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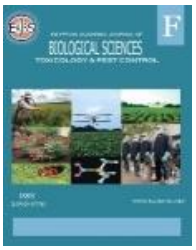

The Joint Action of Entomopathogenic Nematodes Mixtures and Chemical Pesticides on Controlling *Helicoverpa armigera* (Hübner)

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ABSTRACT

Chemical pesticides are characterized by the rapid impact effect in reducing pest population, while, microbial pesticides need along the latent period to cause a limited reduction of the pest population. Low efficiency of biocides may be due to low compatibility with agrochemicals or poor application of biocide, so, the study carried out to evaluate the possibility of mixing five common chemical insecticides and nematicides with five compatible entomopathogenic nematodes (EPNs). For the control of tomato fruit borer (*Helicoverpa armigera*), bioassay revealed that pesticide chlorpyrifos (Pestban 48% EC), chlorpyrifos (Tafaban 48% EC) and flubendiamide (Takumi 20% WG) were applied at the recommended dose. Flubendiamide was less toxic to EPNs infective juveniles compared to fenamiphos (Dento 40% EC). Moreover, steinernematid species were more sensitive than heterorhabditid species to pesticides recording 49.45% and 43.76%, respectively, after 7 days of exposure. The joint action of tested chemical pesticides with IJs of EPNs in controlling the 5th instar larvae of tomato fruit worm showed an additive or antagonistic reaction with no evidence of synergistic action. Antagonism reaction was recorded with all fenamiphos combinations; the combination of abamectin and *S. feltiae* as well as *H. bacteriophora* (Ba-1), in addition to, flubendiamide combinations with *S. feltiae* and *H. bacteriophora* (Ba-1). While, an additive effect was observed in flubendiamide combinations with *S. glaseri*, *S. carpocapsae* and *H. bacteriophora* (HP 88). In the greenhouse experiment, the application of EPNs alone caused mortality ranged from 28 to 36% for 5th instar larvae. Whereas, the highest larval mortality was observed in descending order for combinations between *H. bacteriophora* (HP88 strain) with fenamiphos (64.0%), chlorpyrifos (54%) and abamectin (54.0%), while, local isolate, *H. bacteriophora* (Ba-1 strain) achieved mortality ranged from 40 to 50 % with the tested pesticides. Overall, results indicate the feasibility of the integrated use of these nematode species and chemical pesticides in crop protection.

INTRODUCTION

Tomato crop (*Solanum lycopersicum* Mill) is considered one of the most vegetable crops grown for both local consumption and export. Tomato is a source of several vitamins and capable to decrease the risk of cancer, osteoporosis and cardiovascular

disease (Bhowmik *et al.*, 2012). The crop is infested with many pests during the different stages of plant growth. Among the insect pests, tomato fruit borer *Helicoverpa armigera* (Hübner) is a polyphagous and destructive insect pest distributed over the tropics and subtropics of the world (Fitt, 1989). Management strategies for *Helicoverpa* include multiple control methods are needed. Chemical control is heavily used due to its effectiveness but has a negative effect i.e. rapid disappearance, residual effect, decline efficiency, and health risk. Safe sustainable biopesticides e.g. entomopathogenic nematodes (EPNs) in integrated pest management (IPM) to suppress pest populations is required.

Currently, EPNs are used for controlling scarab larvae, fungus gnats, invasive mole crickets, black vine weevil, and Diaprepes root weevil and other pest insects (Lacey and Georgis, 2012). Foliar spray of EPNs to control insect pests feeding on aboveground parts was carried out by research workers (Arthurs *et al*., 2004; Shapiro-Ilan *et al*., 2006).EPNs kill their insect hosts with the aid of bacteria carried in the nematode's alimentary canal (Poinar, 2018). These nematodes can also provide effective control of some agriculturally important lepidopteran, coleopteran and dipteran pests (Shapiro-Ilan and Gaugler, 2002). Using EPNs alone results in poor to moderate levels of suppression when nematodes were applied to foliage to control *Helicovepra* (Vyas *et al*., 2003). Nematodes and other control agents may be applied simultaneously or within a short time interval of each other to control different pest species or stages of a pest. EPNs are being used more widely in crop-protection strategies and are therefore likely to come into contact with soil amendments and chemical pesticides(Nardo and Grewal, 2003).

Most pesticide formulations e.g. nematicides, insecticides, and acaricide may cause adverse effects on juveniles of *Steinernema carpocapsae* and *S. feltiae* (Rovesti and Deseö, 1990). The high potency of EPNs in controlling many economic pests was recorded, but, IJs tolerance in short-term exposure to different agrochemicals hence providing an opportunity of tank-mixing and application together. The tank mix of traditional agrochemicals with EPNs reduces application time and cost (Vashisth *et al.,* 2013). Also, the parallel and successive applications involved in IPM (Poinar,1990).
Application of EPNs in IPM program required more information about the interaction with chemical pesticides especially that targets a vital system in EPNs nematode to predict the EPNs efficiency after simultaneous or sequential application of chemical pesticides and its effect on EPNs viability. additive or, preferably, synergistic effects on pest mortality (Nardo and Grewal, 2003; Laznik *et al*., 2012). The possible interaction from tank mixing may be additive or, preferably, synergistic effects on pest mortality (Nardo and Grewal, 2003; Laznik *et al.,* 2012). The knowledge about the compatibility between chemical pesticides and EPNs can play a role in developed and improve foliar application

The study aimed to determine the joint action of the biocontrol method (EPNs) with common chemical pesticides to control *Helicoverpa armigera.* The strategy during this study was to use native entomopathogenic nematode previously isolated by EL-Ashry *et al.* (2018) and compare its bioefficacy with imported EPNs when they have applied alone or combined with certain pesticides to define the tank mixability beside the latent effect (survival and viability) on EPNs.

MATERIALS AND METHODS

Pesticides Used:

Five registered commercial formulations of pesticides available in the market and used for controlling insect and nematode pests in Egypt were obtained from the Central Laboratory of Pesticides, Dokki, Giza. The tested pesticides are shown in Table (1).

Table 1 : List of pesticides used in the study.

Pesticides	Common Name	Trade Name	Recommended Dose	Chemical Class
Insecticides	Abamectin	Tervigo 2%SC	3 liters/feddan	Biopesticide
	Chlorpyrifos	Pestban 48% EC Tafaban 48% EC	1 liter/feddan	Organophosphate
	Flubendiamide	Takumi 20% WG	100 g/feddan	Diamide
Nematicide	Fenamiphos	Dento 40% EC	6 liters/feddan	Organophosphate

Rearing of Tomato Plants:

Seeds of tomato plants (*Solanum lycopersicum*) cv. Super Strain B was soaked in sterile distilled water in Petri dishes and kept in an incubator at 26±1 °C. After 48 hours incubation, clay pots **(**20 cm diameter**)** filled with 2 kg autoclaved sterilized sandy soil were used for seeds germination. At the two-leaves stage, seedlings were singly transplanted to plastic pots (20 cm diameter) sterilized with formalin and filled with steam sterilized sandy soil (95.7% sand + 1.2% silt + 3.1% clay). Forty-five days after transplanting (30 cm plant height) each tomato plant was caged using wooden cages covered with nylon nets (30 × 30 × 50 cm) to safeguard the area under experimentation, prevent escaping introduced *Helicoverpa armigera* last instar larvae and trace the emerged adults.

Rearing the Greater Wax Moth, *Galleria mellonella* L.:

Last instar larvae of *G. mellonela* (Lepidoptera: Pyralidae) were reared in glass jars kept at 27 ºC. The larvae of *G. mellonela* were used for storage and nematode isolation/multiplication according to (Kaya and Stock, 1997). The nematodes obtained from *G. mellonella* larvae were kept in aqueous suspension at 16 ± 1 °C and stored for up to one week before being used in the experiments.

Three imported entomopathogenic nematodes (*Heterorhabditis bacteriophora* (HP88 strain), *Steinernema carpocapsae* (All strain) and *S.feltiae* (Filipjev) and one local strain isolated by EL-Ashry *et al*. (2018) by baiting technique of *G. mellonella* modified after Akhurst and Bedding (1975) from Belbies district, Egypt.

The nematodes were multiplied and harvested from greater wax moth larvae (Woodring and Kaya, 1988) and infective juveniles of these nematodes were washed in distilled water three times (Dutky *et al*., 1964).

Laboratory Bioassay:

Viability of EPNs in Combination with Tested Pesticides:

Ten milliliters of recommended dose each tested chemical namely chlorpyrifos (Pestban 48% EC), chlorpyrifos (Tafaban 48% EC) and flubendiamide (Takumi 20% WG) were poured in Petri dishes (9 cm diameter). The IJs of *Steinernema carpocapsae* (All strain), *S.feltiae* (Filipjev), *S. glaseri* (NC strain), *Heterorhabditis bacteriophora* (HP88 strain) and *H.bacteriophora* (Ba-1 strain) were added to the dilution at the rate of 100 nematodes per dish (0.1 ml of the stock nematode suspension). The control treatment consisted of the 100 IJs maintained in 10 ml distilled water free of pesticides. Each pesticide was replicated five times and the dishes were kept at 24±2 °C at the optimum temperature (Dunphy and Webster, 1986). All dishes were sealed tightly with parafilm to avoid solution vaporization. Treatments examination using 0.5 ml pipetted into a Hawksely counting slide by the aid of a research microscope at 100X.

The infective juveniles showing inactive straight posture and did not show any movement after prodding were considered dead (Nardo and Grewal, 2003) while, any other types of movement were scored as alive (Ishibashi and Takii, 1993).

Mortality examination recorded after 1, 2, 4 and 7 days. Nematodes mortality percent was calculated by the following equation:

$$\text{Mortality} \quad (\%) = \frac{\text{Dead larvae}}{\text{Total number of larvae}} \times 100$$

Effect of EPNs on *Heterorhabditis armigera* Viability:

The last instar larvae of *H. armigera* were transferred to Petri-dishes (9 cm diameter) lined with filter paper (Whatman No.1). Each dish was inoculated with each of the five nematode species at various concentrations (0, 50, 100 and 150 IJs/ml). All plates were sealed tightly with a plastic tab and incubated at 24 ± 2 °C. Each treatment was replicated five times. After 72 hours contacting period, the dead larvae were individually transferred to modified white traps (White, 1927) to evaluate nematode infectivity and mortality.

Combination effect of EPNs and Tested Pesticides on *Helicoverpa armigera* Viability:

The last instar larvae of *H. armigera* were transferred to Petri dishes (9 cm diameter) lined with filter paper (Whatman No.1) containing two milliliters of each chemical dilution (5 pesticides). The IJs were added at the rate of 100 nematodes per dish using 0.1 ml of the stock nematode suspension then sealed tightly. The control treatment consisted of the 100 IJs maintained in 2 ml pesticides free distilled water. Each pesticide was replicated five times and the dishes were kept at (24 ± 2 °C). Larvae mortality was counted after three days and percent of dead larvae was calculated by the following equation:

$$\textbf{Mortality}\ (\%) = \frac{\text{No. of Dead larvae}}{\text{Total number of larvae}} \times 100$$

Greenhouse Study:

Combination Effect of Entomopathogenic Nematodes and Tested Pesticides on *H. armigera*:

The experiment was conducted in a greenhouse at 20 20 °C and relative humidity 78%. The selected strains of entomopathogenic nematodes, *Steinernema carpocapsae* (All strain), *S. feltiae* (Filipjev), *S. glaseri* (NC strain), *Heterorhabditis bacteriophora* (HP88 strain) and *H. bacteriophora* (Ba-1 strain) were allowed to acclimate at room temperature for about two hours before application. Tomato plants after the flowering stage (45 days old) cv. Super strain B was transplanted in pots (20 cm diameter). At flowering stage (45 days) were selected for releasing twenty-five, healthy and active last instar larvae of *H. armigera* individually on the leaves of tomato.

Plants were sprayed with 5000 IJs (in 1 ml) of nematodes mixed with 9 ml of the recommended dose of the used pesticides while control treatment was sprayed with the same amount of distilled water. Larval mortality was checked daily for up to 3 days. Dead larvae were removed, rinsed in distilled water and incubated individually in Petri dishes 5 cm diameter lined with moist filter paper or modified white's trap to confirm mortality due to EPNs. After 3 days larvae were examined for signs of nematode infection and placed individually in the modified White traps (White, 1927) to observe nematode emergence. Few larvae, whose color was not altered nematode infection, were dissected to check the presence of nematodes.

Analysis of the Interaction Data of Mixtures:

Interaction data for mixtures were estimated using Limpel's formula reported by Richer (2006) as follows:

$$\mathbf{E} = \frac{(X + Y) - (XY)}{100}$$

Where:

E: The expected additive effect of the mixture.

X: The effect due to component A alone.

Y: The effect due to component B alone.

The expected effect was compared with the actual effect obtained experimentally from the mixture to determine the additive, synergistic or antagonistic effects, according to the equation given by Mansour *et al*. (1966) as follows:

$$\textbf{Co- toxicity factor} = \frac{\text{The observed effect (\%)} - \text{Expected effect (\%)}}{\text{Expected effect (\%)}} \times 100$$

This factor was used to classify results into three categories. A positive factor 20 or more is considered potentiation, a negative factor 20 or more means antagonism and intermediate values between -20 and +20 indicate only additive effect.

Statistical analysis

The experiments were carried out in a completely randomized design in a laboratory while, greenhouse experiment used a completely randomized block design. Data were subjected to analysis of variance (ANOVA) one way or two way using MSTAT version 4 (1987). Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS AND DISCUSSION

Toxicity of the Tested Insecticides and Nematicides To Infective Juveniles of Certain Entomopathogenic Nematodes:

Data in the Table (2) showed the tested nematodes response to different tested chemical pesticides used after exposure for 1, 2, 4, and 7 days. After one day of exposure, *S. glaseri* showed high sensitivity to fenamiphos and abamectin causing mortality 12.4 and 12.2 % with significant difference compared to chlorpyrifos formulations and flubendiamide. The tested chemical pesticides kept up the same data trend with increasing exposure period until the 7th day showing a significant increase in mortality percent of the IJs with fenamiphos (55.8%) followed by abamectin (54.4%) then chlorpyrifos formulations with no significance between formulations. Finally, flubendiamide significantly recorded 33.2% mortality.

Treated *S. carpocapsae* (All strain) with the tested chemical pesticides exhibited a high mortality percentage (14.2 %) in abamectin treatment with a significant difference followed by fenamiphos (12.2%) then chlorpyrifos formulations with no significant difference between the tested formulation, while, flubendiamide caused lowest significant toxicity on tested IJs strain recording 8.2% mortality after one day of exposure. With increasing exposure period, the mortality trend changed after 7 days of exposure, where, fenamiphos (61.4%) exhibited the highest significant toxicity followed by abamectin (55%), while, chlorpyrifos formulations caused 44.4 and 43.6% mortalities with no significance between chlorpyrifos formulations. On the other hand, flubendiamide (39 %) the lowest significant mortality.

The response of *S. feltiae* to abamectin was the highest recording significant mortality (18.6%), followed by fenamiphos with no different significance, while, chlorpyrifos formulations occupied the second significant rank causing mortality 15.8 and 15.6 % with no significance between formulations. Finally, flubendiamide (13.4%) recorded significant mortality after one-day exposure. With increasing exposure period to 7 days, the response of *S. feltiae* raised significantly with fenamiphos (68.6%) followed by abamectin (61.2 %), then, chlorpyrifos (51.8 and 50.6%) formulations with no significance between the tested chlorpyrifos formulations (Pestban and Tafaban). Finally, flubendiamide (44.6%) caused the lowest significant mortality. Abamectin proved to be lethal to *S. feltiae* (Raheel *et al.*, 2017) after fenamiphos. Chlorpyrifos had no effect on *S. feltiae* survival but seriously reduced their virulence after a 48-h exposure at field tank concentrations and overnight (Gutiérrez *et al.*, 2008).

The high sensitivity of *H. bacteriophora* (HP88 strain) to certain chemicals was exhibited with one day of exposure to abamectin (10.4%) and fenamiphos (9.4 %), followed by chlorpyrifos formulations (7.4 and 6.6 %) with no significance mortality between the tested formulations. Finally, flubendiamide recorded 5.6 % mortality with different significance with other treatments except for chlorpyrifos (Tafaban 48% EC) after one-day exposure. Mortality percentages raised gradually after 7th-day exposure, where, the toxicity of fenamiphos (51.8%) raised significantly followed by abamectin (48.6%) then, chlorpyrifos formulations recording 38.4 and 35.8 % for Pestban and Tafaban respectively, with no significance between formulations. Finally, flubendiamidee (30.4%) caused the lowest significant mortality.

On the other hand, *H. bacteriophora* (Ba-1 strain) (20.4%) showed significant high mortality after treating with fenamiphos followed by the rest of the treatments of pesticides which ranged between 12.2 to 14.6 % mortality with no significant difference between abamectin, chlorpyrifos, and flubendiamidee after one-day exposure. After 7 days of exposure to the chemical pesticides, fenamiphos showed (58.0%) significant mortality followed by, abamectin (52.8%) then, chlorpyrifos formulations and finally flubendiamidee significantly recorded 36.8 % mortality.

The experiment showed in the Table (2) was analyzed using two-way ANOVA to elucidate the interaction between EPN tested strains and tested pesticides. Results cleared no interaction between EPNs strain and pesticides but showed a significant difference between each factor ($P < 0.05$). Based on the comparison between the tested pesticides applied in the study after 7 days of exposure, fenamiphos (59.12 %) recorded the highest significant IJs mortality followed by abamectin (54.42 %) then, chlorpyrifos formulation. But chlorpyrifos (Pestban 48% EC) had higher toxicity than chlorpyrifos[1] (Tafaban 48% EC) recording 43.24 and 41.88 % with no significance between formulations. Finally, flubendiamide (36.8 %) recorded the lowest significant mortality as shown in Figure (1). On the other hand, EPNs, tested strains were arranged based on the susceptibility to the tested chemical pesticides in descending order *S. feltiae*, *S. carpocapsae* (All strain), *H. bacteriophora* (Ba-1 strain), *H. bacteriophora* (HP88 strain) and *S. glaseri* (NC strain) recording 55.36, 48.68, 46.52, 41 and 44.36 %, respectively, with a significant difference between all strains of EPNs, as shown in Figure (2).

Steinernematid species and heterorhabditid species showed an equal response to the tested chemical pesticides after one-day exposure. With increasing exposure period, the mortality of steinernematid species increased mortality with accelerated rate comparing with heterorhabditid species which increased with slow rate subsequently need long period for no exhibit the toxic effects after seven days exposure heterorhabditid species showed a high mortality accelerated rate as shown in Figure (3). So, heterorhabditid species were more tolerant than steinernematid species for the tested chemical pesticides. Based on the comparison between nematicide represented by fenamiphos and insecticides represented by abamectin, chlorpyrifos, and flubendiamide, as shown in Figure (4), the nematicide was more toxic to both tested species because of the fenamiphos selectivity and specificity on nematodes. Fenamiphos classified as one the most toxic chemical pesticides tested on *S. carpocapsae* and *S. feltiae* (Rovesti and Deseö, 1990) at or less than the recommended application rates(Rovesti, Heinzpeter, *et al.,* 1988). IJs of the entomogenous nematode *S. feltiae* were adversely affected by, fenamiphos causing partial paralysis with a curled or coiled posture stopping insect infection, but when washed in distilled water they recovered and were infectious to the noctuidae similar to that of untreated ones(Hara and Kaya, 1983). Not only fenamiphos completely suppressed all nictation and body waving behavior in both species *Steinernema carpocapsae* and *S. feltiae* at 10 and 50 µg/ml (Patel and Wright, 1996), but

also, it adversely affected the development and reproduction of *S. feltiae* when *S. exigua* larvae were first infected with the nematode and then treated (Hara and Kaya, 1983). Among all tested insecticides, EPN species were sensitive to abamectin (Raheel *et al.,* 2017; Laznik and Trdan, 2014). The results conflict *H. bacteriophora* sensitivity to abamectin (Laznik and Trdan, 2014). Chlorpyrifos combinations with *H. bacteriophora, Steinernema longicaudum,* and *Heterorhabditis indica* only resulted in additive mortality (YuDong *et al.,* 2012). Three species of EPNs mixed with different formulations were compatible (class 1) under laboratory conditions (Negrisoli *et al.,* 2010). Interaction of *H. indica, S. carpocapsae* and *S. glaseri* with chlorpyrifos (Vexter^{TM} and Lorsban^{TM}) and lufenuron with *S. glaseri* was synergistic(Negrisoli *et al.,* 2010), but depending on the formulation and the tested concentration. In contrast with, the undetectable effect of chlorpyrifos on nematode viability of *S. feltiae* and *H. bacteriophora* (Peters and Poullot, 2004), but susceptibility of Steinernematid species were more affected than heterorhabditid species (Devindrappa *et al.* 2017; Raheel *et al*. 2017; Bajc *et al*. 2017), also, insecticides take the same trend with both tested genus, but in general, heterorhabditid species were more tolerant than steinernematid species.

Table 2 : Mortality percentage of IJs of entomopathogenic nematodes after different exposure periods to the recommended doses of certain chemical pesticides under laboratory conditions**.**

Nematode species	Days after treatment	Pesticide				
		Abamectin	Chlorpyrifos	Chlorpyrifos[1]	Flubendiamide	Fenamiphos
S. carpocapsae **(All strain)**	**1**	14.2 ^{a}	11.4 ^{b}	11.2 ^{b}	8.2 ^{c}	12.2 ^{b}
	2	19.8 ^{a}	18.2 ^{ab}	18.6 ^{a}	12.8 ^{c}	16.4 ^{b}
	4	31.0 ^{a}	24.8 ^{b}	23.8 ^{b}	24.6 ^{b}	31.8 ^{a}
	7	55.0 ^{b}	44.4 ^{c}	43.6 ^{c}	39.0 ^{d}	61.4 ^{a}
S. feltiae **(Filipjev)**	**1**	18.6 ^{a}	15.6 ^{b}	15.8 ^{b}	13.4 ^{c}	18.2 ^{a}
	2	27.6 ^{a}	24.8 ^{b}	24.6 ^{b}	18.4 ^{c}	25.6 ^{b}
	4	38.8 ^{a}	31.2 ^{b}	31.0 ^{b}	31.2 ^{b}	40.6 ^{a}
	7	61.2 ^{b}	51.8 ^{c}	50.6 ^{c}	44.6 ^{d}	68.6 ^{a}
S. glaseri **(NC strain)**	**1**	12.2 ^{a}	8.8 ^{b}	8.6 ^{b}	8.2 ^{b}	12.4 ^{a}
	2	17.8 ^{a}	15.6 ^{bc}	14.6 ^{c}	12.6 ^{d}	16.6 ^{ab}
	4	27.6 ^{a}	21.6 ^{b}	19.4 ^{b}	20.4 ^{b}	26.2 ^{a}
	7	54.4 ^{a}	40.6 ^{c}	37.8 ^{c}	33.2 ^{d}	55.8 ^{a}
H. bacteriophora **(Ba-1 strain)**	**1**	14.6 ^{b}	12.8 ^{b}	13.2 ^{b}	12.2^{b}	20.4 ^{a}
	2	26.6 ^{a}	21.6 ^{b}	20.8 ^{b}	18.4 ^{c}	22.8 ^{b}
	4	32.0 ^{b}	28.6 ^{c}	28.0 ^{c}	32.4 ^{b}	36.4 ^{a}
	7	52.8 ^{b}	42.8 ^{c}	42.2 ^{c}	36.8 ^{d}	58.0 ^{a}
H. bacteriophora **(HP88 strain)**	**1**	10.4 ^{a}	7.4 ^{b}	6.6 ^{bc}	5.6 ^{c}	9.4^{a}
	2	14.2 ^{a}	12.2 ^{bc}	11.2 ^{cd}	10.8 ^{d}	13.2 ^{ab}
	4	25.6 ^{a}	20.2 ^{b}	18.4 ^{bc}	15.8 ^{c}	24.4 ^{a}
	7	48.6 ^{b}	38.4 ^{c}	35.8 ^{c}	30.4 ^{d}	51.8 ^{a}

Chlorpyrifos (Pestban 48% EC); Chlorpyrifos[1] (Tafaban 48% EC); each value is a mean of five replicates; Values followed by the same letter in the same row are not different according to Duncan's multiple-range test ($P < 0.05$).

EPNs survival and efficacy were also affected by host traits e.g. host species, host developmental stage, host's immune system, and molecules emitted by the host (Labaude and Griffin, 2018), so, changing EPN pathogenicity in the presence of chemical insecticides may be due to poor viability or mortality of IJs. The additive effect between *S. carpocapsae* with chlorpyrifos and synergistic effect *S. glaseri* with chlorpyrifos was recorded under laboratory conditions for the control of *Spodoptera frugiperda* (Negrisoli *et al.,* 2010). The response of EPNs varied toward different active ingredient formulation as shown with chlorpyrifos formulations, where, the toxicity of Pestban 48% EC was higher than Tafaban 48% EC. That may be due to the different adjuvants in each

formulation leading to limited different toxicity. EPNs susceptibility toward pesticides showed antagonistic response with the nematicide fenamiphos expressed as incompatibility but the tested insecticides showed additive effect with most combination and antagonism in others.

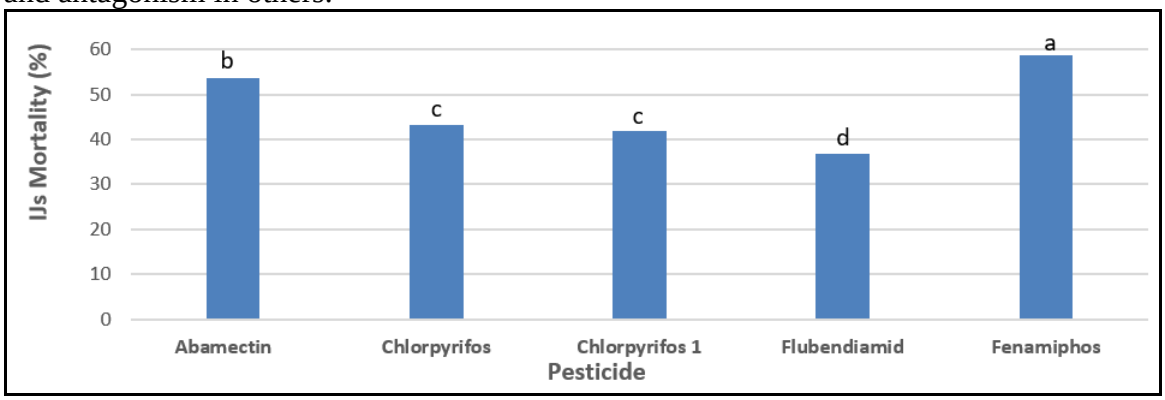

Fig. 1 :Mortality percentages of tested insecticides and nematicides on steinernematid and heterorhabditid species after seven days of exposure.

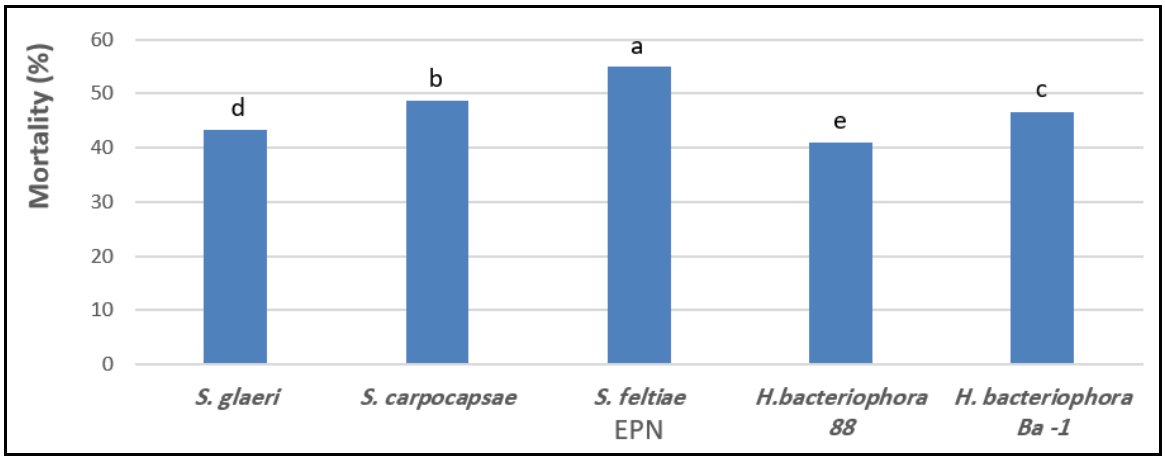

Fig. 2 :Mortality percentages of tested steinernematid and heterorhabditid species after seven days of exposure to insecticides and nematicides.

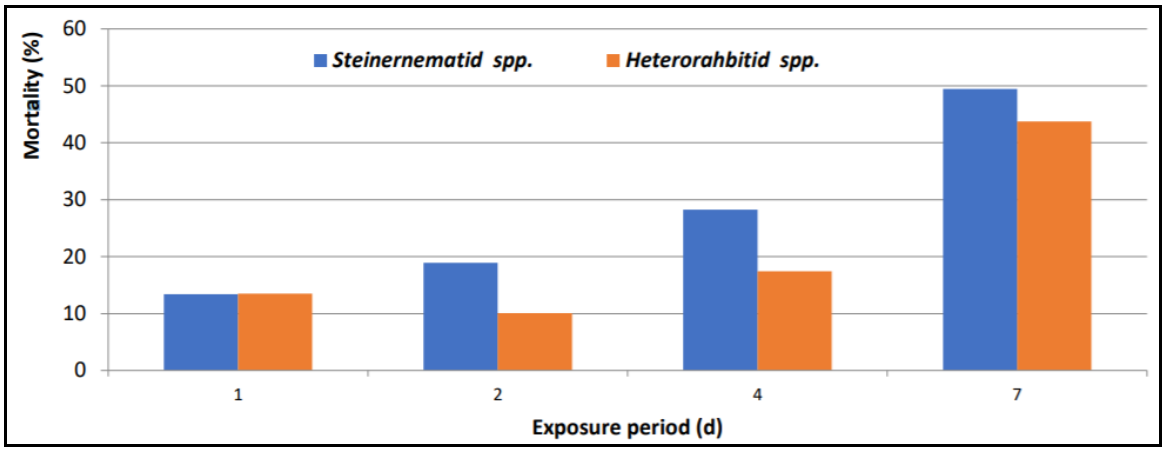

Fig. 3 :Steinernematid and Heterorhabditid species susceptibility after different periods of exposure to the tested chemical nematicide and insecticide.

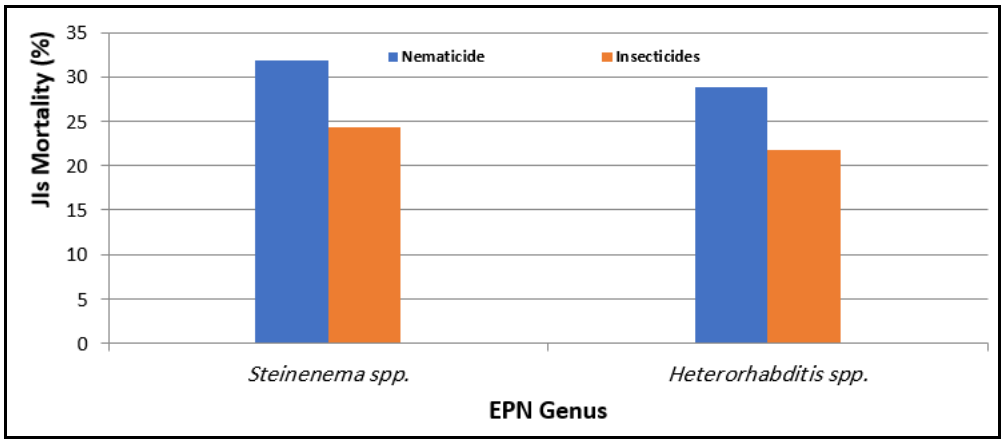

Fig. 4 :Percentage mortality in Steinernematid and Heterorhabditid species resulted from the tested chemical nematicide and insecticide.

Minimum Effective Concentration:

Data in Table (3) showed the mortality percent of the 5th instar larvae of tomato fruit worms infected with the tested EPNs strains after 3 days incubation with tested concentrations 50, 100 and 150 IJs/larvae. Results of 100 and 150 IJs/larva showed close values and a non-significant difference in all tested EPNs strains except *H. bacteriophora* (Ba-1 strain) that showed significant increasing mortality percent resulted from increasing the numbers of IJs per larva. On the other hand, the concentration of 50 IJs/larvae showed significantly lower mortality with all the tested EPNs strains. The descending arrangement of EPNs strains based on the strain infectivity was *H. bacteriophora* (HP88 strain), *S. carpocapsae* (All strain), *H. bacteriophora* (Ba-1 strain), *S. glaseri* (NC strain) and *S. feltiae.*

Many factors influencing EPNs survival and efficacy i.e. EPN species/strain, concentration, temperature, humidity, bacteria hosted, and insect host, etc. (Labaude and Griffin, 2018). The minimum effective concentration of the tested EPNs defined as the lowest concentration of an IJs which causes a considerable mortality rate of treated insect hosts *in vivo* only (McKinnon and Davis, 2004). Mortality rate resulted from increasing IJs concentrations of selected EPN species/strains led to an increase in the infectivity of entomopathogenic nematodes, considering EPN species/strain pathogenicity, insect host susceptibility and mass production cost.

Table 3 :Percentage mortality in 5th instar larvae of *Helicoverpa armigera* after 3 days of application of entomopathogenic nematodes under laboratory conditions.

Nematode species	Nematode Concentrations		
	50 IJs/larva	100 IJs/larva	150 IJs/larva
S. carpocapsae (All strain)	56.0 ^{b}	71.0 ^{a}	71.0 ^{a}
S. feltiae (Filipjev)	29.0 ^{b}	52.0 ^{a}	55.0 ^{a}
S. glaseri (NC strain)	32.0 ^{b}	55.0 ^{a}	58.0 ^{a}
H. bacteriophora (Ba-1 strain)	45.0 ^{c}	61.0 ^{b}	70.0 ^{a}
H. bacteriophora (HP88 strain)	53.0 ^{b}	75.0 ^{a}	76.0 ^{a}

Each value is a mean of five replicates; Values followed by the same letter in each row are not different according to Duncan's multiple range test ($P < 0.05$).

Interactions Between Chemical Pesticides and Entomopathogenic Nematodes:

Interactions between chemical pesticides and different strains of entomopathogenic nematodes in controlling the 5th instar larvae of tomato fruit worm *in vitro* are

demonstrated in Table (4). Abamectin mixed with the tested EPNs strains showed additive interaction with *H. bacteriophora* (HP88), *S. carpocapsae* and *S. glaseri* recording Co-toxicity factor -7.92, -13.09 and -18.24, respectively, whereas, abamectin mixed with *S. feltiae* and *H. bacteriophora* (Ba-1) showed antagonism interaction recording -20.47 and -24.48, respectively. *S. feltiae* infectivity was significantly reduced following exposure to abamectin (Head *et al.*, 2000)

Chlorpyrifos (Pestban 48% EC) showed an additive effect after mixing with *H. bacteriophora* (HP88), *S. carpocapsae, S. glaseri* and *H. bacteriophora* (Ba-1) recording CF= -4.82, -7.63, -11.76 and -19.06 respectively. While Chlorpyrifos mixed with *S. feltiae* (CF= -22.99) (Table 4) showed antagonism interaction.
Chlorpyrifos[1] (Tafaban 48% EC) exhibited additive interaction with *S. carpocapsae, H. bacteriophora* (HP88) and *S. glaseri* resulting (CF= -10.20, -14.91 and -17.05), respectively, but Chlorpyrifos[1] mixed with *H. bacteriophora* (Ba-1) and *S. feltiae* recorded -27.44 and -33.27 and caused antagonism interaction (Table 4).

Although, flubendiamidee considered the lowest toxic active ingredient on EPNs. The antagonism interaction is shown after mixing with *H. bacteriophora* (Ba-1) and *S. feltiae* recording (CF= -32.22 and -38.86). Additive interaction of flubendiamide was performed after mixing with *H. bacteriophora* (HP88), *S. glaseri* and *S. carpocapsae* recording CF= -12.58, -18.10 and -18.13, respectively (Table 4). Fenamiphos exhibited high toxicity and antagonistic effect against *S. carpocapsae, S. glaseri, H. bacteriophora* (HP88), *S. feltiae* and *H. bacteriophora* (Ba-1) recording CF= -26.83, -29.73, -38.39, -40.42 and-42.88, respectively (Table 4).

Table 4 :Interactions between chemical pesticides and different entomopathogenic nematodes strains on mortality of the 5th instar larvae of *Helicoverpa armigera* under laboratory conditions.

Chemical pesticides	Nematode Species	Mortality (%) (Nematodes + pesticides)		Co-toxicity factor (CF)	Response
		Observed	Expected		
Abamectin	***S. carpocapsae***	**80.4**	**92.52**	**-13.09**	additive
	S. feltiae	**72.0**	**90.54**	**-20.47**	antagonism
	S. glaseri	**74.2**	**90.76**	**-18.24**	additive
	***H. bacteriophora* (HP88)**	**86.2**	**93.62**	**-7.92**	additive
	***H. bacteriophora* (Ba-1)**	**70.2**	**92.96**	**-24.48**	antagonism
Chlorpyrifos	***S. carpocapsae***	**84.2**	**91.16**	**-7.63**	additive
	S. feltiae	**68.4**	**88.82**	**-22.99**	antagonism
	S. glaseri	**78.6**	**89.08**	**-11.76**	additive
	***H. bacteriophora* (Ba-1)**	**74.2**	**91.68**	**-19.06**	additive
	***H. bacteriophora* (HP88)**	**88.0**	**92.46**	**-4.82**	additive
Chlorpyrifos[1]	***S. carpocapsae***	**78.8**	**87.76**	**-10.20**	additive
	S. feltiae	**56.4**	**84.52**	**-33.27**	antagonism
	S. glaseri	**70.4**	**84.88**	**-17.05**	additive
	***H. bacteriophora* (Ba-1)**	**64.2**	**88.48**	**-27.44**	antagonism
	***H. bacteriophora* (HP88)**	**76.2**	**89.56**	**-14.91**	additive
Flubendiamide	***S. carpocapsae***	**72.4**	**88.44**	**-18.13**	additive
	S. feltiae	**52.2**	**85.38**	**-38.86**	antagonism
	S. glaseri	**70.2**	**85.72**	**-18.10**	additive
	***H. bacteriophora* (Ba-1)**	**60.4**	**89.12**	**-32.22**	antagonism
	***H. bacteriophora* (HP88)**	**78.8**	**90.14**	**-12.58**	additive
Fenamiphos	***S. carpocapsae***	**66.2**	**90.48**	**-26.83**	antagonism
	S. feltiae	**52.4**	**87.96**	**-40.42**	antagonism
	S. glaseri	**62.0**	**88.24**	**-29.73**	antagonism
	***H. bacteriophora* (Ba-1)**	**52.0**	**91.04**	**-42.88**	antagonism
	***H. bacteriophora* (HP88)**	**56.6**	**91.88**	**-38.39**	antagonism

Chlorpyrifos (Pestban 48% EC); Chlorpyrifos[1] (Tafaban 48% EC); each value is a mean of five replicates.

Combination of Selected EPNs With Tested Insecticides:

Based on the laboratory experiment, *S. carpocapsae*, *H. bacteriophora* (HP88 strain) and *H. bacteriophora* (Ba-1 strain) were selected as the highest compatible strains to control the 5th instar larvae of tomato fruit worm on tomato with tested pesticides under greenhouse conditions (Table 5). Results demonstrated that *S. carpocapsae* mixed with fenamiphos or chlorpyrifos recorded the highest significant mortality 52.0 and 52.0%, respectively. However, abamectin mixed with *S. carpocapsae* (44.0%) caused lower significant mortality, followed by chlorpyrifos[1] (42.0%) or flubendiamide (40.0%). finally, *S. carpocapsae* alone recorded 28% mortality.

Table 5 :Mortality percentage of tomato fruit worm resulted from mixing the selected EPNs strains with the tested chemical pesticides on tomato plants infested with the 5th instar larval of *H. armigera* under greenhouse conditions.

Nematode species	Mortality (%)					
	Control*	Abamectin	Chlorpyrifos	Chlorpyrifos[1]	Flubendiamide	Fenamiphos
S. carpocapsae (All strain)	28.0 ^{c}	44.0 ^{b}	52.0 ^{a}	42.0 ^{ab}	40.0 ^{b}	52.0 ^{a}
H. bacteriophora (HP88 strain)	36.0 ^{c}	54.0 ^{b}	54.0 ^{b}	44.0 ^{c}	42.0 ^{c}	64.0 ^{a}
H. bacteriophora (Ba-1 strain)	30.0 ^{c}	40.0 ^{b}	46.0 ^{ab}	46.0 ^{ab}	44.0 ^{ab}	50.0 ^{a}

* Negative control for chemical pesticides and positive control for Tested EPN species under greenhouse conditions; Chlorpyrifos (Pestban 48% EC); Chlorpyrifos[1] (Tafaban 48% EC); each value is a mean of five replicates. The same letter (s) in each row indicate no significant difference ($P \leq 0.05$) between treatments according to Duncan's multiple range test.

On the other hand, *H. bacteriophora* (HP88 strain) mixed with fenamiphos (64%) recorded the highest significant mortality, followed by significant mortality of *H. bacteriophora* (HP88 strain) (54%) resulted from mixed with abamectin or chlorpyrifos (54 %). *H. bacteriophora* (HP88 strain) mixed with chlorpyrifos1, flubendiamide and EPN alone recorded 44.0, 42.0 and 36.0 % mortality. *H. bacteriophora* (Ba-1 strain) mixed with Fenamiphos caused the highest significant mortality (50%) followed by abamectin mixture (44.0%), then, chlorpyrifos, chlorpyrifos[1] and flubendiamide mixtures recording 46,46 and 44 %. Finally, *H. bacteriophora* (Ba-1 strain) (30%) recorded the lowest significant mortality (Fig.5).

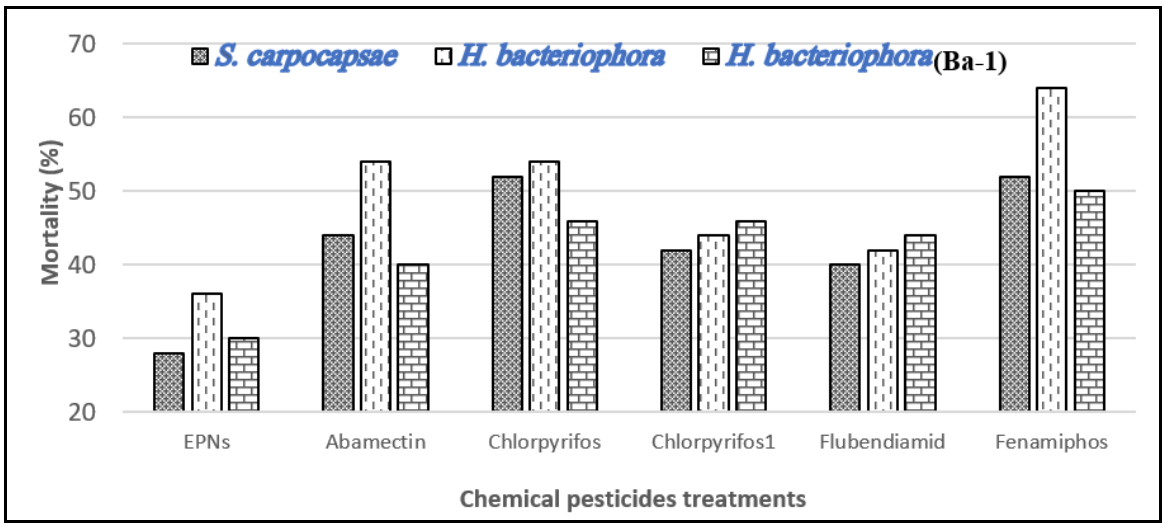

Fig. 5 :Mortality percentage of tomato fruit worm resulted from mixing the selected EPNs strains with the tested chemical pesticides on tomato plants infested with the 5th instar larvae of tomato fruitworm in greenhouse

Fenamiphos mixed with EPNs were the most potent and toxic chemical nematicides against tomato fruit worm resulting from the activity against insects and nematodes, followed by chlorpyrifos, abamectin, chlorpyrifos[1], flubendiamide and finally EPN alone as shown in Figure (6). The most compatible EPN strain was *H. bacteriophora* (HP88 strain) followed by *S. carpocapsae* then *H. bacteriophora* (Ba-1 strain) as demonstrated in Figure (7).

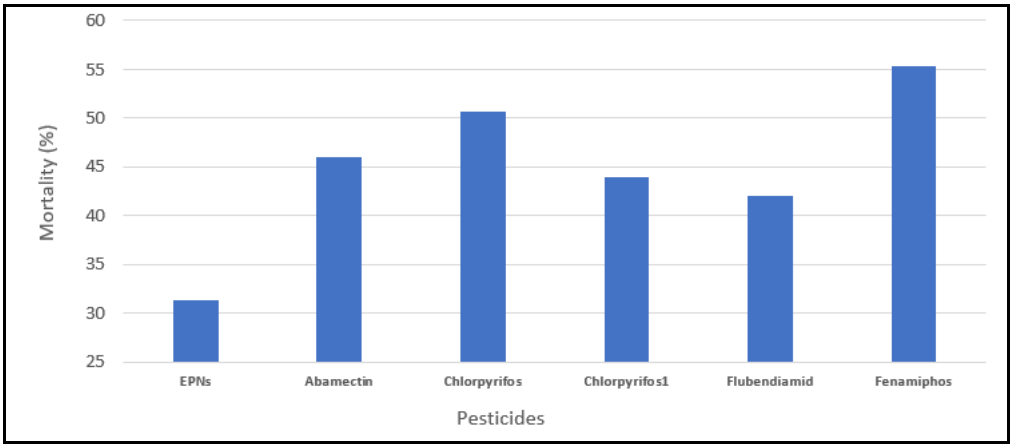

Fig. 6 :Cumulative mortality percentage of tomato fruit borer resulted from mixing chemical pesticides with *S. carpocapsae*, *H. bacteriophora* (HP88 strain) and *H. bacteriophora* (Ba-1 strain)

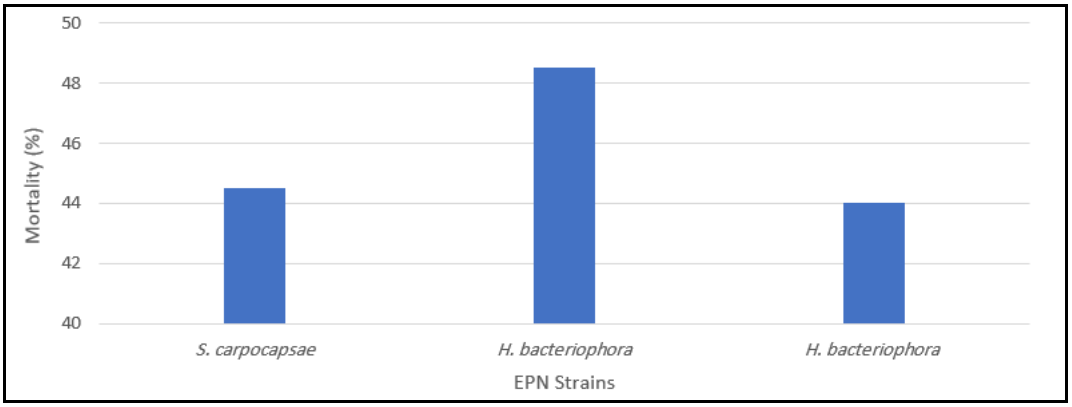

Fig. 7 :Cumulative mortality percentage of tomato fruit borer resulted from the mixtures of selected EPN strains with the tested insecticides.

The synergistic interaction and the negative effect of higher abamectin rates on *Steinernema carpocapsae* were confirmed by (Kary *et al.*, 2018) in a greenhouse experiment on potato plants but abamectin concentration plays the vital role in interaction type, also, previous studies elucidated that the chemical pesticides showed a strong sublethal effect on *S. carpocapsae* and *H. indica* nematode reproductive potential, limiting seriously their possible recycling in the open field (Devindrappa *et al*., 2017) (Gutiérrez *et al.*, 2008). Overall, results indicate the feasibility of combinations and integrated use of these nematode species and chemical pesticides in plant protection (Rovesti and Deseö, 1990). Optimizing EPN dosages and estimating their field recycling depend on interaction results (Gutiérrez *et al*., 2008).

Conclusion:

Based on this research work, it can be concluded that compatibility is not only a species-specific but also a strain-specific characteristic. Chlorpyrifos, chlorpyrifos[1,] and

flubendiamide were less toxic compared to fenamiphos under laboratory conditions, but, flubendiamide was the lowest toxicity against IJs of EPNs. Moreover, steinernematid species were more sensitive than heterorhabditid species to such pesticides. Most tested strains showed additive effect after mixing with the tested insecticides, therefore, as a precaution, mixing can be included when necessary, but it is preferable to use EPN after applying pesticides to avoid adverse effects and also ensure sustainability.

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ARABIC SUMMARY

التأثير المشترك لمخاليط النيماتودا الممرضة للحشرات والمبيدات الكيميائية في مكافحة حشرة دودة اللوز الأمريكية

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تتميز المبيدات الكيميائية بالتأثير السريع في الحد من تعداد الآفة، بينما تحتاج المبيدات الميكروبية لفترة كمون أطول لتحدث خفض محدود في تعداد الآفة. ولعل انخفاض كفاءة المبيدات الحيوية ناجمة عن عدم التوافق مع الكيماويات الزراعية أو سوء التطبيق، لذلك تهدف الدراسة لتعزيز المعرفة حول إمكانية خلط المبيدات الحشرية والنيماتودية الكيميائية الشائعة: أبامكتين (تيرفيجو ٢٪ مركز معلق) والكلوربيريفوس (بستبان ٤٨٪ مركز قابل للاستحلاب) وكلوربيريفوس[١] (تافابان ٤٨٪ مركز قابل للاستحلاب) و الفلوبيدايامايد (تاكومى ٢٠٪ حبيبات قابلة للانتشار في الماء) وفيناميفوس (دينتو ٤٠ % مركز قابل للاستحلاب) مع النيماتودا الممرضة للحشرات المستوردة *Steinernema carpocapsae* كل السلالات و *S. feltiae* و *Heterorhabditis bacteriophora* سلالة HP 88 وعزلة محلية *H. bacteriophora* من بلبيس لمكافحة دودة ثمار الطماطم *Helicoverpa armigera*. أظهرت اختبار الحيوية للطور المعدي للنيماتودا المعامل بالمبيدات الكيميائية بالجرعة الموصي بالمعمل أن المبيدات الحشرية الكلوربيريفوس (بستبان ٤٨٪ مركز قابل للإستحلاب) وكلوربيريفوس (تافابان ٤٨٪ مركز قابل للاستحلاب) وفلوبينديميد (تاكومى ٢٠٪ حبيبات قابلة للانتشار) والفلوبيدايامايد (دينتو ٤٠٪ مركز قابل للاستحلاب) فى الماء كانت أقل سمية ضد الطور المعدي للنيماتودا مقارنة بالفينياميفوس في المختبر. علاوة على ذلك، كانت أنواع *Steinernematid* أكثر حساسية عن أنواع *Heterorhabditid* مسجلة ٤٩,٤٥ ٪ و٤٣,٧٦ ٪ على التوالي بعد التعرض لمدة ٧ أيام. أظهر الفعل المشترك لمبيدات الآفات الكيميائية التي تم اختبارها الأطوار المعدية للنيماتودا لمكافحة يرقات الطور الخامس لديدان ثمار الطماطم تفاعلًا إضافيًا أو تضاد مع عدم وجود دليل على الفعل التنشيطي. تفاعل التضاد سجّل مع جميع مخاليط الفينياميفوس، وأيضًا مخاليط من الابامكتين و*S. feltiae* و *H. bacteriophora* عزلة بلبيس، بالإضافة إلى مخاليط فلوبينديميد و*S. feltiae* و*H. bacteriophora* عزلة بلبيس. في حين لوحظ تأثير إضافي في مخاليط فلندنديميد مع *S. glaseri* و *S. carpocapsae* و *H. bacteriophora* (HP 88). تسبب تطبيق النيماتودا الممرضة للحشرات فقط في قتل ٢٨ إلى ٣٦ ٪ من الأطوار اليرقية المعرضة تحت ظروف الصوبة، حيث سجلت أعلى نسبة موت لليرقات في مجموعات من نيماتودا *H. bacteriophora* سلالة HP88 مع فينياميفوس (٦٤٪) وكلوربيريفوس (٥٤٪) والأبامكتين (٥٤٪)، في حين حققت العزلة المحلية نسبة موت تراوحت من ٤٠ إلى ٥٠ ٪ مع المبيدات المختبرة.