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Compatibility of Egyptian strains of entomopathogenic nematodes (Nematoda: Rhabditida) with insecticides and their activity against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) under laboratory conditions.

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Abstract

Under laboratory conditions, the activity of two entomopathogenic nematodes, *Heterorhabditis bacteriophora* (HP88) and *Steinernema carpocapsae* (AT4), as well as their compatibility with two common insecticide formulations (lambada cyhalothrin and flubendiamide) and one insect growth regulator (lufenuron), were evaluated against *Spodoptera frugiperda*. The nematode IJs were subjected to the LC_{50} and LC_{25} of insecticides, and the viability of infective juveniles (IJs) was measured before being tested against *Galleria mellonella* larvae to determine IJs pathogenicity. Also, the mixtures were evaluated against the 4th instar larvae of *S. frugiperda* to select one mixture can serve in integrated pest management (IPM) in agro-ecosystems.

Results showed that *H. bacteriophora* strain (HP88) was more efficient with higher pathogenicity and virulence against 4th larvae of *S. frugiperda* (LC_{50}= 48.67±0.34IJs/ml^{-1}) than *S. carpocapsae* (AT4) (LC_{50}= 65.88± 3.04). flubendiamidee was the most toxic insecticide (LC_{50} = 3.26±0.52) followed by Lambada cyhalothrin (4.23±0.8PPM). Lufenuron was the least toxic one (5.67±1.53PPM). Both EPN *H. bacteriophora* (HP88) and *S. carpocapsae* viability was not affected by any of tested insecticides (LC_{25} and LC_{50}), while lambada cyhalothrin reduced *S. carpocapsae* pathogenicity with LC_{50} concentration. Lufenuron at the rate LC50 and Lc25, not affect *H. bacteriophora and S. carpocapsae* pathogenicity. All tested insecticides with the two doses were harmless according to IOBC test. All mixtures of insecticides with the two EPN strains were synergistic effect against 4 th larvae of *S frugiperda* except lufeuron with LC_{25} was antagonist. It was explored how these nematode insecticide combinations could be used in maize pest management.

1. Introduction

The fall armyworm (*Spodoptera frugiperda*) is a major invasive pest that has recently spread to many nations throughout the world. It entered Egypt in 2020 [1]. It considered destructive pest for many major crops. It can damage and destroy a wide variety of crops, such as maize, sorghum, forage [2]. The intensive use of insecticides has caused it to acquire resistance to these pesticides [3], their residues harm the environment and human health.[4]. The long persistence of pesticides, loss during the application, leaching, and residual on crops affect public health. So, the use of alternative pesticides to overcome this problem is greatly needed. Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are fatal insect parasites utilized as biological agents against a variety of economically significant insect pests. EPNs can be just as effective as conventional pesticides when conditions like humidity, temperature and UV radiation are ideal. [5]. Combining low-impact insecticides or lower pesticide doses with biological control agents, according to study, may boost biological control agent effectiveness while reducing insecticide toxicity. [6, 7]. Entomopathogenic nematode compatibility with insecticides has the potential to play a significant role in biological insect control. The use of modest amounts of certain insecticides induces physiological weakening of the insect organism and diminishes its resistance to EPNs, according to research. [8-10] They observed that organophosphates and carbamates had sublethal effects on IJs, impaired. [11] observed that (chlorpyrifos, deltamethrin, lufenuron, Deltaphos diflubenzuron, lambda-cyhalothrin, spinosad, cypermethrin, triflumuron, and permethrin) were compatible (class 1) with the three tested nematode species (*Heterorhabditis indica*, *Steinernema carpocapsae* and *Steinernema glaseri*) under laboratory conditions. As a result of the independent position of the different control agents (i.e. EPNs and pesticides), the use of multiple control agents (i.e. EPNs and pesticides) reduces the development of insect resistance. A combination of *Steinernema carpocapsae* and abamectin, for example, proved effective in suppressing *Phthorimaea operculella* Zeller. [12] and Combinations of *Heterorhabditis indica, S. carpocapsae,* and indoxacarb had an additive impact on the control of *S. litura* (Fab). [13]. Integral to integrated pest management (IPM) strategies is the compatibility of entomopathogenic nematodes (EPNs) with pesticides. The goal of this study was to assess the effectiveness of using EPN species and pesticides together to avoid the

infestation with army warm. Three insecticides (lufenoron, lambda-cyhalothrin, and flubendiamide) were examined with EPNs (*Steinernema carpocapsae* (B32), *H. bacteriophora*, and *H. bacteriophora* at concentrations of LC_{50} with LC_{50} and $LC_{25}$of insecticides to see whether it was possible to combine three commonly used chemical insecticides with two compatible entomopathogenic nematodes (EPNs).

2. Materials and Methods.

2.1 Rearing of *Spodoptera frugiperda*

The investigations were carried out at the Plant Protection Dept. Faculty of Agriculture Minia University's laboratories. Initially, fall army worm (FAW) larvae were collected from severely infected maize grown in Derwa village, Mallawi region, and laboratory-reared in a Glass cup at room temperature (26 2°C). Fresh castor bean leaves were fed to the larvae. [14]

2.2. Nematode strains

In the tests, two strains of EPNs, *Heterorhabditis Bacteriophora* (HP88) and *Steinernema carpocapsae* (AT4), were obtained from the laboratory of Center nematode, Faculty of Agriculture, Cairo University and were reared in vivo on full-grown larvae of the greater wax moth, *G. mellonella*. [15]. *G. mellonella* larvae were grown in an insect rearing laboratory on old bee wax at 28±2 Cº and 65% Relative humidity. The emerging infective juveniles (IJs) were collected from nematode traps and stored in sterilized water at 10Cº. [16].

2.3. Insecticide used:

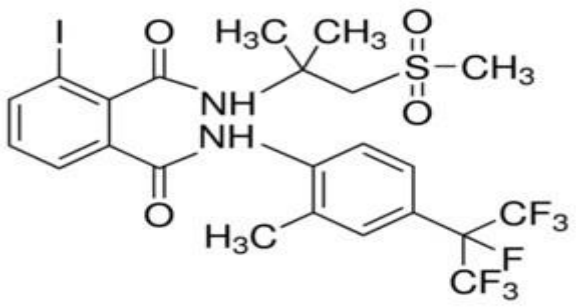
2.3.1. lufenuron, (Match 50% EC)

N– [2, 5–dichloro–4– (1, 1, 2, 3, 3, 3– hexa-fluoropropoxy) – phenyl-aminocarbonyl]–2, 6–difluorobenzamide, a novel acylurea insect growth regulator.

2.3.2. lambada cyhalothrin EC5%: (R)-α-cyano-3-phenoxybenzyl (1S)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and (S)-a-cyano-3-phenoxybenzyl(1R)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2- dimethyl cyclopropane carboxylate.)

2.3.3. Flubendiamide 480 SC (39.35% w/w) Fame Bayer

Flubendiamide is a new generation green insecticide, based on the active ingredient flubendiamide from the novel class of insecticide benzene dicarboxamide (diamide insecticides group). In insects, flubendiamide alters how muscles should work. Flubendiamide triggers the release of intracellular calcium that is ryanodine-sensitive. channels (ryanodine receptors).



Chemical name: N2-[1,1- dimethyl-2-(methyl sulfonyl)ethyl]-3-iodo-N1-[2- methyl1-4-[1,2,2,2- tetrafluoro-1- trifluoromethyl) ethyl phenyl] 1-1,2 bendiamide

2.4. Bioassay of EPNs strains and insecticides against 4th instar larvae of *S. frugiperda*

The bioassay of EPNs strains and pesticides on *S. frugiperda* larvae in their fourth instar was established. 10 cm in diameter and 1.5 cm in height Petri dishes were used for the experiment. Each strain was tested at four different concentrations: 25IJs, 50IJs, 200IJs, and 400IJs/ml-1. To maintain the proper moisture for the tested nematode's action, each concentration was poured onto moistened filter paper. Each replicate's wet filter paper was used to cover five larvae. For each treatment, three replicates were employed. Each insecticide was applied using the dipping method. After 48 hours following treatments, the numbers of dead insects were counted. The control treatment was given 2 mL of distilled water. The virulence of all nematode isolates examined in the laboratory was evaluated, and the adjusted mortality percentage was calculated for each concentration against *S. frugiperda* 4th instar larvae. As a control, a similar concentration of IJs was suspended in pure water. With the dipping method, five concentrations of each insecticide were utilised. The number of killed insects was counted 48 hours after the treatments. According to the Abbott formula, the % mortality in each treatments was corrected for control mortality. [17].

$$\text{Corrected mortality \%} = \frac{(\textit{Mortality \% in treatment} - \textit{Mortality \% in control})}{100 - \textit{Mortality\% in control treatment}} \} \boldsymbol{X\ 100}$$

Lethal concentration (LC_{50}, LC_{90}, and their fiducial limits to control between them [18]. The technique similar to the above described, using the following mixtures concentrations 6 concentrations from each insecticide: (LC_{50} of each EPN strain with LC_{25} and LC_{50} of each tested insecticides) were used to check the virulence of mixtures of EPNs with insecticides against 4th instar larvae of *S. frugiperda*.

2.5. Pesticides' Influence on Infectivity

2.5.1. Insecticide effects on nematode survival

The effects of insecticides on nematode survival were investigated using the [19] method. With modifications to reach LC_{25} and LC_{50} concentrations, insecticide dilutions were made in distilled water and applied to each Petri plate. The insecticide-treated IJ were shaken incubated for 72 hours at 25°C in the dark.

With a density of 500 IJ ml^{-1}, each treatment was reproduced three times (in dishes). As controls, infective juveniles were only treated with distilled water. Following that, three 100 ml samples were taken from each dish, and the IJ mortality was calculated. When infective juveniles did not respond to probing with a needle, they were deemed dead. Pesticides' impact on reproductive capacity were calculated

2.5.2. Pesticides' impact on reproductive capacity

G. mellonella fourth instar larvae were employed as hosts to test the infectivity of IJs treated with pesticides. In test tubes, treated EPNs were put, distilled water was added, and the supernatant was discarded after 30 minutes. This treatment was carried out

three times to completely eliminate pesticides. Following the final rinse, 150 IJs/100 µl was applied to the petri dish containing filter paper. Each petri dish contains last instar *G. mellonella* larva that has been incubated at 25 ±1 °C. Cadavers *G. mellonella* larvae were placed to White's traps immediately after mortality was seen. They were then incubated for 10 days at 25± 1 °C in the dark. The total number of newly formed IJs. The corrected mortality of all tested insecticides was also expressed as the reduction coefficient ***Ex*** for each insecticide. ***Efx*** calculated using the following formula

***E_{mx} = corrected mortality = ((T/C)*100)**

E_{fx} = Reproductive capacity *Efx* = [((Fc-Fx)/ FC) *100]

Where **Fx** is the mean nematode reproductive capacity of each insecticide

Fc is the nematode reproductive capacity recorded in the control

The values **(Ex)** were calculated as following equation [19]

$$EX = \{100[1 - (1 - (Emx/100)][1 - (Efx/100)]\}$$

Where *Ex*= Reduction coefficient, *Emx* = Corrected mortality

Efx = Reproductive capacity calculated before.

and then classified according to the standards of the International Organization of Biological Control (IOBC) which include 4 classes: harmless: *Ex* < or = 30%; slightly harmful: 30 to 79%; moderately harmful: 80 to 99%; harmful: Ex = or > 99%. The experiment was replicated twice and the average was used in calculation

2.6. Application of entomopathogenic nematodes and insecticides against *Spodoptera frugiperda* in laboratory conditions.

To evaluate the joint action of the mixtures of nematodes and insecticides, *against S. frugiperda* in laboratory conditions. Glass Petri dishes with 10-cm diameter were used, five 4th instae larvea of *S. frugiperda* were used in each Petri dish, and then 1 mL of each EPN was added at LC_{50} concentration together with 1 mL of each insecticide at each concentration (LC_{25} and LC_{50}) of each insecticides and 1 mL of distilled water were put on Filter paper, in the dish. The dishes were sealed with Parafilm® and placed at 27 ± 1°C. The mextures used as following treatments all experiments were replicated twice and average of replicates were analyzed.

Combination of each insecticide at LC_{25} + LC_{50} dose of entomopathogenic nematodes; Combination of each insecticide at LC $_{50}$ of the insecticide + LC_{50} of entomopathogenic nematodes; Control treated with 3ml water). The treatments were repeated 2 times. For the evaluations of the treatments with the presence of the nematodes, the larvae were dissected and observed under a stereoscopic microscope to confirm their mortality caused by EPNs. larval mortality was evaluated 96 h post treatments. Before analysis, all mortality data were corrected for control mortality [17]. To determine the type of interaction (synergistic, additive, or antagonistic) the method was first described by [20, 21]. The expected additive mortality *Me* for the EPN/insedcticide combinations was calculated by

$Me = Mn + Mt(1 - (\frac{Mt}{100}))$, where

Mn and *Mt* are the observed proportional mortalities relatively caused

by EPNs (50 %) and insecticide alone(25 or 50%). Chi-square test was calculated

using the following equation

$X2 = (Mnt - Me) \uparrow 2 \div Me$, where *Mnt* represents

the observed mortality for the EPN/insecticide mixture. The calculated value of chi-square was compared with the χ^2 table value for degree of freedom one. If calculated values are greater than the table value (χ^2 1, 0.05 = 3.84), non-additive effects. The synergistic or antagonisticof the mixtures was calculted according to [21]. If the differences *Mnt–Me* was positive value, the interaction was considered synergistic effect, and if the difference was negative, the interaction was considered antagonistic. Corrected mortality values were submitted to analysis of variance (ANOVA), and the difference between the means of the treatments was analyzed using LSD test ($p < 0.05$), and with software Costat.

3. Results and Discussion

3.1. Results of Bioassay

3.1.1. Toxicity of three pesticides against 4th instar larvae of Spodoptera frugiperda

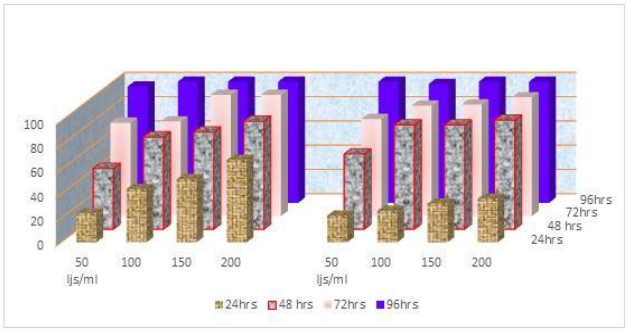
Data in Table (1) showed the toxicity of tested chemical insecticides, **Flubendiamide,** lambada cyhalothrin against 4th instar larvae of fall armyworm (FAW) *S. frugiperda* when applied with immersion technique method. Insecticides were rapid and highly toxic, then EPNs in which compounds gave effects to calculate their LC_{50} values after 24 hours and were (3.26± 0.52 and 04.23±0.85) for flubendiamide and lambada cyhalothrin, respectively without a large variation among LC_{50} values among the two insecticides. Lufenuron was the least toxic insecticide 05.67±1.53 PPM with significant differences when compared its fiducial limits of LC_{50} with flubendiamide and lambada cyhalothrin.

3.1.2. Toxicity of two EPN strains against 4th instar larvae of *Spodoptera frugiperda*

As shown in Table 1 *H. bacteriophora* HP88 showed the highest virulence on ***S. frugiperda***, presenting LC_{50} equal 48.67± 0.34 IJs/ml-1 (28.78 -82.28). Strain *S. carpocapsae* (AT4) was less virulent LC_{50} were (65.88± 3.07 IJs/ml -1) with fiducial limits (27.27-155.33) 59.32) with no significant differences between the two strains.

Based on these results, the isolated strains *H. bacteriophora* (Hp88) and *S. carpocapsae* (AT4) confirmed their efficiency against ***S. frugiperda*** and are promising strains for controlling *it* with concentrations equal to the upper limit of LC99 in the field as shown in Fig 1. [14] showed that *S. frugiperda* larvae were sensitive to (EPNs) *H. bacteriophora* and *S. carpocapsae* under laboratory conditions. [22, 23].

Also indicated that the susceptibility of larvae of *S. frugiperda* to the entomopathogenic nematode *H. bacteriophora* and *strain S.carpocapsae*



H. bacteriophora ***S. carpocapsae***

Fig 1 Efficiency *H. bacteriophora* (Hp88) and *S. carpocapsae* (AT4) against *S. frugiperda* with different concentrations at different time of exposure

3.2. Pesticide effects on entomopathogenic nematode infectivity

3.2.1. The impact of various insecticides on entomopathogenic nematodes' ability to survive

Table (2) show the average corrected mortality of IJ of both two nematodes after treatment with the tested pesticides with two Lethal doses LC50 and LC25 and the exposure time was 72 h. Results showed that S. carpocapsae strain was more tolerant to insecticides flubendiamide and lambada cyhalothrin when treated with LC50 corrected mortality % were 24.99 and 23.00% than H. bacteriophora HP88 strain corrected mortality were (34.09). In addition, there was no significant differences between the strains. We can classified the susceptibility of the strains to insecticide according to IJ survival (highly sensitive, sensitive, moderately sensitive and tolerant) [24]. H. bacteriophora strain was sensitive to lambada cyhalothrin and flubendiamide with concentration of LC50 (means of survived IJs were from 50 to 70%). Corrected mortality was 34.09 and 32.95%, while as the other concentration and treatment were moderately sensitive group ((means of IJs who survived ranged from 70 to 90%).i.e corrected mortality from 10 to 30%) included lufenuron, with LC50, lambada cyhalothrin and flubendiamide with LC25 and the two strains were tolerant to lufenuron with LC25. whereas S. carpocapsae strain was tolerant to lufenuron and lambada cyhalothrin when treated with lethal dose LC25 (corrected mortality were 6.81 and 4.54% respectively (mean survival of IJs was more than 90%). The variations in nematode acetylcholinesterase concentration may be responsible for the variations in adjusted mortality percentages of EPNs as reported by [24]. Furthermore, as reported by [25] the higher survival of S. carpocapsae than H. bacteriophora may be attributed to the difference in acetylcholinesterase and other detoxified enzyme levels in both genera, The varied effects of insecticides and IGRs on IJ survival, on the other hand, could be attributed to the various modes of action on nematode, chemical receptors and respiratory metabolites, as claimed by [26]. Results suggest that these insecticides had lethal effects on nematodes, but less so on S. carpocapsae than H. bacteriophora. Regarding how dose and exposure time affect nematode infectivity.

3.2.2. The impact of various insecticides on entomopathogenic nematodes' on reproductive capacity (Efx) of entomopathogenic nematodes

Following exposure to lethal dosages of the investigated pesticides, Table 2 demonstrates the considerable difference between the nematode strains and the lethal concentrations for reproductive capacity. The percentage of reproductive capacity of the H. bacteriophora strain was higher than that of S. carpocapsae. The reproductive capacity (Efx) was 84.68, 68.86, 77.83, 60.87, 74.08 and 62.67 when Heterorhabditis bacteriophora treated with lufenuron, LC25 and LC50; lambada cyhalothrin with the two doses and flubendiamide with LC25 and LC50 respectively. The overall effect of tested insecticides on the reproductive capacity of G. melonella infected larvae with 500 IJs (in both tested EPN strains) and treated before with LC25 differed significantly more than the reproductive capacity of G. melonella infected larvae with 500 IJs treated with different insecticides with LC50 dose in both tested EPN strains (Table 2). The yield of IJs reduced as the concentration of pesticide used before infection of the host increased. The three insecticides' effects on the survival of infective juveniles and reproductive capacity differed according to the doses administered to H. bacteriophora and S. carpocapse.

3.2.3. The reduction coefficient Ex and IOBC category of the three tested pesticides to Entomopathogenic nematodes.

Reduction coefficient (Ex %) values 96 h. H. bacteriophora and from 0.0 to 29.64 % for S. carpocapse. As shown from results all products with the two concentration LC25 and LC50 were classed as IOBC category 1 (harmless i.e., nontoxic) as calculated as reduction coefficient Ex as shown in Table3

3.3. Joint action of entomopathogenic nematodes and insecticides against Spodoptera frugiperda under laboratory conditions.

Results illustrated in table 4 shows all calculated $\chi 2$ values calculated are less than the value of $\chi 2$ on degree of freedom 1 and probability 0.05 93.84) this means all tested mixtures are additive effects. And the differences between observed corrected mortality and expected mortality of the mixtures were positive except the combination of lufenuron with dose LC25 and the two strains of EPN was negative. These results indicate that all combinations between the three tested insecticide with doses LC25 and LC50 and the two EPN isolated strains are synergistic mixtures except combinations with lefuneron with dose LC25 was antagonistic effect according to [21]. If the differences Mnt–Me was positive value, the interaction was considered synergistic effect, and if the difference was negative, the interaction was considered antagonistic.

3.4. Discussion

In numerous laboratory bioassays throughout the years, different EPN varieties of Heterorhabditis and Steinernema showed different responses to various pests [27-31]. It may be due to the environmental parameters that parasitic bacterium (Xenorhabdus spp. and Photorhabdus spp.) interactions frequently use. The current study's mortality data are

comparable to those from the previous investigation. [32, 33]. The efficiency on fall armyworm larva varied between nematode species, which is not unusual. [31] discovered that when Heterorhabditis sp. and S. arenarium were used to treat with dose 200 IJs/ 5th instant S. frugiperda, the mortality rates were 96.07 and 100.00 percent, respectively. H. Indica and S. surkhetense caused 75.00 percent of mortality 48 hours after incubation, whereas [34, 35] discovered that concentrations of 50 and 100 IJs in S. Diarrhea caused 93.00 and 100.00 percent of mortality, respectively. The current study discovered that H. bacteriophora causes 90% mortality percent in 200 IJs / ml-1 after 72 h. of incubation. and Steinernema carpocapsae gave 96.5% after 72 h. with the dose 200IJs/ ml-1. In our bioassay tests. [28, 34] demonstrated that against S. frugiperda 3rd instar larvae, Steinernema sp. (280 IJs / worm) and H. Indica caused 100 mortalities; Variations in nematode species and this insect's life cycle could explain the small variation in mortality rates and other research. According to our bioassay tests, H. bacteriophora appears to be much more effective in terms of LC50, Table 1, as well as host penetration and cadaver reproduction [34, 36] It was discovered that there was an increase in sickness among isolated EPNs, indicating that climatic conditions were highly variable throughout the study. Pesticides with the highest infectivity and reproductive capacity are the most compatible. Because of differences in nematode and insect physiology and feeding patterns, some insecticides may have different effects on nematodes. The role of nematode physiology in reducing susceptibility to various pesticides should be investigated in this regard.

Finally, future research should look into pesticides' effects on EPNs in the field.. [37]found that several carbamates and organophosphates adversely affected the in vitro development and reproduction of S. carpocapsae, whereas this strain S. carpocapsae was unaffected by methoxychlor and fenvalerate. In our results this species also unaffected with Lambada cyhalothrin, IGRs Lufenuron and flubendiamide when treated with LC25 or LC50 [37]concluded that most insecticides can be used at practical concentrations with S. carpocapsae. According to [38], carbaryl has a significant effect on the reproductive capacity of H. bacteriophora. EPNs are compatible with several different chemical and biological pesticides, according to [39, 40].

Our findings indicate that synthetic pyrethroid lambada cyhalothrin, IGRs lufenuron and flubendiamide at LC25 and LC50 doses can be added to the list of compatible insecticides after 48 and 96 hours, respectively. When mixed with the lethal dose (LC50) of nematodes had no effect on infectivity or reproductive capacity of EPN. This level of compatibility is comparable to imidacloprid's with H. bacteriophora and several other nematode species. This is consistent with [41]. It is difficult to explain why EPNs react differently to different pesticides, but our studies suggest that different nematode species/strains can react differently to the same toxins. The discovered results regarding insecticide interaction effects on nematodes not only make nematode application easier in agro-ecosystems, but also promotes their usage in integrated pest management systems. The findings of this study add to our understanding of EPN-insecticide interactions. By proving that the insecticides used in this investigation are not hazardous to any of the tested worm species. This study demonstrates that nematodes can be successfully integrated into insecticide-based integrated pest management in agro-ecosystems. Knowledge of probable reproduction losses due to pesticide use will aid in predicting the required nematode treatment rate in the field. A variety of factors, including species, strain, application manner and dose, duration, and so on, could explain the sensitivity of infective juveniles (IJs) of EPNs. As a result, determining the interactions of various chemical compounds, EPNs species, and even isolates in both laboratory and field circumstances is critical for establishing local IPM programmes.

Table (1): Toxicity of tested insecticides and strains of Entomopathogenic nematodes against *Spodoptera frugiperda*

Treatments	Slope	LC_{50}± se	Fiducial limits		LC_{25}	LC_{90}	χ^2
			Lower	Upper			
Heterorhabditis bacteriophora HP88	1.49	48.67± 0.34 IJs/ml-1	28.78	82.28	25.71 IJs/ml	349.33	0.72
Steinernema carpocapsae(AT4)	0.73	65.88± 3.07 IJs/ml	27.27	155.33	12.62 IJs/ml	660.87	2.40
Lufenuron	3.56	05.67±1.53 PPM	05.07	06.36	3.66 PPM	012.66	5.15
Lambada cyhalothrin	3.3	04.23±0.85 PPM	03.67	04.88	2.65 PPM	009.35	3.15
Flubendiamide	2.42	3.26± 0.52 PPM	2.59	4.11	1.73 PPM	11.09	4.67

Table (2): Effect of tested insecticides on the infectivity (average ± SE) of *Heterorhabiditis bacteriophora, Steinernema carpocapsa* and their reproductive capacity measured in *Galleria mellonella* after 72 hrs.

Treatments	dose	Entomopathogenic nematodes strain									
		Heterorhabditis bacteriophora HP88					*Steinernema carpocapsae*				
		Avg. no. of 2nd IJIs	(E_{mx})* %	No. IJLs produced	(E_{fx}) ** %	EX	Avg. no. of 2nd IJIs	(E_{mx})%	No. IJLs produced	(E_{fx})%	Ex
Lufenuron	LC_{25}	143.33	2.27±0. 47	1211.33	84.68b	14.86	136.66	06.81±.098	1099.67	79.24b	0.00
	LC_{50}	120.00	16.00± 1.6	987.66	68.86ab	26.16	118.66	19.09±1.34	879.33	63.36ab	29.64
Lambada cyhalothrin	LC_{25}	112.3	23.42±2.6	1115.67	77.83ab	16.97	140.00	04.54±1.78	994.66	71.71ab	27.01
	LC_{50}	96.66	34.09±5.3	870.66	60.87ab	26.12	110.00	24.99±3.22	876.67	63.17ab	27.62
Flubendiamide	LC_{25}	119.66	18.68±1.5	1089.67	74.08ab	21.07	128.33	12.53±1.02	1104.33	79.59b	17.85
	LC_{50}	98.33	32.95±2.5	896.33	62.67ab	25.02	113.00	23.00±2.67	889.00	64.06ab	27.67
Control		146.66	2.22	1430.33	95.35a	0.010	146.66	2.22±1.01	1387.67	92.46a	1.67

***E_{mx} = corrected mortality = ((T/C) *100) ** E_{fx} = Reproductive capacity=*Efx* = [((Fc-Fx)/ FC)*100]**

Table (3): Reduction coefficient E_x and IOBC toxicity classes of tested insecticides to Entomopathogenic nematodes after 10 days post treatment.

Insecticide treatments	dose	*Heterorhabditis bacteriophora*			*Steinernema carpocapsae*		
		EX	IOBC class*	*action*	E_x	IOBC class	action
Lufenuron	LC_{25}	14.86	1	Harmless	00.00	1	Harmless
	LC_{50}	26.16	1	Harmless	29.64	1	Harmless
Flubendiamide	LC_{25}	16.97	1	Harmless	27.01	1	Harmless
	LC_{50}	26.12	1	Harmless	27.62	1	Harmless
Lambada Cyhalothrin	LC_{25}	21.07	1	Harmless	17.85	1	Harmless
	LC_{50}	25.02	1	Harmless	27.67	1	Harmless

E_x = Reduction coefficient

1= Harmless, <30; 2= slightly harmful, >30-<80; 3= moderately harmful, >80-<99 4 = harmful, <99

Table (4): Interactions between the chemical insecticides with the two isolates of *Entomopathogenic nematode* on 4 th instar larvae of *Spodoptera frugiperda*

Treatments	dose	Entomopathogenic nematodes strain							
		Heterorhabditis bacteriophora				*Steinernema carpocapsae*			
		Observed Mortality%	Expected Mortality%	$\square^2$	action	Observed Mortality%	Expected Mortality	$\square^2$	action
Lufenuron	LC_{25}	55.92	62.50	0.6927	Antagonism	59.25	62.5	0.1690	Antagonism
	LC_{50}	75.22	75.00	0.0001	Synergism	75.17	75	0.0003	Synergism
Lambada cyhalothrin	LC_{25}	62.95	62.50	0.0027	Synergism	62.95	62.5	0.0032	Synergism
	LC_{50}	81.47	75.00	0.5581	Synergism	78.51	75	0.1642	Synergism
Flubendiamide	LC_{25}	77.77	62.50	3.734	Synergism	70.36	62.5	0.9884	Synergism
	LC_{50}	92.22	75.00	3.953	Synergism	77.77	75	0.0079	Synergism
Control		10	----------			10	-		

Conclusion

Results indicated that *H. bacteriophora* HP88 gave the highest virulence on ***S. frugiperda***, While *S. carpocapsae* (AT4) strain was less efficiency with no significant differences between the two strains. Flubendiamide was the most toxic insecticide followed by Lambada cyhalothrin Both EPN *H. bacteriophora* (HP88) and *S. carpocapsae* viability was not affected significantly by any of tested insecticides (LC_{25} and LC_{50}), Lufenuron at the rate LC_{50} and LC_{25}, not affect *H. bacteriophora* and *S. carpocapsae* pathogenicity. All tested insecticides with the two doses were nontoxic to the two strains of EPN according to IOBC test. The mixtures of insecticides with the two EPN strains can be used in integrated *S frugiperda* management except Lufeuron with LC_{25}.

Ethics approval and consent to participate.

Not applicable.

Consent for publication

Not applicable

Availability of data and material

The data sets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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