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Evaluation of Entomopathogenic Nematodes, a Commercial Bacterial Bio-insecticide and the Peppermint Oil for the Control the Pink Stem Borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae)

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ABSTRACT

Steinernema carpocapsae strain All and *Heterorhabditis bacterionphora* strain HP88, Dipel-2X (*Bacillus thuringiensis* subsp. *kurstaki*) and peppermint oil (*Mentha piperita*) were assayed for the control of *Sesamia cretica* Led. tested under laboratory and field conditions. The efficacy of *S. carpocapsae* was a concentration dependent. The lower concentrations (25 and 50 IJs/ml) had no effect within one day; while they showed mortality percentages between 60.0 and 73.3 % after two days. The higher concentration levels (100,200 and 400 IJs/ml) caused mortality percentages ranged between 20.0 and 33.3 % within one day. *H. bacterionphora* had no effect at concentrations between 25 and 200 IJs/ml within one day. The insecticidal activity of peppermint oil increased as the applied concentration was increased from 0.13 to 1% inducing larval mortalities from 40 to 93.3%, respectively. Increasing of *B. t. kurstaki* concentration from 0.13 to 1.5% led to increase of mortalities of *S. cretica* larvae from 33.3 to 86.7%, respectively. Some morphological malformations were detected among *S. cretica* pupae and adults after larval treatments by *B. t. var. kurstaki* and peppermint oil. In the field, the intensities of damage after using the four assayed material were statistically insignificant. Both *S. carpocapsae* and peppermint oil had approximately the same level of activity to the dead heart phenomenon. The mean reduction percentages of dead heart plants were 62.5 and 56.3 %, respectively. The entomopathogenic nematode, *H. bacteriophora* showed the lowest level of reduction (28 %) of dead heart plants than control. The yield increased in the treated plants to 3.1 and3.2 tons/feddan by applying the nematode suspension.

Key Words: *Steinernema* sp., *Heterorhabditis* sp., *Sesamia cretica,* Peppermint oil, *Bacillus thuringiensis*, maize.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in Egypt. Plants are subjected to infestation with a variety of insect pests (Tawfik *et. al.*, 1974). Among these pests, *Sesamia cretica* Led. is considered one of the most destructive agricultural pests which cause serious economic damage and reduce the crop yield. This pest species is difficult to be controlled by contact insecticides because the larvae bore into plant tissues shortly after hatching. In addition, pesticide residues in food are becoming increasingly unacceptable to consumers. These constraints have encouraged the search for biological control methods for this pest. Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* may proof promising in this respect. The infective juveniles (IJs) actively seek and invade insects (Lewis *et al.*, 1993). Infected insects die because of septicemia caused by the symbiotic bacteria released by the invading nematodes (Akhurst, 1983).These nematodes are used as biological control agents for soil inhabiting insect pests (Klein, 1990 and Georgis, 1992).

Also, the entomopathogenic bacterium, *Bacillus thuringiensis* is one of the most commonly used biological control agents proving its efficacy against many lepidopterous species with no adverse effect on beneficial species (El-Husseini and Afifi, 1981and Taher *et al.*, 1994). Plants produce a large number of secondary metabolites that proved functional agents in a variety of ecological contexts. Many specialized compounds are toxic and can therefore serve as defense agents against microbial pathogens, insects and herbivores animal (Wittstock and Gershenzon, 2002; Theis and Lerdau, 2003 and Wink, 2003). The Lamiaceae is a large plant family that includes the mints, sages, and basils and is well recognized for the diversity of secondary compounds synthesized and stored in glands found on the surface of leaves, stems, and flowers, (Werker, *et. al.*, 1993; Gang *et al.*, 2001and Iijima *et al.*, 2004).

Allelochemicals, such as essential oils, are part of the chemical defense system of plants against herbivores (Rice and Coats, 1994). Their main volatile compounds have previously been demonstrated to be behaviorally active against several insect pests (Isman, 2000). *Mintha piperita* is known as king of all mints. The essential oil of peppermint (up to 2.5% in the dried leaves), and menthol becomes the dominant monoterpene constituent (Burbott and Loomis, 1969; Croteau and

Martinkus, 1979; Brun and Voirin, 1991).

In this study, *Steinernema carpocapsae* strain All and *Heterorhabditis bacterionphora* strain HP88, *Bacillus thuringiensis kurstaki* beside the peppermint oil (*Mentha pepperita*) were selected for laboratory and field assays against the pink stem borer, *Sesamia cretica* Led..

MATERIALS AND METHODS

1- Target insect

Large numbers of egg- masses and larvae of *S. cretica* were collected (from infested maize plantations in the Experimental farm of Faculty of Agriculture, Moshtohor, Benha University; early summer plantation). Both stages were kept in plastic cups and transferred to laboratory. Egg-masses were placed on fresh succulent rolled corn leaves as suitable food supply for the hatching larvae. Plant leaves were renewed every couple of days and as the larvae grew older; they were fed on tender cuttings of maize stems or young corn ears until reached the desired larval stage.

2- Tested nematodes

Tested entomapathogenic nematodes; *H. bacteriophora* HP88, was obtained from Ulatah, USA., Randy Gaugler, Rutgers University, New Brunswick, NJ, USA. and *S. carpocapse* All, from California, USA., Ramon Georgis, Boisys, Palo Alto, CA, USA.

3- Tested *B.t.*

The commercial preparation Dipel-2x was used. Potency 32000 IU/mg of *B.t.* subsp. *kurstaki* active ingredient 6.4% w/w wettable powders was used at the concentrations, 0.13, 0.25, 0.5, 1.0 and 1.5% where each concentration level equal; 1.43, 2.85, 5.7, 11.4 and 17.1 gm/L, respectively. Manufactured by Chemical and Agricultural Products Division Abbott Laboratories North Chicago, Illinois 60064

4- Tested oil

Peppermint oil (*M. pepperita*) was brought from the market, and used as dilution from commercial formulation (concentration 90% peppermint oil + 10% mineral oil). Manufactured by the Egyptian Natural Oil Company.

5-Bioassay of the tested material

Laboratory experiments were conducted to study the effects of the tested nematodes, *(S. carpocapsae* All and *H. baceteriophora* HP88); Dipel-2X *B. thuringiensis kurstaki* and peppermint oil (*M. pepperita*) on the 3rd instar larvae of *S. cretica*. Three equal pieces of tender parts of maize plant stems were dipped in the desired solution for about two minutes, and then left in shade for about 10 minutes to dry. Test larvae were starved for about 4 hours before offering the treated food to assure rapid ingestion. Larvae were allowed to feed on the treated maize parts of each treatment for 3 days. The total number of treated larvae per treatment was 15 (three replicates of 5 larvae each), five concentration levels (25, 50, 100, 200, and 400 IJs/ml) of each nematode strain, (0.13, 0.25, 0.50, 1.0 and 1.5%) of *B. t. kurstaki* and (0.13, 0.25, 0.50, 0.75 and 1%) of peppermint oil were used. Mortality counts were recorded after 24, 48 and 72 hours for nematode treatments and at the end of larval stage for *B.t.* and the peppermint oil treatments. The efficacy of the two different nematode strains to *S. cretica* larvae was assessed by calculating the LC_{50} and LT_{50} values and number of (IJs) penetrated and developed to adults. The number of nematodes invaded the host was determined by dissecting the dead larvae 6-7 days after initial exposure to infective juveniles. Invasion efficiency is the percentage of the infective stage of nematodes that successfully invaded or established in the host and calculated according to the following formula (Xianqun *et al.*, 1996).

Invasion efficiency (%) =

$$\frac{\text{Nematodes in dead larval body}}{\text{Applied nematodses}} \times 100$$

Survivors from *B. t. kurstaki* and peppermint oil treatments were transferred to other cups containing untreated maize stems that were renewed every 2-3 days until pupation. Pupae were kept in plastic cups until adults' emergence where duration of larvae and pupae were estimated.

Control test was conducted using the same technique of larval feeding; maize stems dipped for about two minutes in water only, then kept for about 10 minutes to dry. All concentrations were prepared in distilled water. Then Tween 20 as emulsifying agent was added to peppermint oil only. The treated and untreated replicates were incubated under constant conditions of 27±1 °C and 65-70% RH. Obtained data were corrected according to Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} values for *S. cretica* at 5% confidence limits and slope regression lines are represented and interpreted using method of (Finney, 1971).

Malformed pupae (due to treatments) of *S. cretica* were counted and their percentages were calculated according to the following formula:

Malformed pupae (%) =

$$\frac{\text{No. of malformed pupae}}{\text{Total inspected pupa}} \times 100$$

Normal pupae were kept in plastic jars until emergence of adults. Percentages of emergence were calculated. Also malformed moths were counted and their percentages were recorded.

6- Field treatments

Field experiments were carried out on maize crop in the Experimental farm of the Faculty of Agriculture, Moshtohor, Benha University (early summer plantation). Maize cross S. C. 10 was chosen to be evaluated under artificial infestation by *S. cretica*. Maize seeds were sown on May 15th. The area was divided into 24 plots of 3.5x3.0 meters each (about 1/400 of feddan). Each plot contained five rows at a distance of 70 cm between rows. Sowing took place on 1st of June. Randomized complete block design was used for each experiment, with 4 treatments (*S. carpocapse* All; *H. baceteriophora* HP88 each at 1000 and 2000 IJs/ml concentration levels, *B. t.* variety *kurstaki* at 1.0 and 1.5 % and peppermint oil at 0.5 and 1%/Liter) and 4 replicates each. Thinning was made 17 days after sowing, leaving 2 plants/hill. All treatments received the normal agricultural practices.

Forty maize plants (23 days-old) free of infestation with *S. cretica* were divided into four replicates and were artificially infested with 3rd instar larvae of *S. cretica*; 4 larvae/plant (Kumar and Saxena, 1992). A volume of about 5 ml of each material was directed in the whorl of each plant. One row was left untreated between every two plots. Treatments started 5 days after artificial infestation. The second treatment was applied 10 days after the first. The effect on the intensity of damage, the percentages of infested plants and percentages of dead hearts were estimated 5 days after 1st and 2nd sprays.

7- Effect of treatments on yields

At harvest time (about 120 day after sowing), maize ears were picked from all plants of each treatment, weighed and percentage of infested ears was measured. Obtained yields were adjusted to the yield/feddan.

8- Statistical analysis

Analysis of variance and "F" tests were used to compare between treatments. Statistical analysis of data was carried out also using the computer software package, "Costat" a product of Cohort software Inc., Berkeley, California. USA. Duncan's multiple range test (Duncan, 1955) was used to differentiate among means.

RESULTS AND DISCUSSION

1- Susceptibility of the test insect to entomopathogenic nematodes

Data presented in Table (1) indicate that the 3rd. instar larvae of *S. cretica* were susceptible and the degree of susceptibility differed in levels according to the concentration level of *S. carpocapsae*. A positive relation was evident between percent mortality and nematode concentration. The lower concentrations (25 and 50 IJs/ml) had no effect within one day; while they showed mortality percentages between 60.0 and 73.3% within two days. The higher concentration levels (100, 200 and 400 IJs/ml) caused mortality percentages ranged between 20.0 and 33.3% within one day. After three days, the mortality ranged between 80.0 and 100% for the same concentrations. LC_{50} value was 8.32 IJs/ml; while LT_{50} value was 26.9 hours when larvae were treated by the concentration 200 IJs/ml. As for *H. bacteriophora* HP88, no mortality occurred at the concentrations between 25 and 200 IJs/ml within one day; while it caused 13.0 to 33.3% mortality at the same concentrations after two days and increased to reach 20.0 to 53.3% after three days. The highest concentration (400 IJs/ml) caused 86.7% mortality within two days. LC_{50} value was 111 IJs/ml and LT_{50} value was 68.2 hours. These results are in accordance with those reported by El-Kholy (2004) and Schroer *et al.* (2005) who found that *S. carpocapsae* achieved a significant reduction of *Plutella xylostella* larvae per plant with >50 % control after 7 days. Data also indicated that higher nematode inoculum levels; however, caused higher and faster mortality than the lower levels. Poinar (1979); El-Kifl (1984) and Glazer & Wysoky (1990) suggested that high concentrations of nematodes will elaborate much more bacteria which in turn kill the insect larvae more rapidly.

2. Establishment of nematodes in the test insect

Invasion rates recorded in Table (1) confirmed that IJs of *S. carpocapsae* All and *H. bacteriophora* HP88 penetrated the 3rd. instar larvae of *S. cretica*. Invasion rates ranged from 7.0-44.7 to 1.7-9.0 nematodes/ cadaver, at concentrations between 25 and 400 IJs/ml; respectively. The *H. bacteriophora* HP88 invasion was generally poorer than that with *S. carpocapsae* All. Highest concentration (400 IJs/ml) resulted the highest number of nematodes established in the host larvae. Obtained results indicated that, the number of IJs that were established in host cadavers were dependent on

nematode species and strain and the inoculum doses. These finding agree with those of EL-Kifl and Sammour (1989) who noticed that the numbers of infective juveniles established per cadaver increased as the rate of infection enhanced. Fan and Hominick (1991) stated that the relationship between dosage of IJs and establishment in a host is an effective method for evaluating the efficacy of different nematode species.

3- Toxicity effects of *B. t. kurstaki* and peppermint oil

Results of the toxicity of *B. t. kurstaki* and peppermint oil to *S. cretica* larvae were calculated at the end of larval stage, and presented in Table (2).

3.1. *B. t. kurstaki*

Data showed that, increasing of *B. t. kurstaki* concentration from 0.13 to 1.5%. led to increase in mortalities of *S. cretica* larvae from 33.3 to 86.7% and the LC_{50} value was 0.29% (4.96 gm/L), while the LC_{90} value was 2.66%(45.49 gm/L). These results agree with many authors, (Abdel-Halim, 1993; Salama *et al.,* 1999; Mohamed *et. al*., 2000 and El- Hefny, 2006) who stated that the accumulative mortality percentages of *S. cretica* larvae treated by (*B. t. kurstaki*) increased as the applied concentration was increased. Also, percentages of pupal mortality were 100% especially at the highest concentration level (1.5%) of (*B. t. kurstaki*).

3.2. Peppermint oil *M. piperita*

The insecticidal activity of peppermint oil was summarized in Table (2). The accumulative mortality percentages among treated *S. cretica* larvae showed a potent and an insecticidal effect against *S. cretica* larvae. Increasing concentration of peppermint oil from 0.13 to 1% increased larval mortalities from 40 to 93.3% and the LC_{50} value was 2.1, while the C_{90} was 12.5%, respectively.

The percentages of pupal mortality were relatively high especially at the highest concentration (100%). Mortality of target insects may be due to the effect of allelochemicals of family *Lamiaceae*, such as essential oils which were demonstrated to be behaviourally active against several insect pests (Isman, 2000).

4- Biological activity of *B. t. kurstaki* and peppermint oil

4.1.1. Effect of *B. t. kurstaki* on the durations of different developmental stages

Increasing in the (*B. t. kurstaki*) concentrations caused increasing in the larval duration, until reached 10.5 days at 1.5% concentration, compared with 5.4 days for control larvae. On the other hand, the pupal duration increased with decreasing concentrations. The average duration was 14.3 days at 0.13% (Table, 2). Low doses of *B. thuringiensis* toxins have been reported to reduce or inhibit larval growth, development and weight, and pupal weight (Salem, 1981; Barker, 1998; Abdel *et al.,* 1999; Deml *et al*., 1999; Liu *et al*., 2001 and Huang, *et al.*, 2005).

4.1.2. Effect of peppermint oil on the durations of different developmental stages:-

Data in Table (2) showed that the peppermint oil treatments caused prolongations of the larval duration being 15 days at 1% concentration, compared to 5.4 days in the control. Statistical analysis showed no significant difference between 0.13, 0.25 and 0.5% concentrations; the mean averages were (9.2, 9 and 9.8 days, respectively). The pupal duration became longer, also, by increasing the concentrations, being 17.3 days at 1%. These results agree with the results of Lu *et al.*

Table (1): Susceptibility of 3rd. instar larvae of *Sesamia cretica* to different concentrations of *Steinernema carpocapsae* All and *Heterorhabditis bacteriophora* HP88 nematodes.

Conc. IJs/ml.	Cumulative mortality (%) at the indicated days after treatment						Mean No. of nematodes established/cadaver ±S.E.		(%) of invasion efficiency	
	1 days		2 days		3 day					
	All	HP88	All	HP88	All	HP88	All	HP88	All	HP88
25	0.0	0.0	60.0	13.3	73.3	20.0	7.0 ± 1.2	1.7 ± 0.3	28.0	6.8
50	0.0	0.0	73.3	26.7	73.3	26.7	10.0 ± 1.2	2.7 ± 0.7	20.0	5.4
100	20.0	0.0	73.3	26.7	80.0	46.7	28.7 ± 3.2	3.3 ± 0.6	28.7	3.3
200	33.3	0.0	93.3	33.3	93.3	53.3	35.0 ± 2.9	3.3 ± 0.6	17.5	1.7
400	33.3	13.3	93.3	86.7	100.0	86.7	44.7 ± 3.2	9.0 ± 0.6	11.2	2.3
LC_{50} (IJs/ml)					8.32	111				
LT_{50} (hours)*					26.9	68.2				

All = *Steinernema carpocapsae* All; HP88 = *Heterorhabditis bacteriophora* HP88

Control mortality was zero % throughout the period of experiment. (*) LT_{50} at concentration 200 IJs/ml.

Table (2): Toxicity and Biological activity of Dipel 2X (*B.t.*) and peppermint oil against 3rd instar larvae of *Sesamia cretica*. Led.

Treatments	Conc. (%)	Larval stage		Pupal stage			Adult stage			Sex Ratio	
		Corrected Mortality (%)	Duration Days ± SE	Corrected Mortality (%)	Duration Days ± SE	Malformed Pupae (%)	Corrected Mortality (%)	Malformed Adult (%)	Adults Emergence (%)	♂	♀
Bacillus thuringiensis var. kurstaki	0.13	33.3	6 ± 0.2 ^{fg}	10	14.3 ± 0.2 ^{bc}	30	16.7	40	33.3	3	2
	0.25	46.7	7.5 ± 0.7 ^{ef}	12.5	14.6 ± 0.2 ^{bc}	25	20	40	20	1	2
	0.5	60	8.1± 0.4 ^{de}	33.3	9.5 ± 0.6 ^{d}	16.7	25	33.3	20	2	1
	1	73.3	9.2 ± 0.2 ^{cd}	50	9.3 ± 0.2 ^{d}	0.0	0.0	50	13.3	1	1
	1.5	86.7	10.5 ±0.4 ^{bc}	100	0.0 ^{f}	0.0	0.0	0.0	0.0	0	0
Mean			8.3		11.9						
Mentha Piperita	0.13	40	9.2 ± 0.4 ^{cd}	0.0	13.5 ± 0.2 ^{c}	44.4	0.0	40	20	2	1
	0.25	53.3	9 ± 0.7 ^{cde}	0.0	15 ± 0.4 ^{b}	42.9	25.0	40	6.7	1	0
	0.5	66.7	9.8 ± 0.2 ^{bc}	20	15.7 ± 0.2 ^{b}	20	33.3	33.3	6.7	0	1
	0.75	80	11.2 ± 0.9 ^{b}	66.7	17.3 ± 0.4 ^{a}	0.0	0.0	0.0	6.7	1	0
	1	93.3	15 ± 0.4 ^{a}	100	0.0 ^{f}	0.0	0.0	0.0	0.0	0	0
Mean		----	10.8	---	12.3	----	---	7.1	100	7	8
Control		0.0	5.4 ± 0.4 ^{g}	0.0	11.5 ± 0.7 ^{e}		0.0				
LSD 5%			1.5		1.3						

Within columns, means followed by a different letter don't differ significantly (LSD test, P<0.05).

	Dipel 2X *B.t.* Kurstaki	Peppermint oil
LC_{50}	0.29% (4.96 gm/ Litter)	0.21% (2.1 ml/Litter)
LC_{90}	2.66% (45.486 gm/ Litter)	1.25% (12.5 ml/ litter)
Slope ± SE	1.33 ± 0.16	1.64 ± 0.19

(1978), who found that the accumulation of the toxic substances in any organism might be affecting the longevity of insects.

4.2. Effect of *B. t. kurstaki* and peppermint oil on malformations of pupae and adults stages

Some morphological malformations were detected among *S. cretica* pupae and adults after larval treatments by *B. t. kurstaki* and peppermint oil (Table, 2 and Fig., 1). Percentage of malformed pupae was increased by decreasing concentrations. The malformed pupae percentages after *B. t. kurstaki* treatment were 30, 25 and 16.7% at the concentrations, 0.13, 0.25 and 0.5%, respectively. These results agree with Moawad *et al.* (1983) on *Earias insulana* treated in the larval stage with Dipel-2X. while with peppermint oil they recorded 44.4, 42.9 and 20% malformed pupae at the same concentrations. In this respect, Matter *et al.* (2002), Mohamed and El-Gammal (2002); Bruce *et al.* (2004) and Abd El-Rady and Osman (2005) mentioned that azadrirachtin played the same role of Juvenile hormone in delaying or prohibiting metamorphosis; and larval and pupal developmental periods were longest with the highest oil concentrations. These malformations of pupae were categorized as, larval-pupal intermediates with larval head; pupae failed in shedding off the larval exuvia and badly deformed pupae.

However, malformed adults increased in number by decreasing concentrations in case of peppermint oil. The morphological malformations appeared in the adults obtained from *B. t. kurstaki* and peppermint oil are shown in Table (2) and Fig. (1). Malformed adult percentages ranged 0- 50%, while in case of peppermint oil, they ranged 0- 40%. Adults' malformations were; failure to emerge from pupa, adult with both fore and hind wings on one side slightly curled and the hind wings being shorter than normal, and poorly developed adults' with twisted wings.

4.3. Effect of *B. t. kurstaki* and peppermint oil on adults' emergence and sex ratio

There was a reduction in adults' emergence percentages, indicating latent effect of both *B. t. kurstaki* and peppermint oil. No adult emerged when *S. cretica* 3 rd instar larvae were treated at the concentrations 1.5 and 1% of both Dipel-2X (*B. t. krustaki*) and peppermint oil. While, the highest percentages (33.3 & 20%) were at the concentration 0.13% of both *B. t. kurstaki* and peppermint oil, respectively. These resultes agree with, Abdel- Halim (1993), and Mohamed *et al.* (2005) who stated that all concentrations of *B. t. kurstaki* caused reduction in pupation and adult emergence percentages of *Spodoptera littoralis* (Boisd.).

Table (3): Averages and reduction percentages of the intensity of damage, percentage of infestation and dead heart maize plants infested with *S. cretica* 3rd. instar larvae 5 days after 1 st application with different materials.

Treatments	Conc.	The intensity of damage Mean ± SE	R (%)	(%) of infestation Mean ± SE	R (%)	(%) of dead heart Mean ± SE	R (%)
Steinernema carpocapsae	1000 IJs/ml	3.2 ± 0.4	20.3	80 ± 2.0	15.8	20 ± 0.0	50
	2000 IJs/ml	1.8 ± 0.1	54.4	65 ± 3.5	31.6	10 ± 0.0	75
Mean		2.5 ^{b}	37.4	72.5 ^{bc}	23.7	15 ^{d}	62.5
Heterorhabditis bacteriophora	1000 IJs/ml	3.0 ± 0.4	25.3	85 ± 2.0	10.5	35 ± 2.9	12.5
	2000 IJs/ml	2.0 ± 0.2	50.6	75 ± 3.5	21.1	22.5 ± 2.5	43.5
Mean		2.5 ^{b}	37.9	80 ^{b}	15.8	28.8 ^{b}	28
Bacillus thuringiensis subsp. *kurstaki*	1 %	3.2 ± 0.4	18.9	80 ± 2.5	15.8	30 ± 2.5	25
	1.5 %	1.9 ± 0.2	51.9	75 ± 2.9	31.6	15 ± 2.9	62.5
Mean		2.6 ^{b}	35.4	77.5 ^{bc}	23.7	22.5 ^{c}	43.75
Mentha piperita	0.5 %	2.9 ± 0.3	27.8	75 ± 2.0	21.1	20 ± 2.5	50
	1 %	1.7 ± 0.3	56.2	55 ± 2.8	42.1	15 ± 2.9	62.5
Mean		2.3 ^{b}	42	65 ^{c}	31.6	12.5 ^{d}	56.3
Control	0.0	4.0 ± 0.3 ^{a}	0.0	95 ± 2.8 ^{a}	0.0	40 ± 2.5	0.0
LSD 5% for treatments		0.6		14.5		5.8	
LSD 5% for concentrations		0.4		9.1		7.3	

R (%) = Reductions than control.

Within columns, means followed by a common letter don't differ significantly (LSD test, P<0.05).

Table (4): Averages and reduction percentages of the intensity of damage, percentage of infestation and dead hearted maize plants infested with *S. cretica* 3rd instar larvae 5 days after 2 nd application with different assayed materials.

Treatments	Conc.	The intensity of damage Mean ± SE	R (%)	(%) of infestation Mean ± SE	R (%)	(%) of dead heart Mean ± SE	R (%)
Steinernema carpocapsae	1000 IJs/ml	1.5 ± 0.4	58.3	32.5 ± 2.5	56.7	5 ± 1.9	88.9
	2000 IJs/ml	0.8 ± 0.1	78	20 ± 0.0	73.3	2.5 ± 1.4	94.4
Mean		1.2 ^{b}	68.2	26.3 ^{d}	65	3.8 ^{bc}	91.7
Heterorhabditis bacteriophora	1000 IJs/ml	1.4 ± 0.4	61.9	45 ± 2.9	40	15 ± 2.0	66.7
	2000 IJs/ml	0.8 ± 0.2	77.5	27.5 ± 2.8	63.3	5 ± 2.0	88.9
Mean		1.1 ^{b}	69.7	36.3 ^{bc}	51.7	10 ^{b}	77.8
Bacillus thuringiensis subsp. *kurstaki*	1 %	1.9 ± 0.4	47.9	47.5 ± 2.5	36.7	15 ± 2.0	66.7
	1.5 %	1.0 ± 0.2	71.8	32.5 ± 2.5	56.7	5 ± 0.0	88.9
Mean		1.3 ^{b}	59.9	40 ^{b}	46.7	10 ^{b}	77.8
Mentha piperita	0.5 %	1.3 ± 0.2	62.5	35 ± 2.9	53.3	5 ± 1.9	88.9
	1 %	0.8 ± 0.1	78	25 ± 2.8	66.7	0.0 ± 0.0	100
Mean		1.1 ^{b}	70.3	30 ^{cd}	60	2.5 ^{c}	94.5
Control	0.0	3.6 ± 0.3 ^{a}	0.0	75 ± 0.2 ^{a}	0.0	45 ± 2.5 ^{a}	0.0
LSD 5% for treatments		0.9		8.8		7.2	
LSD 5% for oncentrations		0.3		5.6		4.5	

R (%) = Reductions than control.

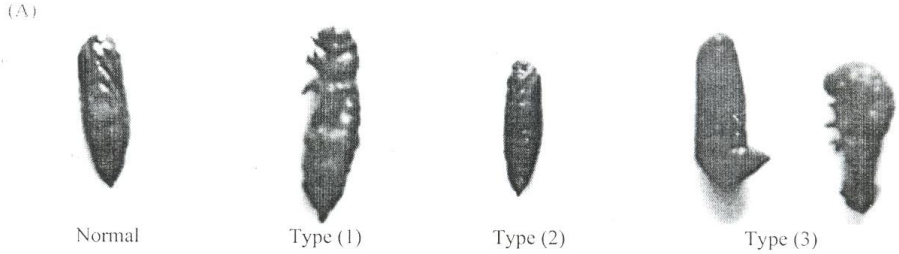
Within columns, means followed by a common letter don't differ significantly (LSD test, P<0.05).

Table (5) Analysis of variance among infested maize after application.

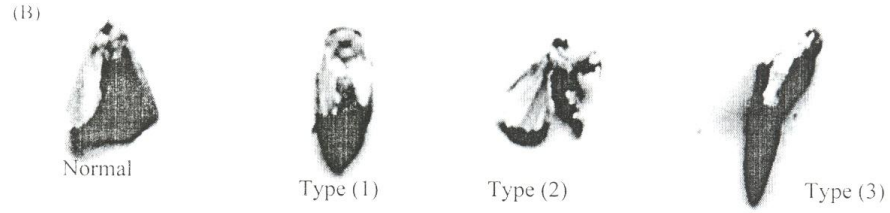
Source of variation	d.f.	The intensity of damage		(%) of infestation		(%) of dead heart		(%) of infested ears	Weight of ears/ row (Kg)
		1st Spray	2 nd Spray	1st Spray	2 nd Spray	1st Spray	2 nd Spray		
Replication	3	0.623	0.099	80.0	275.434	35.833	49.167	275.434	0.247
Treatments	4	3.718 **	9.064 **	985.0 **	411.38 **	537.50 **	2460.0 **	411.377 **	0.972 **
Concentrations	1	9.120 **	2.916 **	1000.0 **	331.2 *	722.50 **	302.50 *	331.20 *	1.901 **
T x C	4	0.609	0.181	125.0	136.38	72.50	40.0	136.377	0.297
Error	27	0.313	0.223	198.519	91.760	32.130	49.167	91.760	0.231
Total	39								

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level

Fig. (1): Malformations among *Seasmia cretica* pupae (A) and adults' (B) resulted after the 3rd instar larval was treated by *B. t.* subsp. *kurstaki* and peppermint oil.



Malformations of pupae were categorized as follows:-
1) Larval- pupal intermediate with larval head
2) Pupa failed in shedding off the larval exuvia
3) Badly deformed pupae



Malformations of adults were categorized as follows:
1) Adult failed to emerge from pupa,
2) Adult with both fore and hind wings on one side slightly curled and the hind wings being shorter than normal.
3) Adult showing poor development with twisted wings.

Table (6): Yield parameters of maize resulted from maize plants infested with 3rd instar larvae of *S. cretica* after being applied with different treatments.

Treatments	Conc.	% of infested ears/row ± SE	(%) Reduction than control	Weight of ears/row (Kg)	weight of yield Ton/feddan	(%) Increase than control
Steinernema carpocapsae	1000 IJs/ml	42.1± 1.4	5.6	1.5 ±0.1	2.6	62.5
	2000 IJs/ml	24.6 ± 3.1	45.3	2.1 ±0.1	3.6	125
Mean		33.4 [b]	25.5	1.8 [a]	3.1	93.8
Heterorhabditis bacteriophora	1000 IJs/ml	47.5 ± 2.8	5.6	1.7 ± 0.3	2.9	81.3
	2000 IJs/ml	42.1 ± 2.5	6.4	1.9 ± 0.2	3.4	112.5
Mean		44.8 [a]	6	1.8 [a]	3.2	100
Bacillus thuringensis subsp. *kurstaki*	1 %	33.3 ± 3.1	26	0.9 ± 0.2	1.7	6.3
	1.5 %	30 ± 2.0	33.3	1.7 ± 0.1	2.9	81.3
Mean		31.7 [b]	29.7	1.3 [b]	2.3	43.8
Mentha Piperita	0.5 %	35 ± 2.0	22.2	1.2 ±0.2	2.1	31.3
	1 %	26.3 ± 2.4	41.55	1.9 ± 0.2	3.4	112.5
Mean		30.7 [b]	31.9	1.6 [a]	2.8	75
Control	0.0	45 ± 3.0 [a]	0.0	0.9 ± 0.3 [b]	1.6	0.0
LSD 5% for treatments		9.83		0.49		----
LSD 5% for concentrations		6.2		0.31		

Within columns, means followed by a common letter don't differ significantly (LSD test, P<0.05).

percentages (100 and 94.4%) in the dead heart plants than the control. Purcell *et al.* (1992) studied the potential of *S. carpocapsae* for the control of *Heliothis zea* in maize borders and determined that the most effective concentrations in the field were 4 x 10^2 and 4 x10^4 IJs/ml of water causing 57.6±5.4 and 74.5±17.4% mortality, respectively and 97% less damage to maize ears than in untreated maize.

5.3. Effect of treatments on maize yield:

5.3.1. Percentages of infested ears

Percentages of infested ears by *S. cretica*/row were 33.4, 44.8, 31.7 and 30.7% after treatment of maize plants by *S. carpocapsae*, *H. bacteriophora*, *B. t. kurstaki* and peppermint oil, respectively, opposed to 45% in the control. Highest percentage of infested ears (47.5%) was associated with plants treated with *H. bacteriophora* at 1000 IJs/ml. On the other hand, the lowest infestation percentage (24.6%) was for *S. carpocapsae* at 2000 IJs/ml. Regarding the data in Table (6), the highest reduction of the infested ears percentage than the control were recorded by *S. carpocapsae* at 2000 IJs/ml and peppermint oil at 1%, being 45.3 and 41.6%, respectively. Ben-Yakir *et al.* (1998) reported that application of the Mexican strain of *S. carpocapsae* to the corn ears under field conditions reduced the economic damage significantly. The high concentrations of all treated materials were more effective than the lower ones in reducing the percentages of infested ears by *S. cretica* except for the plants treated with *H. bacteriophora* at the two concentrations used.

5.3.2. Weight of ears

Maize ears were picked from all plants of each treatment, left to dry and weighed, (Table, 6). Obtained yield/treatment was adjusted to the yield/feddan. The mean averages of ears dry weight/row were 1.8, 1.8, 1.3 and 1.6 kg. for *S. carpocapsae, H. bacteriophora*, *B. t. krustaki* and peppermint oil, respectively. It showed significant increase in weight after nematodes and peppermint oil treatments. Also, the high concentrations of either of the assayed materials caused more increase in the obtained ears yield.

According to the obtained yield and the percentages of increase in this yield from different treatments, it is possible to classify the efficacy of applied materials to the following categories; highly efficient, *S. carpocapsae* and *H. bacteriophora;* moderately efficient, peppermint oil and least efficient, the bio-insecticide Dipel-2X, *B. t. krustak.*

From the obtained results, it could be concluded that all of the applied preparations, which are considered safe to the environment, gave different rates for *S. cretica* control.

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