

Impact of Exposure to Traditional Neurotoxic Insecticides on the Performance of Entomopathogenic Nematode *Heterorhabditis* sp. (Rhabditida: Heterorhabditidae) Against *Galleria mellonella* (Lepidoptera; Pyralidae)

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ABSTRACT

The integration of chemical insecticides with the biocontrol agent, entomopathogenic nematodes (EPNs) belonging to the Heterorhabditidae family, necessitates an understanding of the potential adverse effects of these insecticides on the biocontrol agent. Therefore, the primary objective of this research was to investigate the impact of two organophosphates (profenofos and chlorpyrifos), a carbamate (methomyl), and two pyrethroids (lambda-cyhalothrin and deltamethrin) on the viability and virulence of three strains of EPNs: *Heterorhabditis* sp. NEM 08, *Heterorhabditis* sp. NEM 15, and *Heterorhabditis bacteriophora* HP88. This investigation involved assessing their action on viability and infectivity toward the last instar larvae of *Galleria mellonella* upon exposure to recommended field rates for varying durations: 6 hours, 24 hours, and 48 hours. The results concerning EPN viability revealed that profenofos caused the most significant reduction in EPN viability, ranging from 44.9% to 65.9% after 48 hours of exposure. Additionally, the EPN strains exhibited varying levels of tolerance. Profenofos also had the highest adverse impact on infectivity, resulting in reductions ranging from 65.9% to 82.2% after 48 hours of exposure. Chlorpyrifos followed, which causing reductions in infectivity ranging from 25% to 55.6% after 48 hours of exposure. In contrast, Lambda-cyhalothrin had the least adverse effect on EPN viability and infectivity. Furthermore, it was observed that prolonged exposure time intensified the adverse effects on EPN viability and infectivity. These findings provide valuable insights for the integrated use of these neurotoxic insecticides with EPNs in insect control programs, helping in the selection of the most tolerant EPN strains for this purpose.

Key words: Entomopathogenic nematodes, Heterorhabditidae, Viability, Pathogenicity.

INTRODUCTION

Chemical pesticides have long been employed as a conventional method for controlling insect populations in agriculture, forestry, and horticulture. While these chemical agents have undoubtedly contributed to increasing crop yields and pest management, their indiscriminate use has given rise to a myriad of adverse effects that have raised serious concerns regarding their sustainability and environmental impact. These concerns encompass issues such as groundwater

contamination (Srivastav, 2020), residues in food (Bajwa and Sandhu, 2014), the proliferation of pesticide resistance in target pests (Hawkins *et al.*, 2019), soil and air pollution, secondary pest outbreaks, and the inadvertent harm inflicted upon non-target organisms (Serrão *et al.*, 2022), including beneficial insects and wildlife (Zimmerman and Cranshaw, 1990).

In light of these environmental challenges, there is a growing imperative to explore alternative, more eco-friendly tools for managing insect populations and mitigating the ecological and health risks associated with chemical pesticide usage. Integrated Pest Management (IPM) programs have emerged as a holistic approach that integrates various strategies to minimize pest damage while also reducing the dependency on chemical pesticides. Within this framework, biological control agents have garnered significant attention for their potential to address pest-related challenges in a more sustainable manner (Peshin and Zhang, 2014).

Entomopathogenic nematodes (EPNs), belonging to the families Steinernematidae and Heterorhabditidae, are one such cluster of biopesticides that show immense promise in pest management. These nematodes are lethal parasites capable of infecting and killing a diverse array of economically important agricultural, forestry, and horticultural insect pests (Poinar, 1990; Kaya & Gaugler, 1993; Journey & Ostlie, 2000 and Shahin *et al.*, 2023). What sets EPNs apart are their remarkable attributes, including rapid host mortality (within 24-48 hours), a broad host range, high virulence, chemoreceptors that help them locate hosts, and the capacity for large-scale production, both in living organisms (*in vivo*) and in a controlled laboratory environment (*in vitro*) (Kaya, 1985; Kaya & Gaugler, 1993 and Ehlers, 2001).

The third developmental stage, referred to as infective juveniles (IJ), of these nematodes harbor symbiotic bacteria within their intestinal tracts (Akhurst, 1983 and Glazar *et al.*, 1991). These IJs exhibit an attraction to insects (Poinar, 1990) and gain entry through various natural openings such as the mouth, anus, or spiracles (Mracek *et al.*, 1988). In the case of *Heterorhabditis* IJs, they possess the capability to

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penetrate via the insect's exoskeleton (Bedding and Molyneux, 1982). Subsequently, they infiltrate the insect's hemocoel, releasing the mutualistic bacteria into the insect's circulatory system. The bacteria then undergo a rapid multiplication process, resulting in the demise of the insect host within a span of 24 hours. The bacterium's lethal properties are attributed to the production of proteolytic enzymes (Gaugler, 2018). The nematodes subsequently nourish themselves by consuming the bacteria and metabolizing their byproducts. They proceed to proliferate and reproduce inside insect cadaver, leading to the emergence of numerous newly generated infective juveniles (IJ) within a timeframe of 2 weeks. These IJs are primed and ready.

Despite the promising potential of EPNs, their efficacy can be compromised by various environmental factors, such as low humidity or solar radiation, as well as their susceptibility to certain pesticides (Gaugler *et al.*, 1980 and Koppenhöfer & Fuzy, 2008). This vulnerability necessitates a comprehensive understanding of how chemical pesticides affect the performance of EPNs, particularly their pathogenicity and overall effectiveness in pest control. Therefore, studying the effects of pesticide exposure on these biological control agents is of paramount importance to optimize their integration into IPM programs and ensure their success in sustainable insect population management.

In this context, our study aims to explore the influence of exposure to specific neurotoxic insecticides on the performance of three strains of *Heterorhabditis* nematodes. Furthermore, our research aims to assess how varying exposure durations may affect the pathogenicity and mortality rates of these entomopathogenic nematodes.

MATERIALS AND METHODS

Rearing greater wax moth:

The caterpillars of the greater wax moth, (*L.*), were harvested from damaged beehives from Behira governorate and placed in plastic containers with a 2 kg capacity. They were reared until the adult moths emerged, following the rearing method described by Van Zyl and Malan (2015). The rearing medium consisted of the following components: wheat flour (118 g), wheat bran (206 g), powder milk (118 g), yeast extract (88 g), Macerated bee wax comb (24 g), honey (175 ml), and Sorbitol (175 ml). The diet preparation process is carried out by initially blending the dry components in a single container, followed by the addition of the liquid elements to the mixture, which can then be manually stirred. This resulting blend, referred

to as the diet, is refrigerated until it is ready for feeding *G. mellonella* larvae in the rearing containers.

Soil Sample and Nematode isolation:

Soil specimens were gathered from Mamoura region, Alexandria governorate, Egypt, during April and May 2020 beneath palm trees. Sandy soil was the preferred selection for soil sample collection. Samples were collected from a depth ranging between 10 and 15 cm beneath the soil surface. The collected samples were placed into labeled plastic bags, and subsequently, the soil-filled bags were placed within an icebox and transported to the laboratory.

The nematodes were extracted from the soil samples using the insect-baiting methodology as outlined by Bedding and Akhurst (1975). Each sample was exposed to the presence of ten last larval instar of *G. mellonella* L (5th age of larva). These containers were then upturned and stored in darkness at a temperature of 25± 2 °C and a relative humidity of 75±5%. Over a 7-day incubation period, the samples were regularly inspected to identify any deceased insects.

The cadavers were examined for symptoms of nematode infection, including changes in coloration, according to the criteria established by Woodring and Kaya (1988). The deceased larvae were individually transferred to adapted white traps in accordance with the approach outlined by Kaya and Stock (1997). To validate insect pathogenicity of recovered nematode, the infective juveniles (IJs) were relocated onto damp filter paper within Petri dishes, where live *G. mellonella* larvae were introduced. The subsequent generation of IJs was gathered within a beaker, subjected to two rinses with sterile distilled water, and then preserved at a temperature of 16°C, following the methodology elucidated by Kaya and Stock (1997).

Nematode cultivation:

Two indigenous strains of nematodes (belonging to the species *Heterorhabditis* sp.) were retrieved from the gathered soil samples, along with the introduction of an exogenous isolate, *Heterorhabditis bacteriophora* Pionar (HP88 strain), which was sourced from a stock culture maintained within the Applied Entomology and Zoology Department at the Faculty of Agriculture, Alexandria University, Egypt. These nematodes were cultivated through an *in vivo* culture method utilizing the final-stage larvae of *G. mellonella*, following the procedure described by Woodring and Kaya (1988).

To assess compatibility, the IOBC/WPRS protocol was followed, originally proposed by Vainio (1992). In this context, insecticides solution was prepared at twice the recommended dosage, as illustrated in Table (1).

Table 1. Characteristics of the traditional neurotoxic insecticides used in the experiments

Active ingredient	Commercial name	Formulation A.I %	Chemical group	RC ^a
Profenofos	Telton	72 % EC	Organophosphate	0.75 L
Chlorpyrifos	Dursban	48% EC	Organophosphate	1 L
Methomyl	Copter	90% SP	Carbamate	0.3 Kg
Lambda-cyhalothrin	Katron	5% EC	Pyrethroids	0.375 L
Deltamethrin	Kafrothrin	2.5% EC	Pyrethroids	0.35 L

^a Field recommended rate as manufacturer's guidelines, dose per feddan (=4200 m²) and 200 l as water volume.

Final concentration equivalent to recommended field concentration (RC) was obtained by dilution using tested nematodes suspension and distilled water. The dosage levels were determined in accordance with the manufacturer's guidelines, which specified a solution volume equivalent to 200 liters per feddan. Subsequently, insecticide-nematodes mixture was introduced into flat-bottomed glass tubes, a treatment solely consisting of the nematode suspension in distilled water consider as control. Each treatment was replicated five times, and the entire trial followed a completely randomized design. The glass tubes were then placed in a climatic chamber at a stable temperature of $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ relative humidity (RH) in the dim, for a duration of 24, 48 and 72 hours. It was during this periods that the assessment of viability and infectivity took place.

Tested insecticides:

Five formulated neurotoxic synthetic insecticides, which fall into three chemical categories: Organophosphates, carbamates, and pyrethroids. Organophosphates and carbamates function by inhibiting acetylcholinesterase enzymes, resulting in the buildup of acetylcholine at nerve endings. This excess accumulation leads to nerve and muscle hyperstimulation. In contrast, pyrethroids target sodium channels in nerve cells, causing nerve excitement and paralysis in insects. All these insecticides are widely used in Egypt to combat various insect pests and are officially registered (Agriculture Pesticide Committee, 2020). Additional information about the recommended dosage rates, formulation and active ingredients presumed found in Table (1).

Viability of EPN:

The evaluation of viability was conducted 24, 48 and 72 hours after the beginning of the experiment. by withdrawing 0.1 ml of suspension from each tube. The infective juveniles (IJs) were carefully observed under a stereomicroscope, and both the living and deceased juveniles in the sample were tallied. IJs that exhibited no response to stimulation with a fine-edged scalpel were classified as deceased.

Infectivity of EPN:

To assess the influence of the tested neurotoxic insecticides on nematode infectivity, Petri dish bioassay was conducted following the method described by Caroli *et al.* (1996). The IJs cleaning precede were conducted prior to the viability evaluation using dilution of insecticide exposed IJ's by distilled water and decantation for 30 min at 10°C Supernatant The procedure was repeated three times to eliminate the residues of insecticide. In each 9 cm petri dish, two layers of 9 cm filter paper were placed. Live infective juveniles (IJs), totaling 500 in number per 0.7 ml distilled water were applied on the filter paper in each Petri dishe. Each dish received ten last instar *G. mellonella* larvae. Each treatment was replicated in three separate dishes, which were sealed using Parafilm. Then, they were placed in an incubator at a temperature of $25 \pm 1^\circ\text{C}$ and RH. $70 \pm 10\%$, the effectiveness of the nematodes in infecting the wax moth larvae was quantified by assessing mortality 6, 24- and 48-hours post-incubation.

Statistical analysis:

The survival percentage and infectivity percentage were analyzed using an analysis of variance (ANOVA). The effects of insecticide treatments, nematode strains, exposure time, and their interactions on the survival and mortality rates of *G. mellonella* larvae, were assessed using PROC GLM (SAS version 9.0; SAS Institute). When the ANOVA yielded significant results, comparisons of the relevant means were conducted using Tukey's test with a significance level of 5%.

RESULTS AND DISCUSSION

RESULTS:

Two EPNs isolates were obtained from soil samples collected from Al Mamoura region using *G. mellonella* bait technique of Bedding and Akhurst (1975) named *Heterorhabditis* sp. NEM 08 isolated from soil under palm trees and *Heterorhabditis* sp. NEM 15 isolated from soil sample under citrus trees . The transformation of the Galleria larvae, infected by the two EPN isolates, resulted in a distinctive dark reddish hue, suggesting that these nematodes are classified within the *Heterorhabditis* genus. *Heterorhabditis bacteriophora*

HP88 strain was used as exogenous isolates for comparison purpose.

Impact of EPNs infective juveniles exposure to tested insecticide (Table 1) at their application rate for different times 6h, 12h and 48 hours of exposure was divided into two trials; the first one is the survivability of EPNs strain after expose to tested insecticides, the second was the infectivity of a live EPNs strains toward *G. mellonella* last instar larvae after expose to the tested insecticides at different times.

Impact of insecticides on EPN viability:

The viability of EPNs following a 6-hour exposure to tested neurotoxic insecticides is presented as survival percentages (means \pm SE) in Table (2). The data reveal highly significant differences in the impact of the tested insecticides on the viability of infective juveniles from three EPN strains: *Heterorhabditis* sp. NEM 08 (F = 26.2; df = 5; P < 0.0001), *Heterorhabditis* sp. NEM 15 (F = 36.6; df = 5; P < 0.0001), and *Heterorhabditis bacteriophora* HP88 (F = 21.52; df = 5; P < 0.0001).

Profenofos resulted in the highest significant reduction in survival rate, causing a decrease of 16.9% in the NEM 15 strain and 11.2% in both the NEM 08 and HP88 strains, respectively. Methomyl also had a notable impact, leading to a reduction percentage ranged between 4.8% to 5.8% in the survival rate of the EPN strains. In contrast, lambda-cyhalothrin had the lowest impact, with a reduction in survival rate ranging from 2% to 3.6% across all EPN strains. The EPNs strain exhibited statistically significant differences in survival rates when exposed to different insecticides for 6 hours, as indicated by the analysis of variance (F = 9.11, df = 2, P < 0.05).

Tables (3 and 4) present the survival percentages (means \pm SE) of EPN strains after exposure to tested insecticides for 24 hours and 48 hours, respectively. The results revealed significant differences in the impact of the tested insecticides on the survival rate of

Heterorhabditis sp. NEM 08 after 24 hours of exposure (F = 56.36; df = 5; P < 0.0001) and 48 hours of exposure (F = 62.02; df = 5; P < 0.0001). Similarly, there were significant differences in survival rates after 24 hours of exposure (F = 192.74; df = 5; P < 0.0001) and 48 hours of exposure (F = 201.98; df = 5; P < 0.0001). For *Heterorhabditis bacteriophora* HP88, significant differences were also observed after 24 hours of exposure (F = 88.71; df = 5; P < 0.0001) and 48 hours of exposure (F = 185.69; df = 5; P < 0.0001).

Following the trend observed at the 6-hour mark of insecticide exposure, Profenofos emerged as the insecticide causing the most significant reduction in the survival rate of the tested EPNs. Among these, *Heterorhabditis* sp. NEM 08 exhibited the highest sensitivity, experiencing a substantial 65.5% reduction in survival rate after 48 hours of exposure, while *Heterorhabditis* sp. NEM 15 displayed least sensitivity with a 44.9% reduction in survival rate. Methomyl followed Profenofos in terms of adverse effects, resulting in a 14.2% reduction in the survival rate of *Heterorhabditis* sp. NEM 08 after 48 hours of exposure. In contrast, *Heterorhabditis* sp. NEM 15 and *Heterorhabditis bacteriophora* HP88 exhibited more resilience to Methomyl, with reductions in survival rates of 9.2% and 9.5%, respectively, after 48 hours of exposure. On the other hand, Chlorpyrifos caused a 13.4% reduction in the survival rate of *Heterorhabditis bacteriophora* HP88 after 48 hours of exposure. *Heterorhabditis* sp. NEM 08 and *Heterorhabditis* sp. NEM 15 demonstrated notable tolerance to Chlorpyrifos exposure, with reductions in survival rates of only 6.8% and 8.7%, respectively, after 48 hours of exposure.

The EPNs strain exhibited statistically significant differences in survival rates when exposed to different insecticides for 24 hours (F = 5.82, df = 2, P = 0.0003) and 48 hours (F = 14.3, df = 2, P < 0.0001), as indicated by the analysis of variance.

Table 2. Mean survival percentages (\pm SE) of infective juveniles from three Heterorhabditidae nematode strains after a 6-hour exposure to the recommended field rate of the tested neurotoxic insecticide, maintained at (25 \pm 1°C) and (RH 70 \pm 10%)

Treatments	Survival (%) ^a		
	<i>Heterorhabditis</i> sp. ENM 08	<i>Heterorhabditis</i> sp. ENB 15	<i>H. bacteriophora</i> HP88
Control	99.6 \pm 0.24 ^{Aa}	99.4 \pm 0.24 ^{Aa}	100 \pm 0.00 ^{Aa}
Profenofos	88.4 \pm 1.36 ^{Abd}	82.6 \pm 2.01 ^{Bc}	88.8 \pm 1.28 ^{Ac}
Chlorpyrifos	96.8 \pm 0.58 ^{Aabc}	95.2 \pm 0.66 ^{Abb}	94.0 \pm 0.71 ^{Bb}
Methomyl	94.0 \pm 0.63 ^{Ac}	93.6 \pm 0.51 ^{Ab}	95.2 \pm 0.66 ^{Ab}
Lambda-cyhalothrin	97.6 \pm 0.60 ^{Aab}	95.8 \pm 0.58 ^{Aab}	97.0 \pm 0.71 ^{Aab}
Deltamethrin	94.4 \pm 0.68 ^{Abc}	93.2 \pm 0.37 ^{Ab}	95.2 \pm 0.86 ^{Ab}

^a means \pm standard error within row followed by the same capital letters are not significantly different at 0.05 significance level, means in the same column followed by the same lowercase letters are not significantly different at 0.05 significance level (Tukey HSD test)

Table 3. Mean survival percentages (\pm SE) of infective juveniles from three *Heterorhabditidae* nematode strains after a 24-hour exposure to the recommended field rate of the tested neurotoxic insecticide, maintained at ($25 \pm 1^\circ\text{C}$) and (RH $70 \pm 10\%$)

Treatments	Survival (%) ^a		
	<i>Heterorhabditis</i> sp. ENM 08	<i>Heterorhabditis</i> sp. ENB 15	<i>H. bacteriophora</i> HP88
Control	98.2 \pm 0.37 ^{Aa}	97.8 \pm 0.86 ^{Aa}	98.8 \pm 0.58 ^{Aa}
Profenofos	69.2 \pm 2.73 ^{Ac}	57.8 \pm 2.13 ^{Bd}	72.0 \pm 1.84 ^{Ac}
Chlorpyrifos	92.4 \pm 0.93 ^{Abb}	95.2 \pm 0.66 ^{Abc}	92.0 \pm 0.84 ^{Bb}
Methomyl	89.6 \pm 0.93 ^{Bb}	93.6 \pm 0.51 ^{Ac}	92.6 \pm 0.51 ^{Ab}
Lambda-cyhalothrin	93.6 \pm 0.60 ^{Bab}	93.8 \pm 1.02 ^{Ab}	95.6 \pm 0.40 ^{ABab}
Deltamethrin	90.4 \pm 0.93 ^{Bb}	93.2 \pm 0.37 ^{ABbc}	94.0 \pm 1.14 ^{Ab}

^a means \pm standard error within row followed by the same capital letters are not significantly different at 0.05 significance level, means in the same column followed by the same lowercase letters are not significantly different at 0.05 significance level (Tukey HSD test)

Table 4. Mean survival percentages (\pm SE) of infective juveniles from three *Heterorhabditidae* nematode strains after a 48-hour exposure to the recommended field rate of the tested neurotoxic insecticide, maintained at ($25 \pm 1^\circ\text{C}$) and (RH $70 \pm 10\%$)

Treatments	Survival (%) ^a		
	<i>Heterorhabditis</i> sp. ENM 08	<i>Heterorhabditis</i> sp. ENB 15	<i>H. bacteriophora</i> HP88
Control	97.2 \pm 0.73 ^{Aa}	96.8 \pm 1.07 ^{Aa}	98.2 \pm 0.73 ^{Aa}
Profenofos	53.6 \pm 2.62 ^{Ad}	33.0 \pm 2.83 ^{Bc}	44.6 \pm 2.54 ^{Ad}
Chlorpyrifos	90.6 \pm 1.12 ^{Ab}	88.4 \pm 1.08 ^{Abb}	85.0 \pm 1.41 ^{Bc}
Methomyl	83.4 \pm 1.36 ^{Bc}	87.6 \pm 1.33 ^{Abb}	89.2 \pm 1.39 ^{ABc}
Lambda-cyhalothrin	91.8 \pm 1.36 ^{Aab}	91.4 \pm 1.63 ^{Aab}	93.2 \pm 0.73 ^{Aab}
Deltamethrin	85.6 \pm 3.31 ^{Aab}	88.8 \pm 1.43 ^{Bb}	90.0 \pm 0.89 ^{ABbc}

^a means \pm standard error within row followed by the same capital letters are not significantly different at 0.05 significance level, means in the same column followed by the same lowercase letters are not significantly different at 0.05 significance level (Tukey HSD test)

Impact of insecticide exposure on infectivity:

Infectivity of EPN infective juveniles towards last instar *G. mellonella* larvae following exposure to tested neurotoxic insecticides for various durations is illustrated in Figures (1 to 3), corresponding to *Heterorhabditis* sp. NEM 08, *Heterorhabditis* sp. NEM 15, and *Heterorhabditis bacteriophora* HP88, respectively. The results indicate that after 6 hours of exposure to insecticides, there was no significant effect observed in infectivity for *Heterorhabditis* sp. NEM 08 ($F = 2.55$, $df = 5$, $P = 0.072$) and *Heterorhabditis* sp. NEM 15 ($F = 2.36$, $df = 5$, $P = 0.071$). However, there was a slight significance observed for *Heterorhabditis bacteriophora* HP88 ($F = 2.98$, $df = 5$, $P = 0.032$). For longer exposure durations, statistical significance was evident between the tested neurotoxic insecticides for all EPN strains. Profenofos exhibited the most adverse effect on EPN strains, with its impact gradually

increasing with exposure time, leading to an infectivity reduction of up to 82.2% in *Heterorhabditis bacteriophora* HP88 after 48 hours of exposure. In contrast, *Heterorhabditis* sp. NEM 08 was the least affected EPN strain, with an infectivity reduction of 65.9%. Chlorpyrifos followed Profenofos in terms of adverse effects on EPN infectivity, causing reductions of up to 55.6% in *Heterorhabditis bacteriophora* HP88. *Heterorhabditis* sp. NEM 15 exhibited less impact, with a 25% reduction in infectivity after 48 hours of exposure to Chlorpyrifos.

Lambda-cyhalothrin had the lowest impact on EPN infectivity, resulting in reductions ranging from 6.8% to 11.4% across the tested EPN strains. Methomyl caused infectivity reductions from 13.6% to 18.2% after 48 hours of exposure, while deltamethrin had a slight impact, leading to reductions of 18.2% to 22.2% in infectivity for the treated EPN strains.

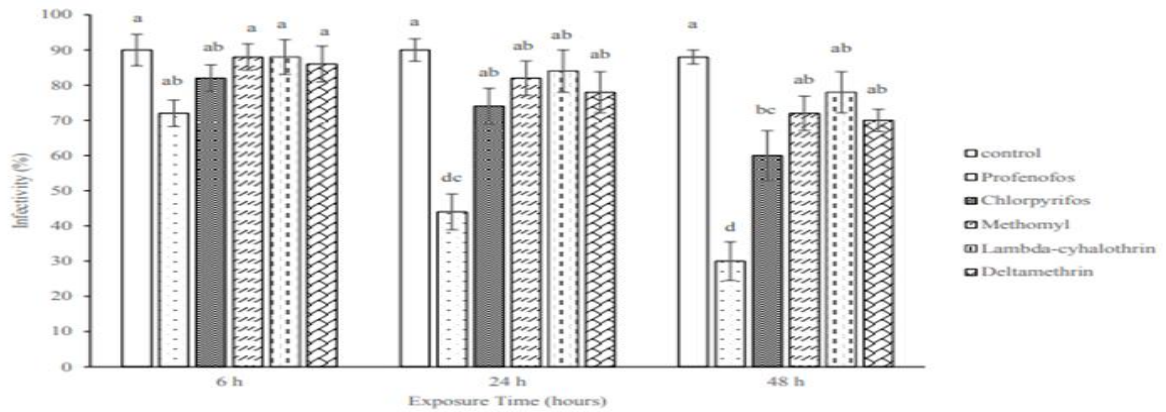


Fig. 1. Infectivity percentage (mean ± SE) of *Heterorhabditis* sp. ENM 08 strain on *G. mellonella* larvae after exposure to tested neurotoxic insecticides at their field recommended rate for different times. Bars with the same letters are not significantly different (Tukey HSD test, $P \leq 0.05$)

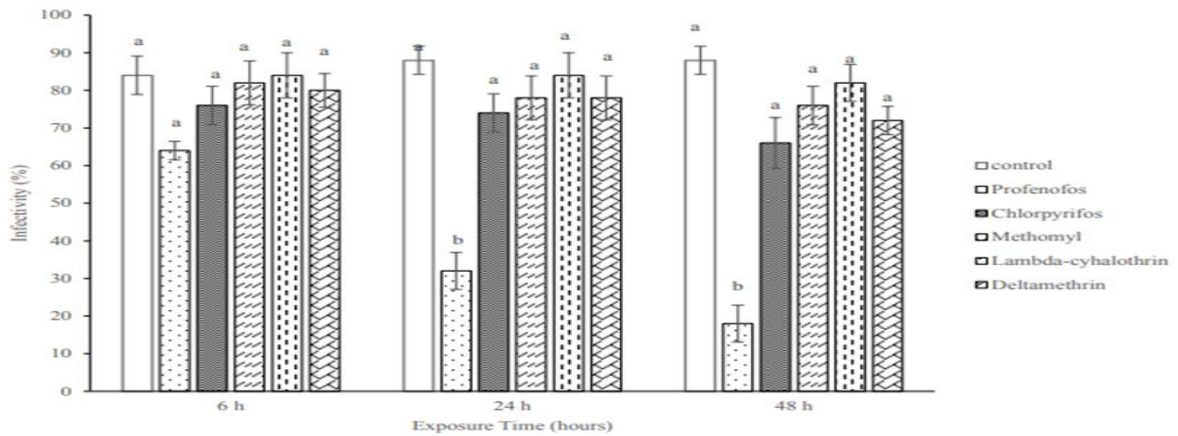


Fig. 2. Infectivity percentage (Mean ± SE) of *Heterorhabditis* sp. ENM 15 strain on *G. mellonella* larvae after exposure to tested neurotoxic insecticide at their field recommended rate for different time. Bars with the same letters are not significantly different (Tukey HSD test, $P \leq 0.05$)

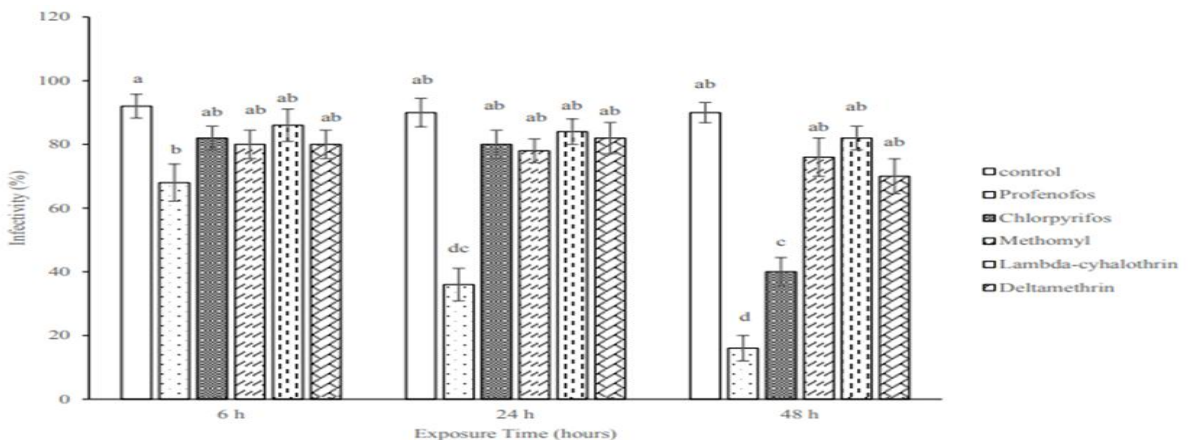


Fig. 3. Infectivity percentage (Mean ± SE) of *Heterorhabditis bacteriophora* HP88 strain on *G. mellonella* larvae after exposure to tested neurotoxic insecticide at their field recommended rate for different time. Bars with the same letters are not significantly different (Tukey HSD test, $P \leq 0.05$)

DISCUSSION:

The utilization of biological control agents, such as EPNs, in conjunction with insecticides is a well-established and crucial component of integrated pest management (IPM) programs aimed at controlling numerous agricultural pests (Koppenhöfer and Grewal, 2005). To investigate the potential harmful effects of insecticides on natural enemies, including EPNs, it is essential to assess the compatibility of EPNs with these insecticides. This is particularly significant because insecticides represent one of the primary agricultural inputs that farmers commonly rely on when pests reach economically damaging levels (El-Wakeil, 2013).

The majority of studies focusing on the compatibility of EPNs and pesticides have typically addressed specific groups of pesticides, often those employed against particular pests (Head *et al.*, 2000; Negrisoni Jr *et al.*, 2010). These studies have also commonly concentrated on pesticides within the same chemical category, such as carbamates (Gordon *et al.*, 1996), or those sharing similar biological activities, like nematicides (Hara and Kaya, 1982). However, it is important to note that EPNs inhabit regions where a variety of pesticides are applied. Hence, a significant strength of this research lies in its comprehensive evaluation of pesticides, encompassing all major categories widely utilized in pest management.

A study conducted by Borges *et al.* (2023) aligns with our own research, indicating that Profenofos was incompatible with *H. amazonensis* MC01 and *Steinernema feltiae* nematodes. Furthermore, Lambda-cyhalothrin exhibited compatibility with the *H. amazonensis* MC01 strain, resulting in a significant effect with 98.6% viability of infective juveniles after 48 hours of exposure and inducing an 84% infectivity rate in treated *Tenebrio molitor* larvae, which corresponds with our findings. Conversely, their study suggested incompatibility of Methomyl and Chlorpyrifos with *H. amazonensis* MC01. However, in our research, Chlorpyrifos demonstrated compatibility with EPN strains for up to 24 hours, both in terms of viability and infectivity. After 48 hours of exposure, it led to a substantial reduction in infectivity (up to 55.6%) in *H. bacteriophora* HP88, while the survival rate remained high (up to 85%) in the same EPN strain. On the other hand, our findings suggest that Methomyl exhibits compatibility with the tested EPN strains.

Consistent with our findings, the toxicity of Profenofos insecticide was previously documented by Zhang *et al.* (1994) highlighting its status as one of the most lethal insecticides. Their study reported a 57.1% mortality rate after 48 hours of exposure to Profenofos at a concentration of 100 $\mu\text{g ml}^{-1}$. Furthermore, profenofos exhibited adverse effects on the

pathogenicity of the exposed EPN strain against *Spodoptera Litura* larvae.

In a research investigation led by Negrisoni Jr *et al.* (2008), it was noted that deltamethrin had a limited effect on viability, with a mere 5.6% mortality rate among infective juveniles (IJs) following a 48-hour exposure. Nevertheless, its detrimental impact became more evident as it led to a 40% reduction in infectivity among last instar caterpillars of *G. mellonella*, in contrast to the 52% infectivity observed in untreated IJs of *H. bacteriophora*. In contrast, Rovesti and Deseö (1990) discovered that methomyl was highly toxic to *Steinernema carpocapsae* and *S. feltiae* in their study. Furthermore, Heungens and Buysse (1987) reported that methomyl exhibited toxicity to Heterorhabditis nematodes, while chlorpyrifos and Endosulfan had slight toxicity.

In a study conducted by Mohamed *et al.* (2017), it was discovered that the *H. bacteriophora* EM2 strain exhibited elevated levels of the AChE enzyme, which displayed a notable insensitivity to inhibition by methomyl insecticide, which explain the low toxic effect of methomyl on Heterorhabditis strains viability.

In the study conducted by Negrisoni Jr *et al.* (2008) it was observed that pyrethroids resulted in a higher mortality rate for *S. carpocapsae* (28.4%) compared to *H. bacteriophora* (5.6%) when exposed to deltamethrin. In our own research, we found that tested Heterorhabditis strains displayed greater resilience in terms of survival rates and infectivity when exposed to lambda-cyhalothrin and deltamethrin insecticides. These findings emphasize the influence of EPN species on insecticide tolerance.

CONCLUSION

our findings indicate that native isolates of entomopathogenic nematodes, which were isolated from local soil samples collected in Al Mamoura, Alexandria, Egypt, exhibit similar performance to exogenous isolates when tested on *G. mellonella* larvae. However, exposure to selected neurotoxic insecticides, primarily organophosphate-based profenofos, has adverse effects on both viability and pathogenicity. Furthermore, prolonged exposure exacerbates these adverse effects on the performance of native and exogenous EPN strains from the Heterorhabditidae family. It is worth noting that pyrethroid insecticide lambda-cyhalothrin and methomyl demonstrate compatibility with the tested EPN strains. IPM programers should put the gained results in mind when planning IPM strategies.

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الملخص العربي

تأثير التعرض للمبيدات الحشرية التقليدية عصبية التأثير على أداء النيماطودا الممرضة للحشرات

***Heterorhabditis* sp. (Rhabditida: Heterorhabditidae)**

ضد حشرة *Galleria mellonella* (Lepidoptera; Pyralidae)

نعمة عبد الحكيم عامر

أظهرت النتائج أن البروفينوفوس تسبب في أكبر انخفاض في حيوية النيماطودا، حيث تراوحت نسبة الخفض من ٤٤,٩٪ إلى ٦٥,٩٪ بعد ٤٨ ساعة من التعرض. بالإضافة إلى ذلك، أظهرت سلالات النيماطودا الممرضة للحشرات مستويات متفاوتة من التحمل تجاه المبيدات المختبرة. كما كان للبروفينوفوس أكبر تأثير سلبي على القدرة المرضية لعزلات النيماطودا، مما أدى إلى انخفاضات تتراوح من ٦٥,٩٪ إلى ٨٢,٢٪ بعد ٤٨ ساعة من التعرض. تبعه الكلوربيريفوس، مما تسبب في انخفاضات في القدرة على الإصابة تتراوح من ٢٥٪ إلى ٥٥,٦٪ بعد ٤٨ ساعة من التعرض. في المقابل، كان للامبادا سيهالوثرين أقل تأثير سلبي على حيوية وقدرة النيماطودا الممرضة للحشرات على الإصابة. علاوة على ذلك، لوحظ أن إطالة وقت التعرض يزيد من التأثيرات السلبية على حيوية وقدرة النيماطودا على الإصابة. تقدم هذه النتائج رؤيه لأمكانية التكامل بين المبيدات العصبية مع النيماطودا في برامج مكافحة الحشرات، وتساعد في اختيار السلالات الأكثر تحملاً.

أن التكامل بين استخدام المبيدات الحشرية الكيميائية مع النيماطودا الممرضة للحشرات كأحد العوامل البيولوجية في مكافحة الآفات، يتطلب فهماً للتأثيرات السلبية المحتملة للمبيدات الكيميائية على تلك العوامل البيولوجية. لذلك، كان الهدف من هذا البحث هو دراسة تأثير مبيدات ذات سمية عصبية تشمل مبيدين من مجموعة مبيدات الفوسفور العضوية (بروفينوفوس وكلوربيريفوس) ومبيد من مجموعة الكاربامات (ميثوميل) ومبيدين من مجموعة البيثرويد (لامبادا سيهالوثرين و دلتامثرين) على ثلاث عزلات من النيماطودا الممرضة للحشرات والتابعة لعائلة Heterorhabditidae وهي Heterorhabditis sp. و Heterorhabditis sp. NEM 08 و Heterorhabditis bacteriophora HP88 و NEM 15 وشملت الدراسة تأثير تعرض عزلات النيماطودا للتركيز الحقل للمبيدات لمدة ٦ و ٢٤ و ٤٨ ساعة على حيويتها والقدرة المرضية ليرقات *Galleria mellonella*.