15.11 mg /100 gm when the population density of mites was maximum. Concerning the relation between the population levels and the previously mentioned components, the calculated correlation coefficient values were significantly positive in case of 2total phenol as the corresponding r values is (1), while reversal relationships were detected with protein, chlorophyll and carbohydrate as the correlation coefficient values were -0.99, -1 and -0.99, respectively.

Concerning Brencessa; it was clear that its leaves were infested with 8.6 and 321.11 individuals /leaf of the spider mite at the beginning and at the peak of infestation, respectively, and by evaluating some of the phytochemical components in Brencessa leaves; the result revealed that it contained 20.98, 121.33 and 13.91 mg /100 gm of protein, chlorophyll and carbohydrate at the beginning of infestation which decreased at the end of infestation and became 18.95, 114.65 and 10.23 mg /100 gm of the previous components, respectively. Meanwhile, for the repellent component, the phenol, the data showed a low value 10.98 mg /100 gm at the beginning of infestation when compared to those of peak infestation, 12.82 mg /100 gm. Statistically, the correlation coefficient clearing the fact by increasing the population density of the spider mites on cultivar leaves; its contents of protein, chlorophyll and carbohydrate decreased significantly as their values were (-1) of the three compounds, while total phenols affected positively on population density of the spider mites i.e. the population increased by increasing the content of phenols as the correlation value was (1).

With respect to Eskandarani, which classified as the highest susceptible one of the three cultivars, it was observed that its leaves were infested with 14.34 and 427.11 individuals/leaf throughout the beginning and at the peak of infestation. Its leaves possessed a high value of nutrient contents recorded 21.85, 124.55 and 14.87 mg /100 gm of protein, chlorophyll and carbohydrate, respectively, during the beginning of infestation and those components were decreased by increasing the population of the pest and reach its peak which became 18.23, 113.22 and 10.85 mg /100 gm. While total phenols were 9.14 mg /100 gm when the population of the pest at the beginning of infestation which increased to 11.58 mg /100 gm at the end of infestation.

By concerning the correlation coefficient calculated to clear the relation between the densities of spider mites and those components. Results indicated positive relationships in case of total phenol (r = 1), while its defense secretion increased in the cell sap. On the other direction by increasing the population density of spider mites, the amount of protein, chlorophyll and carbohydrates were significantly decreased, their r values were (-1) in the three components.

These results revealed that the lowest variety infested by spider mite was Mabroka. The chemical analysis showed that this cultivar had a high content of phenol and protein at the beginning of infestation (12.05 and 23.96 mg /100 gm, respectively) compared to the highest variety of infestation (9.14 and 21.85 mg /100 gm) of (Eskandarani) at the end of the season.

Data showed that Mabroka cv. was the lowest squash cultivar to spider mite infestation. It had the least number of motile stages of the pest (113.55 individuals) at the peak infestation. The second rank of mite infestation was occupied by Brencessa which its corresponding value was 321.11 individuals of the pest. However, the third rank of infestation recorded the highest pest infestation (427.11 individuals) belonged to Eskandarani cv. i.e. an increase in the population density of spider mite, the amount of protein, chlorophyll and carbohydrates were significantly decrease, while an increase in the amount of phenol, the cultivar acquires resistance and the pest the spider mite decreases. This result was coincided with that of Tomczyk and Kropczynska (1985) they proved that proteins, carbohydrates, phenolic compounds can be influenced by mite feeding as the reduction of total protein was occurred, the injured leaves protein can be degenerated or its synthesis is prohibited. In addition, changes in the concentration of soluble sugars and amino acids not only create better physical conditions for feeding but also provide a higher nutritive value of the food for spider mites. Also, Tomczyk *et al.* (1987) studied the amino acids and soluble proteins in resistance of four cucumber to the tetranychid mite, *T. urticae*. They indicated that the increases in photosynthetic activity and growth in plants as a result of mite feeding sometimes led to increase in yield. Moreover, Ahmed (1994) proved that the susceptible cucumber cultivars to infestation with *T. urticae* contain more protein, total amino acids, than resistant varieties. In addition, Mahgoob (2004) studied the effect of chemical constituents and anatomical structure of some host plants leaves against the two-spotted spider mite *T. urticae*. He observed that the mite resistance could be related to the high phenolic content as in *Dodonaea viscosa* and *Citrus aurantium*. Taha and EI-Raies (1996) showed that, the increasing of salinity levels increased leaf sodium, and decreased nitrogen, in consequence, the mite infestation was decreased. Abdallah *et al.* (2009) studied the correlation between the phytochemical contents and the rate of mite infestation higher number of phytophagous mites which was associated with higher levels of sugar content and this indicates a positive significant relationship with the population densities and sugar content. While the reverse was true with total phenol (which indicates a negative significant relationship with the population densities. El-Saiedy *et al.* (2011) stated that infestation with the spider mites may be affected by its chemical contents; a positive relationship occurred between mite infestation levels and total soluble sugars in watermelon cultivars while negative relationship found with total phenol compounds.

3.2. Effect of leaf anatomy on the density of spider mite on the three cultivars of squash:

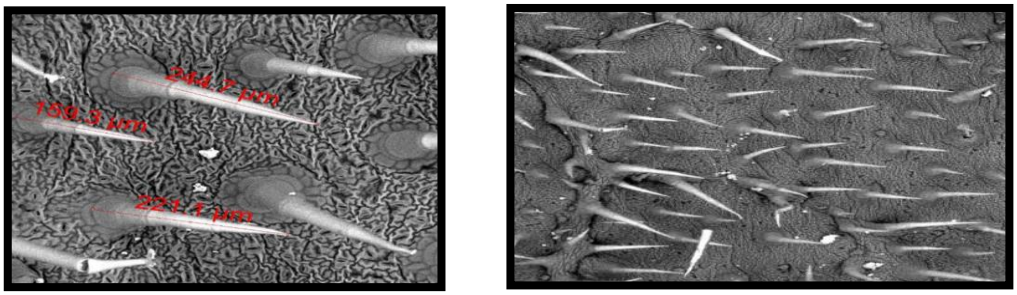
The susceptibility of squash plants to infestation with affected by plant leaf morphological structure. Therefore, the morphological leaves of the three squash cultivars, Mabroka, Brencessa and Eskandarani were studied (Figure, 5).

The number of trichomes (Table, 4) in the lower surfaces in mm were 29, 39 and 61 setae for Mabroka, Brencessa and Eskandarani, respectively. The length of the longest seta was 285.2 µm, for Brencessa followed by 267.4 µm, for Eskandarani and then 174.6 µm for Mabroka. The length of the smallest seta was in Eskandarani of 37.5 µm followed by Mabroka of 50 µm and then Brencessa of length 56.25 µm; setae of Mabroka spine in shape, short with 2 segments; setae of Brencessa, flagellate with two segments; setae of Eskandarani flagellate with three segments. The anatomical structure of some host plants leaves of three cucurbit crops, Sudanian watermelon, snake watermelon and squash on their susceptibility to the spider mite infestation (Ibrahim *et al.*, 2008). Data show that the spider mite population increased as dense of hairs increased and vice versa.

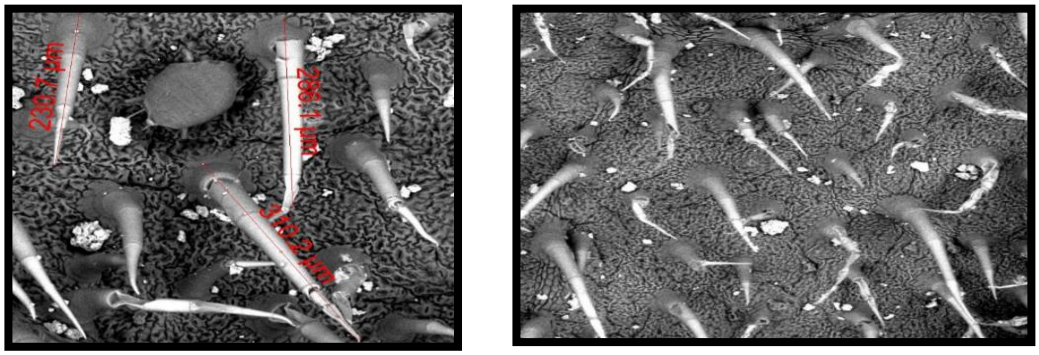
Table (4) : Measurements of some morphological characters of squash leaves in three cultivars.

Cultivar	Density of trichomesin 1mm	Length of smallest trichome in µm			Length of longest trichome in µm		
		length with base	length without base	size base	length with base	length without base	size base
Mabroka	29	106.25	50	56.25	244.7	174.6	70.1
Brencessa	39	112.5	56.25	56.25	310.2	285.2	25
Eskandarani	61	106.25	37.5	68.75	309.3	267.4	41.9

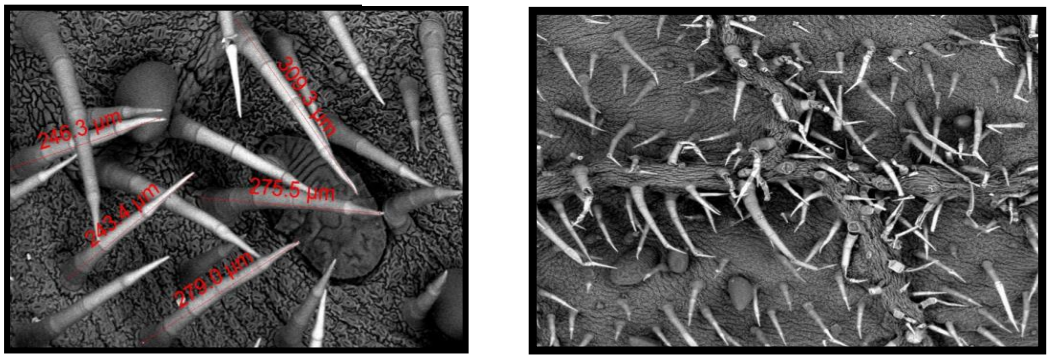
The newly developed setae with base size are 56.25, 56.25 and 68.75 µm for Mabroka, Brencessa and Eskandarani, respectively (Table 4). Base of old trichome of Mabroka cv. increased in size and became 70.1 µm and decreased in Eskandarani cv. became 41.9 µm, while in Brencessa cv., the base diminished more than half recorded 25 µm. The obtained data indicated that there were correlations between the phytochemical contents and the rate of mite infestation.



Mabroka



Brencessa



Eskandarani

Figure (5): Scanning electron micrograph of lower surface leaves showing trichomes.

There is a positive relationship between the infestation levels and the total sugar contents in the squash cultivars, while there is a negative relationship between the infestation rate and the total phenol as well as the free amino acids contents in each cultivar.

The results confined that the increasing of total sugar content lead to an increase in the population of phytophagous mite species on the tested cultivars, while a negative relationship was detected with total phenol and free amino acids. The obtained results are in agreement with that recorded by many authors; Kielkiewiez *et al*. (1983) stated that the phenol content distribution in the infested tissues of the resistance leaves is considered as an important factor in the defense reactions of plants against mite attacks. El-Saiedy (2003) and Mahgoub (2004) stated that, the mite resistance could be related to the high phenolic content in the infested leaves. El-Saiedy (2003) concluded that the high infestation of the mite may be related to the high sugar content exhibited.

It was evident from the above results that the Mabroka cultivar was the most resistant cultivar to the spider mite pest as its leaves harbored the lowest mean numbers and produced higher average of yield than that of Eskandarani cultivar in both villages, followed by Brencessa cultivar which occupied a moderate mean numbers of the spider mite in spite of produced higher total of yield production than that of Eskandarani cultivar. Which suffered the highest infestation of the spider mite population during both seasons so its production of fruit yield was very weak.

It could be classified to three degrees, the severe infestation as represented by (Eskandarani cv.), the moderate infestation (Brencessa cv.) and the last of low infestation (Mabroka cv.). It is concluded that growing the Mabroka cultivar better than the other two tested cultivars.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Biological control of two spotted spider mite, *Tetranychus urticae* (Acari: Phytoseiidae) with releases of predatory mite, *Neoseiulus californicus* (Acari: Phytoseiidae) in strawberries

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Abstract:

The two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests responsible for yielding losses to many agricultural crops. In this study, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) has been evaluated as a natural predator for *T. urticae* infesting strawberry. The results indicated that reduction in number of *T. urticae* after releasing the predator *N. californicus* were in the first week, 31.9% and 51.3% in single and double releases, respectively. The reduction in the second and third weeks reached to 70.8 &80% and 71.7 and 89.3% for single and double releases, respectively. It is concluded that the repeated releases of *N. californicus* was the best for preventing *T. urticae* to exceed the economic threshold level.

Introduction

The cultivated plants in green house particularly strawberries are greatly affected by the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) (Rhodes *et al.*, 2006). The high rate of *T. urticae* fecundity enables it to complete the life cycle within one week at quite high temperature ≥32°C. In that context, the growers frequently use acaricides which might reduce the infestation and its consequences for a short run. However, the use of these chemicals for a long run might lead to the development of *T. urticae* resistant population (Fraulo and Liburd, 2007). *T. urticae* is feeding on the lower surface of the strawberry leaves leading to change in shape and color and leaving fine webbing. For its fast growing, high reproductive potential and under favorable growing conditions, mites could rapidly reach damaging population levels at which berry number significantly reduced (Walsh *et al.*, 1998 and Sato *et al.*, 2007).

For their bad effect on the long run, coast and for environmental concerns it became necessary to find an alternative to acaricides to overcome such obstacles. The use of the predacious mites, family Phytoseiidae (Acari: Mesostigmata), which commercially available are commonly used for the control of *T. urticae* on various vegetable and ornamental crops (Palevsky *et al.*, 2008).

The predatory mite, *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) is polyphagous in nature and it could survive in a wide range of temperature and humidity. This predator is currently mass

produced to be used as a bio-control agent against spider mites (Gerson and Weintraub, 2007). It has a high predation capacity of about 15-20 spider mite eggs per day. Additionally, it could feed on pollen, other mites, thrips and aphids, thus surviving for days without the presence of the prey in the field (de Moraes and Flechtmann, 2008 and Marafeli *et al.*, 2014). It may face the challenge of food limitation or its absence in the field during storage or during transportations (Ghazy *et al.*, 2015). For field application of *N. californicusis* to be successful, it is very important to adjust the prey/predator ratio and to maintain adequate long-term control of *T. urticae*, combined treatment of Acramite with *N. californicus* may be an effective strategy to reduce the of *T. urticae* in commercially grown strawberries (Rhodes *et al.*, 2006).

The purpose of this study is to determine the effectiveness of *N. californicus* release as a bio-control agent against *T. urticae* infesting strawberry fields.

Materials and Methods

Commercial strain of *N. californicus* that was obtained from bio-log Company. The experiment was conducted in strawberry field in Belkas city, Dakahlia Governorate in an experimental field with dimensions 15x20 meter. The field has a low infestation with *T. urticae*. The area was divided into 3 equal parts each one with 5x20 m dimensions. The first part was assigned to once release with *N. californicus* and the second with double release 2 times per week with *N. californicus* and the last one served as a control. It was applied (50 individuals /m^2) starts from 1st of February and calculate the reduction in number of *T. urticae* by randomly collecting 20 leaves (3 leaflets) before and after 1,2 and 3 weeks after releasing and examined by stereomicroscope in laboratory. The reduction percentages of mites were calculated by using the Henderson -Tilton formula (Henderson and Tilton, 1955).

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment X n in T after treatment}}{\text{n in Co after treatment X n in T before treatment}}\right) \times 100$$

Data were analyzed by one way analysis of varience (ANOVA) and the means were separated using Duncan's Multiple Range Test (Snedecor, 1980).

Results and Discussion

The effect of releasing of the predatory mite *N. californicus* in single and double times are represented in Table (1) the mean number of *T. urticae* after 1 week of single release was 5.4 (individuals/ leaflet) which increased to 6.9, 8.1 (individuals /leaflets after 2 and 3 weeks), respectively. Meanwhile, in double release of *N. californicus* the starting number of *T. urticae* was 6.2 individuals/ leaflets and slightly decreased after 1 week to 5.3 and significantly decreased to 4.3, 3.2 after 2 and 3 weeks of treatment. Reduction in number of *T. urticae* in the first week was 31.9% and 51.3% in single and double releases, respectively. The reduction in the second week reached to 70.8, 80% and in the third week reached to 71.7, 89.3% for single and double releases, respectively.

The number of predatory mite *N. californicus* (Figure,1) counted also in single, double and in control replicates which the mean number was 1.2, 1.7 in single and double releases but in control (no release) it was Zero individuals/leaflets. The mean number of *N. californicus* increased at 2 weeks after release to 2.7, 4 and 0.7 individuals/leaflets in single, double releases and control (no release), respectively. While in 3 weeks after release, the numbers were 3, 5.4 and 1.6 individuals/leaflets in single, double and control (no release), respectively.

The time of release and environmental conditions are important in the use of *N. californicus* as it never achieved control in the late release plots (Fraulo and Liburd, 2007 and Audenaert *et al.*, 2014).

Application of *N. californicus* could attain season-long control of *T. urticae* with substantial economic saving for growers compared with current recommendations acaricides. (Fraulo and Liburd, 2007). The current experiment has been done when the number of *T. urticae* was higher than injury threshold level because once harvest begins, strawberry plants become more tolerant to mite feeding and treatment thresholds increase to an average of 15–20 mites per mid-tier leaflet (Fraulo and Liburd, 2007 and Iwassaki *et al.*, 2015). In this study, *N. californicus* showed the ability to maintain numbers of *T. urticae* in the treated plants compared with the increasing numbers of this pest in the untreated plants.

Table (1): Effect of single and double release of *Neoseiulus californicus* on *Tetranychus urticae* population.

Date	Times of release	No. of *T. urticae*/leaflet	Reduction %	LSD
Pre-count	Single	5.7 ± 0.47	-	
	double	6.2 ± 0.64	-	
	No release	7 ± 0.17	-	
After 1 week	Single	5.4 ± 0.57 [b]	31.9	0.7605
	Double	5.3 ± 0.76 [b]	51.3	
	No release	12.4 ± 0.95 [a]	-	
After 2 weeks	Single	6.9 ± 0.63 [c]	70.8	0.595
	Double	4.3 ± 0.34 [b]	80	
	No release	27.9 ± 1.78 [a]	-	
After 3 week	Single	8.1 ± 0.21 [c]	71.7	2.27
	Double	3.2 ± 0.45 [b]	89.3	
	No release	33.5 ± 3.2 [a]	-	

The same letters in a raw are not-significantly different (ANOVA, P < 0.05)

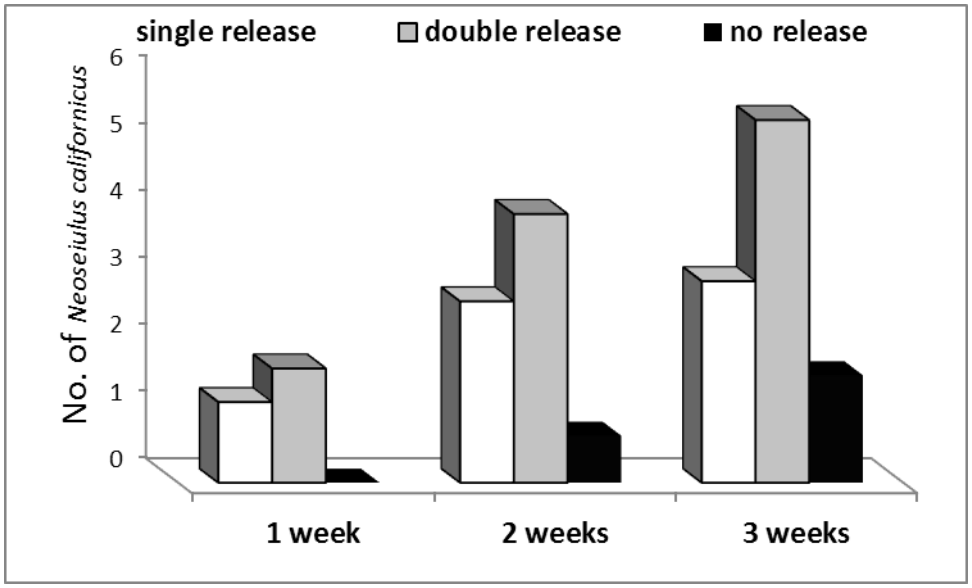

Figure (1): The number of *Neoseiulus californicus* after one, two and three weeks of release.

Compared with single release, double release treatment showed 89.3% reduction in mite population after three weeks. Greco *et al.* (2005) have contradicting results, they stated that *N. californicus* was very effective in limiting pest densities (*T. urticae*) at a 7-day period after releasing and within the range of pest-predator ratios and absolute densities used in this study.

The results here indicated that reduction in number of *T. urticae* after releasing of the predator *N. californicus* were in the first week, 31.9% and 51.3% in single and double releases, respectively. The reduction in the second and third weeks reached to 70.8, 80% and 71.7 and 89.3% for single and double releases, respectively. These data concluding that *N. californicus* keeps the balance between the numbers of predator to the number of prey in the rate limiting factor for such experiment to succeed. The same results conducted by Fraulo and Liburd, 2007 as they stated that *N. californicus* when was released at several times reduced *T. urticae* significantly.

It is concluded that *N. californicus* keeps the balance between the numbers of predator to the number of preys in the rate limiting factor for such experiment to succeed. The repeated releases of *N. californicus* was the best for preventing *T. urticae* to exceed the economic threshold level.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Evaluation of FEEDBEE® under Egyptian condition

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Key words: *FEEDBEE®, honey bee, proteins, pollen yield, pollen traps and Egypt.*

Abstract:

Proteins are an important determinant of the growth of many organisms. Social insect such as honey bees provide protein sources to their individuals and stored them, lake of protein sources is negative reflacting of brood rearing in honey bee colonies, worker's population and colonies activities. So, under condition of lack pollen yield or using pollen traps, it necessary to compensation of colonies with pollen substitutes materials, such as FEEDBEE®. Form evaluation data at spring season under Egyptian condition, powder FEEDBEE® 5gm/L of sugar syrup (1:1), was perfect to provide colonies with pollen substitutes with high significant effects on worker's population, brood rearing areas, and showed lowest significant effect in stored beebread.

Introduction

Worker bees do not have substantial protein reserves in their bodies; therefore, they require a daily diet of about 3.4 – 4.3 mg of pollen, depending upon their age, to make up this nutritional deficiency. A typical 10-combs covered with bees consumes between 13.4 and 17.8 kg of pollen annually (Crailsheim *et al.*, 1992). Pollen is a food of complex chemical makeup, the protein being the ingredient of the greatest importance for bees. Breaks of prolonged duration in the supply of that food to bee colonies may negatively affect the development and the functioning of a bee colony (Rogala and Szymaoe, 2004). In such cases pollen collected with bee traps during high pollen flow or pollen substitutes should be fed to colonies (Doull, 1980 a,b ; Peng *et al*., 1984 and Chambers, 1990). The basic food for honey bee is represented by honey as energetic source and pollen that rich in protein, vitamins, enzymes, mineral and lipids, etc. Which is necessary for the growth, development and activity of honey bees, when brood-rearing was limited for long periods when pollen was available in the field and that it ceased completely in the absence of pollen (Parker, 1926) and fed colonies with pollen substitutes increase of brood production of 43 and 73% in colonies fed substitute comparative with colonies fed equivalent amounts of syrup instead (Wille and Schafer, 1970). It is almost necessary to indemnity honeybee when colonies lacking natural pollen yield with pollen supplies or substitutes material that survival honeybee colonies strength. The activity of honey bee colonies to rear a brood is highly dependent on the contribution of a suitable protein in food, as well as on its quality, to activate their hypopharyngeal glands (Mostafa, 2000). Larvae are especially dependant on protein and brood production is strongly affected by shortages of this nutrient. The

number of larvae reared may be reduced to maintain the quality of remaining offspring. The quality of developing workers also suffers under conditions of larval starvation, leading to slightly affected workers. Larval starvation, alone or in combination with other stressors, can weak colonies. The potential of different diets to meet nutritional requirements or to improve survival or brood production is outlined (Brodschneider and Crailsheim, 2010).

The aim of this work is to evaluate FEEDBEE® as pollen substitute under Egyptian environmental condition.

Materials and Methods

The evaluation of FEEDBEE® as pollen substitute carried out in apiary of Beekeeping Research Department, Plant Protection Research Institute, Agriculture Research Centre, Giza, Egypt. Assessment was done for nine weeks started in 23 Feb.2017 and finished 29 April 2017.

1. FEEDBEE® concentration:

For evaluation FEEDBEE® powder (FB.) using three concentrations (5, 6 and 7gm/L) in sugar solution (1:1) 500ml/colony two time a week. FEEDBEE® substitute (FB.S.) which content minimum protein 33.0%, 2.0% fat, 28.0% carbohydrate, 20.0% sugars and maximum 4.0% fiber, 4.0% mineral 0.15% calcium, 0.50% phosphorous, 23.0% moisture; was evaluated in the same time 500 gm/colony

2. Colonies preparation:

Fifteen honeybee's colonies equal strength were chosen and headed by new sister under this experimental, four treats triplicate and comparative with control negative (-) and all honeybee colonies nearly were the same strength under the same condition.

3. Measurements:

Evaluation was after twelve days, included, bee population, brood Area, and beebread area, and all areas measured using a typical longstroth frame divided into sq.inch, at 12days intervals.

Results and Discussion

1. Activities of bee population:

As a number of combs completely covered with honey bee worker. Statistical analysis from Table (1), showed that, no significant differences between all treatments from the beginning of evaluation at zero time read depend on replication similarity and colonies near the same strength. Slightly differentiation appeared after 24 days of treatments for worker population with no significant effect, with mean ranged from (6.17 to 7.33 combs covered with bees), during the first 5 or 6 days of adult life, worker bees consume large amounts of pollen to obtain the protein and amino acids required to complete their growth and development. A larva is regularly inspected by nurse bees and fed if necessary, so that it is always sufficiently provided with food (Robert and Karl, 2010), so after 36 days of treatments according to data of worker populations that depended on number of combs covered with honey bee workers gradually increased and recorded means 7.75, 8.00 and 8.69 for treatments FB.S., FB. 7gm/L, FB. 6gm/L and FB. 5gm/L, respectively, with no significant effects.

2. Brood areas:

If young adult worker bees do not consume needed proteins, their hypopharyngeal glands (brood food glands) will not develop completely, and their royal jelly will not support normal growth and

development of worker larvae or egg production in the adult queen. (Standifer *et al.*,1977). Highly significant effect appeared of brood areas using FEEDBEE® 5gm/L with mean 731.67 inch^2 after 12days and decreased significant with FEEDBEE® 6 and 7gm/L with means 538.33 inch^2 and 688.33 inch^{2,} respectively. FEEDBEE® substitute showed no significant effect with mean 464.00 inch^2. So, to improved honeybee physiological conditions its necessary addition of protein to the carbohydrate food (Perl'son, 1961).

On the other side, honey bees mix pollen with regurgitated nectar, honey and glandular secretions to produce bee bread, which differs from freshly collected pollen, in having a lower pH and less starch (Herbert and Shimanuki, 1978 and Ellis and Hayes, 2009). The shift in the quality of pollen stored in the colony (bee bread) is attributed to microorganisms associated with the honey bee (Gilliam, 1997). After 36 days of treatments according to data of brood areas highly significant effects showed in brood areas with concentrations 5 gm/L and 7gm/L with means 935.00 and 839.00 inch^{2,} respectively, less brood received only with carbohydrate diet (Standifer *et al*. ,1971). Followed by concentration FB. 6gm/L with mean 626.00 inch^2, finally, FB.S. was the lowest significant with mean 531.00 inch^2.

3.Stored pollen "Beebread":

In the colony, honey bees mix pollen with regurgitated nectar, honey and glandular secretions to produce bee bread, which differs from freshly collected pollen, in having a lower pH and less starch (Herbert and Shimanuki,1978 and Ellis and Hayes, 2009). Pasquale *et al.* (2013) found that both bee physiology and tolerance to a parasite varied depending on the type of pollen diet, suggesting that not only does the availability but also the quality of environmental resources matter. The shift in the quality of pollen stored in the colony (bee bread) is attributed to microorganisms associated with the honey bee (Gilliam, 1997). The basic principle of an artificial diet should be that it contains all the ingredients, texture, and consistency that are acceptable to the honeybees (Herbert and Shimanuki, 1979; Schmidt *et al*., 1987; Wilson *et al.*,2005 and Saffari *et al*., 2010). It must have nutritional values and be free from anti-nutritional factors (Schmidt *et al*., 1987; Herbert, 2000; Wilson *et al*., 2005 and Saffari *et al*., 2010). Vásquez and Olofsson (2009) suggested that lactic acid bacteria from the honey bee stomach belonging to the genera *Lactobacillus* and *Bifido bacterium* are involved in the fermentation process of beebread and may be responsible for improving the nutritive value by producing vitamins (Ellis and Hayes, 2009). More brood than those received only carbohydrate diet (Standifer *et al*.,1971).

Stored pollen at the first read showed not significant effect between all treatments and ranged from (34.00 to 89.00 inch^2), while low significant showed at the second read after 24 days and it recorded 19.67 inch^2 for the con. FB. 5gm/L,48.33 and 29.00 inch^2 for the con. FB. 7gm/L and FB.S., respectively, followed by 67.33 inch^{2}for the con. FB. 6gm/L against control 116.50 inch^2.

Table (1): FEEDBEE® effects on honey bee worker population "as a number of covered combs with workers, brood area and beebread area, reflacting of four concentration.

	Treatment	Zero time	1 st Read	2 nd Read	3 rd Read	Mean
worker population	**FEEDBEE® 5gm/L**	4.67[a]	5.33 [a]	7.33 [a]	8.69 [a]	6.51 [a]
	FEEDBEE ®6gm/L	4.67 [a]	5.33 [a]	6.17 [a]	8.00 [a]	6.04 [a]
	FEEDBEE ®7gm/L	4.83 [a]	5.50 [a]	7.00 [a]	8.00 [a]	6.33 [a]
	FEEDBEE®SUBSTITUTE	4.75 [a]	5.50 [a]	6.75 [a]	7.75 [a]	6.19 [a]
	control(-)	4.75 [a]	5.50 [a]	6.50 [a]	7.17 [a]	5.98 [a]
brood area	**FEEDBEE®5gm/L**	251.33 [a]	593.33 [a]	731.67 [a]	935.00[a]	627.83[a]
	FEEDBEE® 6gm/L	296.00 [a]	463.33 [a]	538.33[bc]	626.00[b]	480.92[bc]
	FEEDBEE® 7gm/L	386.67 [a]	570.67 [a]	688.33[ab]	839.00[a]	621.17[ab]
	FEEDBEE®SUBSTITUTE	330.00 [a]	445.00 [a]	464.00[c]	531.00[bc]	442.50[c]
	control(-)	407.50 [a]	511.50 [a]	500.00[c]	454.50[c]	468.38[c]
beebread area	**FEEDBEE® 5gm/L**	14.67 [a]	46.67 [a]	19.67[c]	22.12[c]	25.78[b]
	FEEDBEE® 6gm/L	51.67 [a]	89.00 [a]	67.33[b]	43.00b[c]	62.75[b]
	FEEDBEE® 7gm/L	28.67 [a]	43.00 [a]	48.33[bc]	58.00[b]	44.50[b]
	FEEDBEE®SUBSTITUTE	21.00 [a]	46.50 [a]	39.00[bc]	48.00b[c]	38.63[b]
	control (-)	15.00 [a]	34.00 [a]	116.50[a]	438.00[a]	150.88[a]

Pollen pellets from 15 species were identified as providing protein levels below those acknowledged to satisfy honey bee dietary requirements when they are the only source of pollen available to the honey bee colony (Somerville and Nicol, 2006), so stored pollen in the colony "beebread", while absent of protein source its stimulated colonies to gathered pollen to complete food chin for honey bee as a natural protein. Control recorded high significant effect than all treatments with mean 438.0 inch^2 after 36 days of FB. evaluation but the lowest significant effect recorded for powder FB. 5gm/L with mean 22.12 inch^2, and other treatments FB. 6gm/L, FB.S. and FB. 7gm/L ranged between 43.0, 48.0 and 58.0inch^2, respectively.

Generally, for three parameters; worker's population increased in evaluation time increased, but weren't significant effect it may will be appear next 12days. While in the brood areas data approved that concentration of FB. 5gm/L was the high significant effect with mean 627.83 inch^2, followed by concentrations FB. 6 and7gm/L with a few mortalities in bee feeder. While FB.S. showed low effective and beebread areas, showed low significant effect with FB.S. against control it may be related to colonies necessary needs. Obviously, there is an inverse relationship between increasing brood areas and beebread areas. Perhaps it related to specific requirement of amino acids for normal growth and development, reproduction, and brood rearing. The protein and amino acid requirements of larval and adult queens are unknown, but we have a fairly comprehensive knowledge of the chemical constitution of their basic food (Standifer *et al.*,1977).

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Development and reproduction of the cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) in laboratory

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Abstract:

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is an invasive pest species that has appeared in different parts of the world. The aim of this study is to investigate the influence of four seasons temperature and relative humidity on the development and reproduction of the cotton mealy bug. The results revealed that, incubation period significantly decreased from 7.43 ±0.51 hours in winter at 15.43°C with 52%RH to 1.90±0.18 hours in summer at 29.6°C with 51.24% RH. The developmental period of nymphal stage tented to be shortened with the corresponding temperature. Life period of first, second and third nymphal instars of female was decreased from 17.59, 16.02 and 17.01 days in winter at 15.43°C with 52% RH to 4.26, 4.50 and 5.004 days in summer at 29.6°C with 51.24% RH, while, the two nymphal instars (1st and 2nd instars) of male decreased from 17.89, 17.11 days to 4.50, 6.033 days, respectively, in winter and summer at the same temperature and humidity. Pupae duration decreased from 19.00 days in winter at 15.43°C with 52% RH to 6.00 days in summer at 29.6°C with 51.24% RH. The total female life cycle decreased from 102.52 days in winter at 15.43°C with 52% RH to 38.43 days in summer at 29.6°C with 51.24% RH. The total male life cycle decreased from 56.20 to days in winter at 15.43°C with 52% RH to 17.50 days in summer at 29.6°C with 51.24% RH. There was significant effect of temperature on fecundity of adult female, the mean of female fecundity was 495±0.46 eggs under perfect conditions in summer at 29.6°C with 51.24% RH. Total life cycle (both male and female) was prolonged at lower temperature and shortened at higher temperature. Optimum temperature for development of the pest was in summer at 29.6°C with 51.24% RH. It is concluded that the study *P. solenopsis* biology, give the understanding of mode and degree of its population growth. Hence, this information will be helpful during the development of successful integrated pest management program (IPM) for *P. solenopsis*.

Introduction

The cotton mealybug, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) is a major threat to agriculture and horticulture in many tropical and subtropical countries which was found to attack large number of plant species including crops, vegetables, ornamental plants and weeds (Wang *et al*., 2010 and Abbas *et al*., 2010). However, a decade ago, the evidence of mealybug was reported from Uttar Pradesh, Madhya Pradesh and Karnataka (Bambawale, 2008 a and b). Further detail studies support the strong evidences for its presence in India (Hodgson *et al.*, 2008). The newly world species of mealybug, *P. solenopsis* has emerged as a serious pest of cotton in Pakistan and India and is now being as a serious threat to cotton in China. It has been reported from 173 species in 45 families and from 26 countries in different ecological zones (Abbas *et al*., 2010). *P. solenopsis* cause crinkling, twist and condense flower, bud, bolls growth and finally it cause yield loss (Sahito *et al*., 2009).The mealybug insects has many traits make it a serious pest like the body covered with mealy wax section reduced the insecticide effects and save it from natural enemies attack, as well as, the highly spreading because diversity of reproduction manners and various host plant (Al-Rubeae and Al-Obaidi, 2014). In Egypt, this pest was recorded for the first-time infesting *Hibiscus* sp. in September, 2009 by Abd-Rabou *et al*. (2010). This pest spread rapidly on different host plants to the extent that recorded it on 29 host plant species belonging to 16 plant families including field crops (3), vegetables (3), ornamentals (7), weeds (13) and fruits (3) (Abdel-Razzik *et al.*, 2015).

The present work was conducted to determine the effect of temperature and relative humidity of seasons on the biology of the cotton mealybug at different sets of climatic conditions.

Materials and Methods

1.Collection and rearing of insects:

To have a culture of the cotton mealybug, *P. solenopsis*, ovi-positing females were collected from infested ornamental plant, *Hibiscus* sp. and reared on sprouting potato tubers under laboratory conditions, Plant Protection Research Institute, Giza, Egypt. The collected mealybug was identified at Scale Insects and Mealybugs Research Department, Plant Protection Research Institute, Agriculture Research Center.

To establish initial culture of *P. solenopsis*, steams of the host plants infested with adult females were brought to the laboratory individuals were separated and inoculated on sprouting potato in carton cylindrical boxes (8 cm long and 12 cm diameter) and reared in the laboratory. After about three days, the female mealybugs settled on sprouting potato and started egg laying. The crawlers emerged out and started feeding on the sprouting potato tubers. For that individual sprouting potato of same size and did not exposed to any previous pesticide applications and free from any infestations, were washed with tap water, shade dried and used as food. Each sprouting was infested with an adult female mealybug individual and was individually transferred to sprouting in carton cylindrical boxes (8 cm long and 12 cm diameter).

2. Data collection:

When the newly emerged crawlers settled for feeding on sprouting potato, the crawlers were marked by drawing a circle around them and were observed daily in the morning till they attained adult stage for further aspects of biology. The eggs laid by females of *P. solenopsis* were examined under binocular microscope for color, shape and size. The time of egg laying was noted, freshly laid eggs were counted and transferred to fresh sprouting potato. Time taken for egg hatching was recorded to obtain the incubation period. The freshly emerged nymphs were marked individual characteristics shape, size and color on the sprouting potato and observed daily under

microscope to note moulting process. The moulting was confirmed by the presence of exuivum on the sprouting potato or on the posterior end of nymphs. Data on morphological characters and duration for relative development at various sets of temperature and humidity were recorded. The pupal stage duration, shape, color and size was observed and recorded twice a day. Adult stage characteristics, size, shape, color, duration, longevity, fecundity, pre-oviposition period, oviposition period and post- oviposition period were observed and recorded twice day.

The study was conducted between January 2015 to December 2015 in the laboratory when mean temperature and mean relative humidity of the study seasons ranged from 15.43, 22.50, 23.34 and 29.60°C and 52.00, 47.10, 51.24 and 57.60 % RH, respectively. Thermo-hygrograph was used to maintain temperature and humidity of experimental units. Data were statistically analyzed for analysis of variance and mean by Duncan test (P=0.05) using Costat (2005) software.

Results and Discussion

1.The eggs:

The cotton mealybug eggs were very small, oval in shape, yellowish green color with 0.4x0.1mm size. The eggs were present in the pouch made up of silken thread in the last abdominal segments of female insect. The egg duration differed at different seasons. It was 7.43 ±0.51 hours in winter at 15.43°C with 52%RH; 5.43± hours in spring at 23.34°C and 47.07%RH; 1.90±0.18 hours in summer at 29.6°C with 51.24%RH and 4.93±0.33 hours in autumn at 22.5°C and 57.5%RH (Table,1). The results are similar to those of Amjad *et al*. (2012) who reported that eggs of the cotton mealybug were small, having yellowish green color with 0.3x0.1mm size and were present in the ovisac. The data showed the decreasing incubation period from 7.43 hours to 1.90 hours from 15.43to 29.6°C, respectively. The findings confirm with this of Chong *et al.* (2008) who found prolonged duration at lower temperature and shorted at higher temperature.

Table (1): Effect of various seasons temperature and relative humidity on different stages of *Phenacoccus solenopsis* female under laboratory conditions.

Stags	Duration(days) in various seasons							
	Winter		Spring		Summer		Autumn	
	Temp. °C	%RH	Temp. °C	%RH	Temp. °C	%RH	Temp. °C	%RH
	15.43	52	23.34	47.07	29.6	51.24	22.5	57.6
Egg (LSD=0.991)	7.43±0.51[a]		5.43±0.17[b]		1.90±0. 18[c]		4.93±0.33[b]	
1st instar (LSD=0.921)	17.89±0.21[a]		7.74±0.18[b]		4.26±0.27[c]		8.17±0.47[b]	
2nd instar (LSD=0.793)	16.02±0.12[a]		7.65±0.31[b]		4.50±0.[30c]		8.16±0.20[b]	
3rd instar (LSD=0.737)	17.01±0.18[a]		8.24±0.28[b]		5.04±0.25[c]		9.04±0.25[d]	
Nymphal stage (LSD=1.203)	51.00±0.16[a]		23.62±0.38[b]		13.78±0.63[c]		25.38±0.41[d]	
Pre-ovipositing (LSD=0.848)	20.16±0.35[a]		8.47±0.25[b]		7.00±0.25[c]		8.00±0.251[b]	
Ovipositing (LSD=0.903	25.00±0.31[a]		13.16±0.30[b]		12.26±0.39b[c]		13.03±0.37[c]	
Post-ovipositing (LSD=0.898)	6.40±0.18[a]		4.26±0.37[a]		5.42±0.34[b]		6.48±0.15[c]	
Adult longevity (LSD=1.2)	51.52±0.61 [a]		25.65±1.00 [c]		24.66±1.00 [c]		27.20±0.77 [b]	
Fecundity (LSD=98.196)	107.20±20.14[a]		318.80±49.2[b]		495.46±32.1[c]		306.10±22.8[b]	
Total life cycle (days) (LSD=2.478)	102.52±2.49[a]		49.51±1.71[b]		38.43±1.79[c]		52.58±1.69[d]	

Mean in row sharing similar letter are not significantly different by Duncan test at P= 0.5

2.The first nymphal instar:

The 1st nymphal instar of the cotton mealybug was small and yellowish green in color, their size varied from 6 mm to 2 mm. There was no wax coating on the 1st instar. The 1st instar was very fast crawlers and they quickly searched the food and settled on the host. The

duration of 1st instar of the cotton mealybug differed at different seasons, temperature and relative humidity. However, it declined with increase in temperature and decrease in relative humidity. The duration of 1st instar was 17.89±0.21days in winter at 15.43°C with 52 %RH; 7.74±0.18 days in spring at 23.34°C with 47.07%RH; 4.25±0.26 days in summer at 29.6°C with 51.24 %RH and 8.17±0.47days in autumn at 22.5 °C with 57.6 %RH, respectively (Table,1). The present study is in conformity with those of Aheer *et al.* (2009) who reported that the 1st instar of the cotton mealybug was yellowish green in color and fast crawler. The results indicated that survival of 1st instar was decreased at higher temperature and are agree with those of Ahree *et al.* (2009) and Amjad *et al.* (2012) they reported that duration of 1st instar 7-9 days at 25°C. Also, the present results can be compared with those of Child (2007) who reported that increase in damage to agriculture crop was observed due to increased environmental temperature.

3. **The second nymphal instar:**

The 2nd nymphal instar of *P. solenopsis* was light green in color and its size varied from 0.8-.41 mm. It has two black dots on the thorax and abdomen. They found on the twigs of host plants and waxy layer developed on the body surface through a few hours after moulting. The duration of 2nd instar of the cotton mealybug varied at different seasons. The duration of 2nd instar female 15.02±0.12 days in winter at 15.43ºC with 52%RH; 7.65±0.31 days in spring at 23.34 ºC with 47.07%RH; 4.50±0.29 days in summer at 29.6ºC with 51.24%RH and 8.16±0.201days in autumn at 22.5 ºC with 57.6%RH, respectively (Table, 1).

The duration of the 2nd instar male was 17.11±0.183 days in winter at 15.43ºC with 52%RH; 9.1±0.3 days in spring at 23.34°C with 47.07%RH; 6.033±0.34 days in summer at 29.6ºC with 51.24% RH and 10±0.35days in autumn at 22.5 ºC with 57.6 %RH, respectively (Table,2). These results occurred that life duration of the 2nd instar both female and male decreased as the temperature increased. Thus, increase in temperature lead to rapid growth of the cotton mealybug of 2nd instar of both sexes. These findings are in conformity with those of Aheer *et al.* (2009) who reported light green color of 2nd instar and variation in duration of both sexes i.e. 4 to 9 days in male and 3 to 4 days in female. Similar results were also obtained by Amjad *et al.* (2012) who revealed that the duration of 2nd instar (female) was 4.6 days at 20 ºC with 75%RH which gradually reduced to 3days at 40 ºC with 40%RH.

Table (2): Effect of various seasons temperature and relative humidity on different stages of *Phenacoccus solenopsis* male under laboratory conditions.

Stags	Duration(days) in various seasons							
	Winter		Spring		Summer		Autumn	
	Temp °C	%RH	Temp. °C	%RH	Temp. °C	%RH	Temp. °C	%RH
	15.43	52.00	23.34	47.07	29.60	51.24	22.50	57.60
Egg (LSD=0.991)	7.43±0.51[a]		5.43±0.17[b]		1.90±0.18[c]		4.93±0.33[b]	
1st instar (LSD=0.921)	17.89±0.209[a]		8.34±0.42[b]		4.26±0.268[d]		8.17±0.47[c]	
2nd instar (LSD=0.859)	17.11±0.18[a]		9.05±0.29[b]		6.033±0.338[c]		10.00±0.354[b]	
Pupae (LSD=0.932)	19.00±0.32[a]		8.00±0.36[b]		6.00±0.351c		8.13±0.13[b]	
Adult longevity (LSD=0.356)	2.20±0.11[a]		2.14±0.26[a]		1.60±0.16[a]		2.74±0.16[b]	
Total life cycle (LSD=1.472)	56.20±3.88[a]		27.50±1.33[c]		17.51±1.12[d]		28.72±1.11[b]	

Mean in row sharing similar letter are not significantly different by Duncan test at P= 0.5

4. The third nymphal instar:

The 3rd nymphal instar of the cotton mealybug was light green in color with present two dots on the thorax and

abdomen. White waxy layer appeared on 2nd day. The third instar appeared only in the female specimens while in male specimens pupae were formed. The duration of 3rd instar was 17.01±0.18 days in winter at 15.43ºC with 52%RH; 8.24±0.29 days in spring at 23.34 ºC with 47.07%RH; 5±0.25 days in summer at 29.6ºC with 51.24%RH and 9±0.25 days in autumn at 22.5 ºC with 57.6%RH, respectively (Table,1). The results showed that life period of the 3rd instar female ranged from 5.00 to 17.01 days at season temperature range of 15.4 ºC to 29.6 ºC, respectively. Thus, lower temperature prolonged life span of the insect on opposite the higher temperature. Aheer *et al.* (2009) they recorded that the third instar female was light green in color and completed its life in 6.5 to 8 days at 25 ºC and 70% RH. Amjad *et al*. (2012) reported that low temperature resulted in longer development period.

5. The pupae:

Pupae were white in color enclosed in silken sac made up of white threads. Pupae was light green; its size was 1.70 and 0.40mm. The duration of pupae was 19.00±0.3 days in winter at 15.43ºC with 52% RH; 8.00±0.36 days in spring at 23.34 ºC with 47.07% RH; 6.00±0.35 days in summer at 29.6ºC with 51.24%RH and 8.13±0.13 days in autumn at 22.5ºC with 57.6% RH, respectively (Table, 2). The present findings are similar to Aheer *et al*. (2009) who reported that male pupae were formed after 2nd instar and pupae duration was 7 to 8 days at 25ºC. Amjed *et al*. (2012) who reported the pupae duration decreased from 9.57 days at 20ºC to 3.78 days at 35ºC.

6. The adult female:

The adult female was wingless and oblong in shape with light green in color and converted into dark brown when died, there were two pairs of dark black spots on dorsal surface of female body, it size varied from 1.60 mm to 2.25 mm. As shown in Table (1), the adult female longevity was divided into pre-oviposition, oviposition and post–oviposition periods . In general, the adult longevity for the cotton mealybug female differed at reached different temperature and relative humidity. The findings are in conformity with those of Shaymaa *et al*. (2017) who reported the female was wingless, oblong in shape and having two pairs of black spots on dorsal side of body region.

7. The pre-ovipostion period:

Results showed that the female passed through the pre-ovipositing period were varied from one season to another. Duration of pre-ovipositing was 14.36±0.16 days in winter at 15.43ºC with 52 %RH; 8.47±0.253 days in spring at 23.34 ºC with 47.07% RH; 6.99±0.25 days in summer at 29.6ºC with 51.24 % RH and 7.68±0.25 days in autumn at 22.5 ºC with 57.6 % RH, respectively (Table, 1).

8. The oviposition period:

This period was found to be the longest of adult female's life throughout all seasons. The duration of this period was varied from one season to another. Duration of oviposition period was 25.00±0.31 days in winter at 15.43ºC with 52% RH; 13.16±0.30 days in spring at 23.34 ºC with 47.07% RH; 12.26±0.39 days in summer at 29.6ºC with 51.24% RH and 13.03±0.37 days in autumn at 22.5 ºC with 57.6 %RH, respectively (Table, 1).

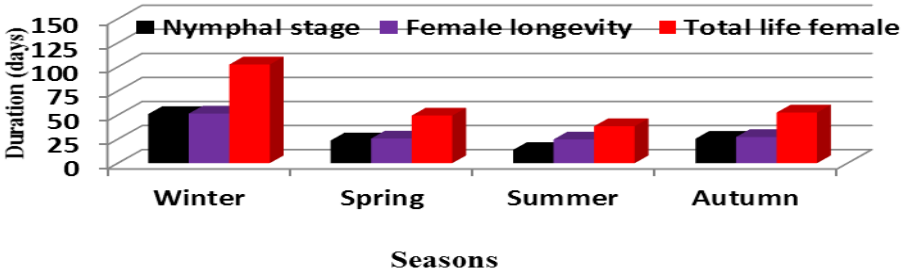
9. The post-oviposition period:

This period was elapsed after female had stopped oviposition till the death. The post-oviposition period was found to be the shortest one. Mean durations of this period were 6.40±0.18 days in winter at 15.43ºC with 52%R.H.; 4.26±0.73 days in spring at 23.34 ºC with 47.07 R.H.%; 5.45±0.34 days in summer at 29.6ºC with 51.24 R.H.% and 6.48±0.15 days in autumn at 22.5 ºC with 57.6 R.H.%, respectively (Table, 1).

10. The adult female longevity:

The duration of the female longevity differed at reached different temperature and relative humidity. Mean durations of this period were 51.52±0.61days in winter at 15.43ºC with 52% RH; 25.65±0.1 days in spring at 23.34 ºC with 47.07 % RH; 24.66±1 days in summer at 29.6ºC with 51.24 % RH and 27.20±0.77 days in autumn at 22.5 ºC with 57.6 % RH, respectively (Table,1 and Figure,1). The adult female longevity period decreased significantly with increase of temperature and vice versa. Nikam *et al*. (2010) who found that longevity of female was 33.67±1.19 days at temperature and relative humidity range from 25 to 30ºC and 75 to 80% RH, respectively. Similarly, studies on the biology of mealybug *P. solenopsis* under laboratory condition on potato sprout at Hayana Agriculture University, Hisar found that the total life span was 39.12±2.85 and 18.60±1.5 days (Kedar *et al*., 2011). Nikam *et al*. (2010) reported that longevity of female was 33.67±1.19 days at temperature and relative humidity ranged from 20 to 30ºC and 75 to 80%RH, respectively. Shaymaa *et al*. (2017) and Shehata (2017) who reported that the adult female longevity of *P. solenopsis* was shorter 30ºC.than 20ºC.

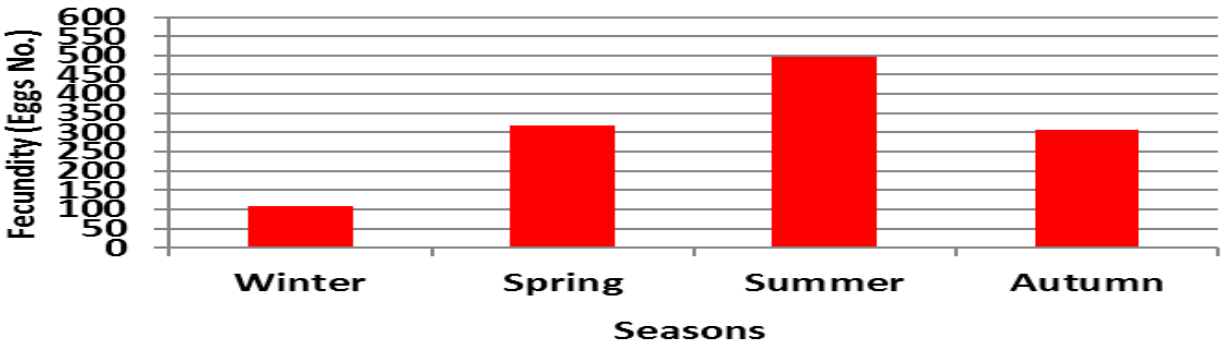
Figure (1): Effect of various seasons temperature and relative humidity on different stages of *Phenacoccus solenopsis* female under laboratory conditions.



11.Fecundity of adult female:

The results showed that the total number of deposited eggs/females differed at different seasons, temperature and relative humidity during its life cycle. Data given in Table (1) and illustrated in Figure (2) showed the total number of deposited eggs/female during its ovi-position period was 107.20±20.14 days in winter at 15.43ºC with 52% RH; 318.80±49.2 days in spring at 23.34 ºC with 47.07 %RH; 495.46±32.1 days in summer at 29.6ºC with 51.24% RH and 306.10±22.8 days in autumn at 22.5 ºC with 57.6 %RH, respectively. The results clearly indicated that the highest number of egg in summer at 29.6ºC. It could be concluded that the temperature may be considered as the thermal optimum for egg laying activity.

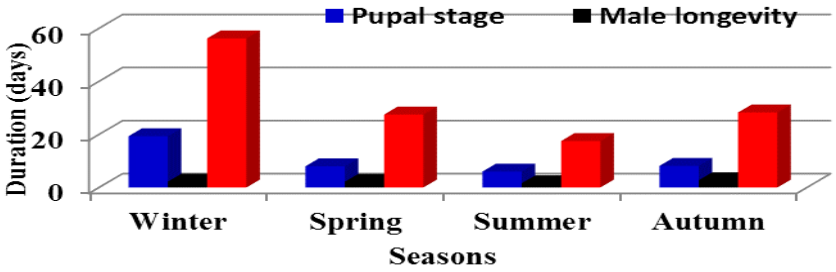
Figure (2): Effect of various seasons temperature and relative humidity on fecundity of *Phenacoccus solenopsis* female under laboratory conditions.



12.The longevity of adult male:

The adult male of the cotton mealybug was blackish brown in color and had four waxy filaments at the end male body and winged, wings were transparent. Size of adult male was 1.3 mmx0.4 mm. The duration of the adult male was varied from one season to another. Duration of the adult male was 2.20±0.11 days in winter at 15.43°C with 52% RH; 2.14±0.26 days in spring at 23.34°C with 47.07% RH; 1.60±0.16 days in summer at 29.6°C with 51.24% RH and 2.74±0.16 days in autumn at 22.5 °C (Table, 2 and Figure, 3). Prishanthini and Vinobaba (2013) found that longevity of *P. solenopsis* male ranged from 1 to 2 days with an average of 1.50±0.5 days. Also, Aheer *et al.* (2009) reported that adult male is blackish brown in color, with transparent wings and four abdominal segments. They further reported that adult survived 1-2 days at 25°C with 75% RH.

Figure (3): Effect of various seasons temperature and relative humidity on different stages of *Phenacoccus solenopsis* male under laboratory conditions.



13.Total nymphal stage duration:

The results in (Table, 1 and Figure,1) indicate that duration of total nymphal stage tented to be shortened with the corresponding raise of temperature. Under any of the temperature of season, the total nymphal stage of *P. solenopsis*s as a whole varied greatly. The duration of total nymphal stage was 51.00±0.16 days in winter at 15.43°C with 52%RH; 23.62±0.62 days in spring at 23.34 °C with 47.07% RH; 13.00±0.63 days in summer at 29.6°C with 51.24%RH and 25.38±0.41 days in autumn at 22.5 °C. The obtained results are in agreement with (Aheer *et al.*, 2009 and Amjad, 2012) who reported that decreased in temperature increased the duration of nymphal stage and increase in temperature decreased the duration of nymphal stage.

14.Total life cycle of female:

The results in (Table, 1and Figure, 1) showed that duration of total life cycle of female varied significantly with increases in temperature and relative humidity. The duration of total female life was 102.52±2.49 days in winter at 15.43°C with 52% RH; 49.51±1.71 days in spring at 23.34°C with 47.07% RH; 38.43±1.79 days in summer at 29.6°C with 51.24%RH and 52.58±1.69 days in autumn at 22.5°C. These results are similar to those of (Aheer *et al.*, 2009 and Amjad, 2012) who reported that duration of female life cycle reduced on increasing temperature and vice versa.

15. Total life cycle of male:

The total life cycle of male of the cotton mealybug duration varied significantly with increase in temperature and relative humidity. The duration of the life cycle male was varied from one season to another. Duration of the total life cycle male was 56.20±2.88 days in winter at 15.43°C with 52%RH; 27.50±1.33 days in spring at 23.34 °C with 47.07%RH; 17.51±1.12 days in summer at 29.6°C with 51.24%RH and 28.72±1.11 days in autumn at 22.5°C, respectively in (Table,

2 and Figure, 2). Also, Aheer *et al*. (2009) reported that decrease in temperature increased the duration while increase in temperature resulted in decreased duration of insect.

It is concluded that the study *P. solenopsis* biology, give the understanding of mode and degree of its population growth. Hence, this information will be helpful during the development of successful integrated pest management program (IPM) for *P. solenopsis* which is considered as major polyphagous pest in the world.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Toxicity and development of resistance in *Tribolium castaneum* and *Sitophilus oryzae* to certain selected insecticides

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Key words: *Tribolium castaneum*, *Sitophilus oryzae*, toxicity resistance and cross-resistance.

Abstract:

The rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) are considered the most important insect species which destruct the stored grain. Application of synthetic insecticides for stored grain protection has been created resistance and cross-resistance in storage insects. Laboratory bioassays were undertaken to assess the toxicity, resistance and cross-resistance of insecticides, chlorpyrifos, chlorpyrifos-methyl, malathion, pirimiphos-methyl, cyfluthrin, cypermethrin and methomyl, to *T. castaneum* and *S. oryzae.* Two methods, exposure to treated surface and mixing with wheat grain as well as selective pressure and cross-resistance studies using LC_{50} were used. Results obtained after 24h by thin film method revealed that pirimiphos-methyl was the premier insecticide to *T. castaneum* with LC_{50} of 0.007 µg/cm^2. While methomyl showed low toxicity. On the other hand, chlorpyrifos-methyl was the most toxic compound to *S. oryzae* with LC_{50} of 0.007 µg/cm^2. For mixing with medium the order of toxicity obtained in this technique was different from that in thin film residue method. The development of resistance in *T. castaneum* indicated that methomyl failed to build up resistance after 4 generations since its resistance factor was 5 fold. The highest level of resistance was induced by malathion followed by pirimipho-methyl with resistance factor, R.F., of 214.4 and 67.4-fold, respectively. Selective pressure with chlorpyrifos for 4 generations did not succeed in building up more than 10.9 fold. Data also, presented that all of the tested Op-resistant strains of *T. castaneum* show only low level of cross-resistance to the pyrethroid insecticides cyfluthrin and cypermethrin at the LC_{50} level (R.F. values ranged from 2.55 to 6.4 fold). These resistant strains were more susceptible to the insecticide, chlorpyrifos-methyl than the susceptible strain (RF values ranged from 0.29 to 0.66). It is concluded that the common insecticides, malathion and pirimipho-methyl have created high level of resistance compared to methomyl which was rarely used in grain protection.

Introduction

Stored products are subjected to attack by numerous of insects which reduce weight and quality. These are the rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae, which cause misfortune wastages to wheat grains and their products. Rice weevil has lately become the number one pest of whole grains in modern commercial storage system (Kljajić *et al*., 2006). Conventional pesticides have been used as the major tools for stored grain and food protection. Actually, whatever the efficiency of the chemical insecticides for protecting stored grains, but it has created some problems involving pesticide resistance, toxic residues, health hazards and pest resurgence. Pesticide resistance is an increased tolerance to a pesticide that has a genetic basis. As a heritable trait, the development and spread of resistance will be influenced by the selective pressures of pesticide use, fitness costs associated with individuals carrying resistance gene, and movement of pests on geographical scales. Since the mid-20th century insecticides have been drawn mainly from the organophosphates (OPS) (malathion, chlorpyrifos, methomyl and dichlorvos), the pyrethroids (bioresmethrin, deltamethrin, and beta-cyfluthrin) and the Juvenil hormone analogues (JHAs) (methoprene and hydroprene) (Opit *et al*. ,2012). Cross - resistance is when resistance to a given pesticide causes resistance to another pesticide without the insect having been exposed to the latter pesticide (Scott *et al*., 1990). For example, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) that is resistant to one organophosphate has a tendency to be resistant to other organophosphates. A similar situation occurs with pyrethroid resistant *T. castaneum* and *R. dominica* (Collins, 1990; Guedes *et al.,* 1996 and Daglish *et al.,* 2003). Many authors established many cases of insect resistance against more registered insecticides which belong to different groups over worldwide. Resistance to malathion in *S. oryzae* has been reported from Egypt (Toppozada *et al*., 1969), Asutralia (Rimes and Moulden, 1979) and USA (Halisack and Beeman, 1987 and Irshad and Jillani, 1992). The development of resistance became ordinary phenomenon especially in grain stored because of the suitable conditions. To prevent or minimize the resistance and cross-resistance the current study dealt with their extent notably with *T. castaneum* and *S. oryzae* to some chemical insecticides (chlorpyrifos, chlorpyrifos-methyl, cyfluthrin, cypermethrin, malathion, methomyl and pirimiphos-methyl) through the selective pressure bioassay pattern and how to suppress it.

Materials and methods

1. Insecticides:

2. Chlorpyrifos:

Chemical name: O. O-diethyl-O-3, 5, 6-trichloro-2 pyridyl phosphorothioate.

48% E.C. produced by Dow Chemical Co.

Trade marks: Dursban, Brodan and Dowco 179.

1.2. Chlorpyrifos-methyl:

Chemical name: O,O, dimethyl O- (3, 5, 6-trichloro-2 – pyridyl) phosphorothioate.

E.C. 50% Dow Chemical Company.

Trade marks: Dowco 214, Reldan and Zertell.

3. Malathion:

Chemical name: O.O-dimethyl phosphorodithioate ester of diethyl mercaptosuccinate.

E.C. 57% produced by Sumitomo Chemical Company.

Trade marks: Charbophos, Chemathion and Malaspray.

1.4. Pirimiphos-methyl:

Chemical name: 2-diethyl-amino –6 – methyl pyrimidin – 4 – yl O.O-dimethyl phosphorothioate.

Trade marks: Actellic, Blex and Silosan

1.5. Cyfluthrin:

Chemical name: Cyano (4-fluoro- 3 – phenoxyphenyl) methyl 3- (2,2 –

dichloroethenyl) -2, 2 – dimethyl – cyclopropanecarboxylate.
5% E.C. Bayer AG of West Germany. Being developed in the U.S. by Mobay chemical corp.
Trade marks: Baythroid and Bay-FCR-1272.

1.6. Cypermethrin:

Chemical name: (±) alpha-cyano-3-phenoxybenzyl (±) cis. trans 3-(2,2-dichlorovinyl)-2, 2-dimethyl cyclopropane-carboxylate.
E.C. 30% ICI, FMC, Sumitomo and Shell Chemical Co.
Trade marks: Ripcord, Ammo and Arrivo.

1.7.Methomyl:

Chemical name: S- methyl – N – (methyl carbamoyl oxy) thioacetimidate.
Oxime carbamate produced by Shell Chemical Co.
(90% soluble liquid).
Trade marks: Lannate, Nu. Bait and Nudrin.

1. Insects:

2.1. The rust red flour beetle, *Tribolium castaneum*:

The adults of rust red flour beetle, *T. castaneum* were collected from Kafr El-Sheikh rice and wheat mills companies. Insects were reared in a mixture of wheat seeds and wheat flour under laboratory conditions of 26±1°C and 65±5% R.H. The media contained also 5% dried yeast. Insects were reared for two years with the same above conditions. Adults of 2-3 weeks old were selected for toxicity evaluation tests, resistance and cross-resistance

2.2. The rice weevil, *Sitophilus oryzae*:

The adults of the rice weevil, *S. oryzae* were collected from Kafr El-Sheikh rice mills Co. they were reared on wheat grains, under laboratory conditions of 26±1°C, 65±5% R.H. insects were reared for two years in the Department of stored product pest Research, Sakha Agricultural Research station, adults of 16-21 days old were used for toxicity evaluation tests.

3. Bioassay procedures:

3.1. Exposure to treated surface (thin film technique):

Stock dilution (w/v) of each compound was prepared by dissolving the desired quantity in acetone and series of dilutions were prepared. Toxicity tests were carried out on films achieved by spreading aliquot of one ml of each concentration at the bottom of a Petri dish of 9 cm in diameter and left to dry. After complete dryness of the insecticide film, ten adult beetles from each of the tested insects were placed in each of the treated Petri dishes. The same number of insects also was confined on Petri dishes treated with acetone only and served as control. Mortality was recorded after 24 h of exposure and corrected by **Abbott's formula (1925)**. The LC_{50} values for all insecticides were calculated by the method of **Litchfield and Wilcoxon (1949)**.

3.2. Exposure to treated wheat grains (mixing with feeding medium):

Batches of uninfected wheat grain (of moisture content 9%) were weighed and placed in wide-mouth glass jars. The insecticides were diluted in water and added to the grains at rates which give the required concentrations. Jars were mechanically shaken for adequate and fixed time to ensure complete mixing process. Serial concentrations were made. The treated grains were allowed to dry at room temperature. For each concentration, twenty grams of treated grains were placed in Petri dishes (9-cm diameter) and this was replicated four times. Ten adults of the tested insects (2-3 weeks old) were transferred to each dish. The same numbers of insects were transferred to Petri dishes containing non-treated grains. Mortality counts were recorded after 24 h and corrected by **Abbott's formula (1925)**. LC_{50} values were calculated by the method of **Litchfield and Wilcoxon (1949)**.

3.3. Selective pressure and cross - resistance studies:

Adults of the laboratory strain *T. castaneum* were exposed to the LC_{50} of each of the tested insecticides pirimiphos-methyl, malathion, chlorpyrifos and methomyl for 24 h using thin film technique. The survival insects were transferred into clean Jars

containing clean and sterilized medium which was preheated in an oven at 70°C for 2 h. After 15 days the parent adult individuals were eliminated. After 60 days the adults of the new generation were treated in the same manner as mentioned before and so on up to four generations. At every generation, LC_{50}'s of the tested insecticides were determined using thin film technique. To determine the cross -resistance patterns, concentrations response tests for each resistant strain to each insecticide were carried out and compared with these of susceptible strain.

Results and Discussion

The toxic potency of tested insecticides was evaluated against *T. castaneum* and *S. oryzae* adults using to methods of bioassay, thin film residue and mixing with feeding medium.

1.Thin film residue:

Log-dosage probit regression lines were drawn and statistically analyzed according to the method of Litchfield and Wilcoxon (1949). Values of LC_{50}, confidence limits and slopes were calculated and recorded in Table (1). Based on the LC_{50} values, pirimiphos methyl was the most toxic compound to *T. castaneum* with LC_{50} value of 0.007 µg/cm^2 followed by chlorpyrifos, malathion, chlorpyrifos-methyl, cyfluthrin and cypermethrin. Insecticide, methomyl showed low toxicity (LC_{50} 1.1 µg/cm^2). On the other hand, chlorpyrifos- methyl was the premier insecticide to *S. oryzae* with LC_{50} of 0.007 µg/cm^2 followed by pirimiphos-methyl, chlorpyrifos, malathion, cypermethrin, cyfluthrin and methomyl with LC_{50} values of 0.02, 0.031, 0.132, 0.236, 0.283 and 0.472 µg/cm^2, respectively.

Tablel (1): Toxicity of insecticides against *Tribolium castaneum* and *Sitophilus oryzae* after 24h exposure to treated surface.

Insecticides	*Tribolium castaneum*			*Sitophilus oryzae*		
	LC50 (µg/cm2)	Confidence limits	slope	LC50 (µg/cm2)	Confidence limits	slope
Chlorpyrifos	0.036	0.05-0.025	1.39	0.031	0.04-0.028	5.6
Chlorpyrifos- methyl	0.088	0.12-0.036	1.5	0.007	0.009-0.006	1.7
Cyfluthrin	0.197	0.28-0.140	1.43	0.283	0.65-0.123	0.83
Cypermethrin	0.220	0.39-0.130	1.8	0.236	0.34-0.16	1.45
Malathion	0.066	0.08-0.060	2.6	0.132	0.15-0.12	5.26
Methomyl	1.100	1.77-0.680	1.23	0.472	0.66-0.34	2.2
Pirimiphos-methyl	0.007	0.01-0.005	1.6	0.020	0.03-0.015	2.6

The red flour beetle, *T. castaneum* is one of the most damage insect species invading warehouses and mills around the world (Rees, 2004; Almaši, 2008; Mahroof and Hagstrum, 2012). Regarding the control of that and some other stored product pests, a variety of factors decide the effectiveness of contact insecticides, the most important of which is insect resistance (Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2005, 2006; Kljajić *et al.*, 2009; Boyer *et al.*, 2012 and Opit *et al.*, 2012).

2. Exposure to treated wheat grain:

The toxicity of the tested insecticides was also determined by exposure of *T. castaneum* and *S. oryzae* to treated wheat grains. The order of toxicity obtained in this technique was different from that in treated surface method. Data recorded in Table (2) showed that, insecticides can be arranged according to their toxicities to *T. castaneum* in the following descending order: Chlorpyrifos- methyl> chlorpyrifos > pirimiphos-methyl > cypermethrin >

cyfluthrin > malathion > methomyl. Against *S. oryzae*, the order of toxicity was slightly changed as follows: chlorpyrifos > chlorpyrifos- methyl > pirimiphos-methyl > cypermethrin > malathion > cyfluthrin > methomyl. Differences in the potencies of insecticides obtained according to the method of application may be explained. In treated surface method, insecticide act as a contact poison whereas in exposure to treated grains, insecticide act as a contact and/or stomach poison. Practically, exposure to treated grain method is largely preferred as a criterion for evaluating insecticides potency. Also, differences in the responses of the tested insects to insecticides may ascribe to the behaviouristical and alimentary habituations.

Generally, from the obtained results, organophosphorus compounds (OP) were the most effective insecticides against both insects. For pyrethroid insecticides, cypermethrin was more potent than cyfluthrin and both exhibited a considerable toxicity to *T. castaneum* and *S. oryzae*. The carbamate insecticide, methomyl showed very low toxicity particularly when mixed with grains and tested against both insects. On the other hand, the rice weevil, *S. oryzae* was more susceptible to the tested insecticides than the red flour beetle, *T. castaneum* when both were exposed to treated grains.

Abbassy *et al.*, 1977 and Masoud *et al.*, 1982 tested various insecticides against *Tribolium confusum* Duval (Coleoptera: Tenebrionidae), *T. castaneum* and *S. oryzae*, they found that, chlorpyrifos and pirimiphos methyl were the most effective compounds followed by certain pyrethroid insecticides e.g. Cypermethrin. Cyfluthrin was reported to be a promising insecticide for the control of pests that attack stored products and packing materials (Behrenz *et al.* 1983). The organophosphorus compound, chlorpyrifos-methyl was reported to have potent effects against many insects of stored products e.g. *S. oryzae, T. castaneum, R. dominica, Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae) (Quinlan *et al.*, 1979, Samson and Parker 1989 a, b; Samson *et al.*, 1989 and Daglish *et al.*, 1993).

Table (2): Toxicity of insecticides against *Tribolium castaneum* and *Sitophilus oryzae* after 24h exposure to treated wheat grain.

Insecticides	*Tribolium castaneum*			*Sitophilus oryzae*		
	LC_{50} (mg/g grain)	Confidence limits	slope	LC_{50} (mg/g grain)	Confidence limits	slope
Chlorpyrifos	0.0034	0.0034-0.0027	2.5	0.00070	0.0084-0.00058	3.3
Chlorpyrifos- methyl	0.003	0.0037-0.0025	2.6	0.0011	0.00136-0.0081	2.3
Cyfluthrin	0.023	0.0304-0.0212	1.9	0.028	0.0384-0.0204	1.67
Cypermethrin	0.0125	0.0178-0.0089	1.4	0.009	0.0124-0.0064	1.7
Pirimiphos-methyl	0.008	0.0099-0.0065	2.92	0.0028	0.00304-0.00257	6.7
Malathion	0.078	0.1053-0.0578	1.75	0.0185	0.0259-0.0132	1.7
Methomyl	>0.50	--- ---	---	0.50	0.5964-0.4167	3.8

3.Resistance and cross -resistance studies: 3.1. Development of resistance to four of the tested insecticides in *Tribolium castaneum*:

The development of resistance in laboratory strain of *T. castaneum* after selective pressure by pirimiphos-methyl, malathion, chlorpyrifos and methomyl for 4 generations was studied. Results are recorded in Table (3). Selective pressure with methomyl failed to build up resistance in the insect after 4 generations since its resistance factor was 5 fold. The highest level of resistance was induced by malathion followed by pirimiphos-methyl with resistance factors, R.F., of 214.4 and 67.4-fold, respectively. Selective pressure with chlorpyrifos for 4 generations did not succeed in building up more than 10.9 fold resistance indicating low level of resistance. This result revealed that, the development of resistance against chlorpyrifos is slower than that of pirimiphos-methyl or malathion.

Table (3): Comparative levels of resistance in *Tribolium castaneum* selected by different insecticides for 4 generations.

Insecticides	Generations											
	1			2			3			4		
	LC_{50} (µg /cm^2)	Slope	RF	LC_{50} (µg /cm^2)	Slope	RF	LC_{50} (µg /cm^2)	Slope	RF	LC_{50} (µg /cm^2)	Slope	RF
Chlorpyrifos	**0.267**	**3.30**	**7.42**	**0.283**	**2.11**	**7.86**	**0.346**	**1.92**	**9.61**	**0.393**	**2.00**	**10.90**
Malathion	**3.3**	**2.94**	**50**	**6.76**	**1.50**	**102.4**	**12.26**	**2.80**	**185.8**	**14.15**	**2.30**	**214.4**
Methomyl	**1.57**	**2.14**	**1.43**	**1.60**	**1.48**	**1.45**	**2.04**	**1.32**	**1.85**	**5.52**	**1.23**	**5.02**
Pirimiphos-methyl	**0.173**	**2.00**	**24.7**	**0.204**	**1.56**	**29.1**	**0.236**	**2.10**	**33.7**	**0.472**	**2.13**	**67.4**

The red flour beetle, *T. castaneum* is one of the most abundant and injurious pests of stored grain and flour in warehouses and mills. This insect has been subjected to considerable selection pressure with pesticides as a result of established control practices in the storage environment. As a consequence of the selection pressure, these beetles have developed resistance to many of the commonly used pesticides (Zettler, 1991). Among the insecticides involved in resistance of this insect is malathion. Malathion resistance in *T. castaneum* populations has become common in many regions of the world (Champ and Dyte, 1977) and was frequently reviewed by many authors (Osman and Rejesus, 1981; Navarro *et al.*, 1986; Picollo de Villar *et al.*, 1987; Halliday *et al.*, 1988; Subramanyam *et al.*, 1989; Beeman and Wright, 1990; Collins, 1990; Herron, 1990; Sayaboc and Acda, 1990; Zettler and Cuperus, 1990 and Zettler, 1991).

3.2. Cross-resistance:

Pirimiphos-methyl, chlorpyrifos and malathion resistant strains of *T. castaneum* were tested to determine their susceptibility to chlorpyrifos-methyl, cypermethrin and cyfluthrin. Table (4) revealed that all of the tested OP-resistant strains of *T. castaneum* show only low level of cross -resistance to the pyrethroid insecticides cyfluthrin and cypermethrin at the LC_{50} level (RF values ranged from 2.55 to 6.4 fold). On the other hand, these resistant strains were found to be more susceptible to the insecticide, chlorpyrifos-methyl than the susceptible strain (RF values ranged from 0.29 to 0.66).

Similar results were obtained by many authors. Organophosphorus-resistant strains of *Oryzaephillus surinamensis* L. (Coleoptera: Silvanidae), Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) and *T. castaneum* did not show cross-resistance to chlorpyrifos-methyl (Attia and Frecker, 1984; Summer *et al.*, 1988 and Subramanyam *et al.*, 1989). In malathion-resistant of *T. castaneum*, cross-resistant to the pyrethroid, permethrin was not detected (Zettler and Jones, 1977). Attia and Frecker (1984) reported that, OP-resistant strain of *O. surinamensis* showed low level of resistankce to dichlorovos, bioresmethrin and pyrethrins (<10-fold). Bansode and Campbell (1979) found that, malathion resistant strain of the red flour beetle *T. castaneum*, did not show cross-resistance to 4 other organophosphorus insecticides but exhibited tolerance to these insecticides (0.8-1 fold). Generally, in order to have effective control methods available in the eventuality that resistance in insects increases to the extent that present chemical controls are no longer effective, it is important to test new materials and methods against strains of insects. The tested OP compound, chlorpyrifos-methyl might satisfy the criteria of these materials against the red flour beetle in this respect. No indication of resistance to chlorpyrifos-methyl was found in different strains of *T. castaneum* (Halliday *et al,.* 1988; Zettler and Cuperus 1990 and Zettler, 1991). For pyrethroids, the situation is probably more different. Resistance to α-cyano pyrethroid insecticides e.g. cyfluthrin and cypermethrin in *T. castaneum* has highly significant implications for the grain storage, industry (Collins, 1990). These compounds were regarded as the likely future replacements for the organophosphorus insecticides. However, result of Collins (1990) showed that if the pyrethroids are introduced into the field at currently proposed application rates they will ultimately fail to control *T. castaneum*. How long this will take depends on a combination of factors such as rates of selection and the inherent characteristics of the pyrethroid-resistance gene. On the other hand, this new resistance does not jeopardize current organophosphorus grain protectants nor future materials including methacrifos and methoprene. Andric *et al*. (2015) determined the possible alteration in susceptibility of two field strains of *T. castaneum* in a warehouse to dichlorvos, malathion, chlorpyrifos-methyl, deltamethrin and bifenthrin after previous selection with the LD_{80} of pirpmiphos-methyl and deltamethrin using topical application method. Data obtained showed that chlorpyrifos-methyl was the most toxic insecticide to *T. castaneum* adults, while malathion was the weakest. Also, the selection changed / reduced significantly the toxicity of deltamethrin and bifenthrin, increasing their resistance ratios (RR) at the LD_{50} from 1.1 to 1.8 (bifenthrin) and from 0.9 to 2.2 (deltamethrin). Kljajić and Perić (2007) determined the toxicity of six contact insecticides to local populations of granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) after selection with pirimiphos-methyl and deltamethrin. They found that the population that underwent three selections with the LD_{50} of deltamethrin, the resistant ratios of that insecticide increased significantly, so that the initial 7.0 and 7.2 at the LD_{50} and LD_{95} levels increased to 32.1 and 51.9, respectively. Galleya (1999) studied the deltamethrin resistance in *R. dominica*. Mribeiro *et al.* (2003) surveyed insecticide resistance and synergism in Brazilian population of *S. zeamais* using the discriminating concentrations established from LC_{95}'s estimated for a standard susceptible population against chlorpyrifos-methyl, malathion and pirimiphos-methyl and three pyrethroids (cypermethrin, deltamethrin and permethrin). Collins *et al*. (2003) investigated the resistance that had emerged against fumigants and protectants.

Tablel (4): Cross-resistance patterns in chlorpyrifos, malathion and pirimiphos-methyl resistant strains of *Tribolium castaneum*.

Insecticides	Chlorpyrifos- LC_{50} µg/cm^2			Malathion-resistance LC_{50} µg/cm^2			Pirimiphos-methyl - LC_{50} µg/cm^2		
	S	Rc	R.F	S	Rm	R.F	S	Rp	R.F
Chlorpyrifos-methyl	0.088	0.038	0.43	0.088	0.025	0.290	0.088	0.058	0.660
Cyfluthrin	0.197	1.260	6.40	0.197	0.550	2.800	0.197	0.500	2.550
Cypermethrin	0.220	1.160	5.30	0.220	0.790	3.570	0.220	0.880	4.000

Andric *et al.* (2015) stated in insecticides choices need to be made carefully, considering the target species of stored product insects and their susceptibility to malathion and other insecticides: Attention should also be focused on a crucial role of insecticide selection of different stored grain insect populations in order to enable predictions of resistance evolution in individual populations and, based on such knowledge, sound choices of the most adequate resistance management strategy. Ultimately, a key to the successful management of resistance to insecticides is its early detection and proper characterization. All resistance data are being stored in an integrated database for future reference on trends and frequencies of resistance.

The present study suggests application of sequence of different groups to prevent or delay the resistance and cross-resistance to certain insecticides specially that used in the current study.

It is concluded that the common insecticides, malathion and pirimipho-methyl have created high level of resistance compared to methomyl which was rarely used in grain protection. Furthermore chlorpyrifos-methyl succeeded in covering the resistance in the resistant strains tested.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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Instruction to authors

Original articles are full length papers describing original research. The paper should not exceed 15 pages of double-spaced typed text (including abstract, tables, figures and references). One double-spaced typed page contains approximately 300-350 words. Reviews are normally by invitation from the Editor-in-Chief. However, authors are encouraged to submit a tentative title and a table of contents of a proposed review for consideration. These reviews should not exceed 20 pages of double-spaced typed text (including abstract, tables, figures and references). Scientific notes and short communications to the Editor usually on matters of general concern to plant protection, are welcome but should not exceed 4 typed pages. The decision to publish submitted scientific notes and short communications with the Editor-in-Chief.

Manuscripts should be written in clear, concise, and grammatically correct English. Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else. Authors should submit their manuscripts online. To the Email: shaabanabdrabou59@yahoo.com

Manuscript preparation

Title: The title should reflect the most important aspects of the article, in a preferably concise form of not more than 150 characters and spaces. By-line The authors' names should be followed by affiliations and addresses.

Abstracts are required for all the manuscripts. They should be typed in one paragraph and limited to max.150- 200 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords: Most important words of paper. Should be from 4 to 6 words.

Text: Main text should contain (1) an Introduction (2) a Material and Methods (3) a Results (4) a Discussion (5) a Conclusion (6) a References

Text formatting: Use a normal, plain font (e.g., 14 Point Times Roman) for text.

Abbreviations should be defined at first mention and used consistently thereafter. Authors should adhere to the rules governing scientific nomenclature to the International Code of Zoological Nomenclature. All biotica (crops, plants, insects, birds, mammals, etc.) should be identified by their scientific names including authors (and Order: Family) when the English term is first used in the main text, with the exception of common domestic plants and animals. Scientific names should be as follows: In the Title only give the Latin name but No authority or (Order: Family); **i**n the Abstract all Latin names should be accompanied with the correct authority and with (Order: Family)**;** in addition, at the first mention in the body of the text - and only then - these data should be given; authority, the order, family, should also go in the Key Words list.

Footnotes

Footnotes on the title page are not given reference symbols. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data).

References

The list of References should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Cite references in the text by name and year in parentheses. Some examples:

• Negotiation research spans many disciplines (Abd-Rabou, 1996).

• This result was later contradicted (Simmons and Abd-Rabou, 2009).

• This effect has been widely studied (Abd-Rabou ,1998; Simmons *et al.*, 2002; Evans and Abd-Rabou, 2005and Abd-Rabou *et al.*, 2005).

List style

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal article

Abd-Rabou, S. (1998): The efficacy of indigenous parasitoids in the biological control of *Siphoninus phillyreae* (Homoptera: Aleyrodidae) on pomegranate in Egypt. Pan-Pacific Entomolgists, 74 (3): 169-173.

Evans and Abd-Rabou (2005): Two new species and additional records of Egyptian Aphelinidae. Zootaxa,833:1-7.

Simmons, A. and **Abd-Rabou, S. (2006):** Whitefly populations in vegetables crops with different fertilizers. 52nd Annual meeting of the South Carolina Entomological Society, Mc Cormick, Sc., October 19-20.

Abd-Rabou, **S. and Simmons, A. M. (2012):** *Bemisia tabaci* (Hemiptera: Aleyrodidae) whitefly as a pest in Egypt. Advances In Agricultral Research In Egypt, 10 (1): 1-82.

Figures Line-drawing should be clear and of high quality. Cite all figures in numerical order in the manuscript.

Tables The title should be self-explanatory and include enough information so that each table is intelligible without reference to the text or other tables. The title should summarize the information presented in the table without repeating the subheadings. Subheadings should be brief, nonstandard ones can be explained in footnotes. Cite tables in numerical order in the manuscript. Information presented in a table should agree with that in the text.