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Article

Combine Usage Possibilities of Entomopathogenic Nematodes with Insecticides against Mediterranean Corn Borer *Sesamia nonagrioides* (Lefebvre)

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Abstract: The Mediterranean Corn Borer (MCB), *Sesamia nonagrioides*, poses a significant threat to maize crops, necessitating effective pest management strategies. This study investigates the compatibility of two entomopathogenic nematode (EPN) isolates, *Steinernema feltiae* KV6 and *Heterorhabditis bacteriophora* EO7, with four registered insecticides for MCB control: deltamethrin, flubendiamide, spinetoram, and betacyfluthrin. The impact of these insecticides on EPN mortality, infectivity, and reproduction was assessed. Results indicate that deltamethrin exhibits the lowest toxicity to EPNs, with mortality rates of 1.3% for *S. feltiae* and 0.63% for *H. bacteriophora* at field dose (FD) after 24 hours, and 4.63% and 1.96% respectively after 48 hours. In contrast, spinetoram and flubendiamide showed higher toxicity, with mortality rates of 38.04% and 14.17% for *S. feltiae* at FD after 48 hours. The infectivity assays demonstrated that deltamethrin-treated EPNs caused up to 100% mortality in MCB larvae, while the reproduction capacity varied significantly between the EPN species and insecticides. *H. bacteriophora* exhibited higher progeny production, especially in the presence of Deltamethrin (87,900 IJs/larva). The findings suggest that integrating EPNs with selective insecticides like deltamethrin can enhance pest control efficacy and support sustainable agricultural practices. This study provides valuable insights for developing integrated pest management (IPM) strategies aimed at mitigating MCB infestations in maize while minimizing environmental impacts.

Keywords: entomopathogenic nematodes; Mediterranean Corn Borer; insecticides; compatibility

1. Introduction

Corn (*Zea mays* L.) is one of the most important cereal crops globally, yielding higher productivity per unit area compared to other cereals like wheat and barley [1]. In the Mediterranean region, corn ranks as the second most significant agricultural product; however, its production is threatened by the Mediterranean Corn Borer (MCB), *Sesamia nonagrioides* (Lefebvre) (Lepidoptera: Noctuidae) [2]. The MCB is a major pest in Mediterranean countries, causing substantial damage to maize crops [3–5]. This pest exhibits a polyphagous nature, infesting a variety of host plants including corn, sorghum, millet, rice, sugar cane, grasses, melon, asparagus, palms, and banana [6,7]. Originating from the Mediterranean region, the MCB has spread extensively across Europe, North Africa, and the Middle East due to its adaptability to different climates and broad host range [2,8].

Studies have shown that MCB infestations can cause significant yield losses, particularly in late and second crop maize productions, with potential losses reaching up to 100% if not adequately controlled [4]. Chemical insecticides are often recommended and applied multiple times throughout the growing season to manage this pest; however, even with intensive insecticide use, yield losses can still exceed 30% during severe outbreaks [9]. Additionally, MCB infestations lead to secondary issues such as fungal infections at feeding sites, further compromising crop quality and safety [10]. Despite the implementation of various control methods, including frequent use of chemical

insecticides, damage from MCB remains pervasive. However, chemical insecticides, while effective in reducing pest populations, pose significant threats to the environment and human health.

Entomopathogenic nematodes (EPNs) are soil-inhabiting organisms known for their capability to parasitize and eliminate insect pests [11–13], making them valuable biocontrol agents against *Sesamia nonagrioides*. These nematodes, particularly from the genera *Steinernema* and *Heterorhabditis*, display efficient host-seeking behavior through their infective juvenile stage, which actively locates and invades insect hosts [14,15]. Upon entering the host, EPNs release symbiotic bacteria (e.g., *Xenorhabdus* spp. for *Steinernema* and *Photorhabdus* spp. for *Heterorhabditis*), which proliferate, produce toxins, and ultimately cause insect death by septicemia within 24–48 hours [16]. EPNs' effectiveness in targeting soil-dwelling stages of pests, such as the larvae of the MCB, has been well documented [17–20]. Their use is particularly advantageous in integrated pest management (IPM) programs due to their minimal impact on non-target organisms, including beneficial arthropods and soil microbiota, and their compatibility with organic farming practices [21,22]. Additionally, EPNs' resilience to various environmental conditions enhances their potential as a sustainable and reliable pest control method [23].

Recent studies have also highlighted the synergistic potential of combining EPNs with other biocontrol agents or compatible chemical insecticides, which can lead to improved pest management outcomes while reducing the reliance on conventional pesticides [24,25]. Such integrated approaches not only improve the efficacy of pest control strategies but also align with the increasing demand for environmentally sustainable agricultural practices [26,27]. The integration of entomopathogenic nematodes (EPNs) with chemical insecticides offers a synergistic approach to enhancing pest control efficacy while reducing reliance on conventional pesticides. Numerous studies have investigated the compatibility of EPNs with various chemical agents, including pesticides, fertilizers, and microbial control agents [12,13,28–38].

The findings from these studies have been mixed, with some indicating negative effects of various pesticides on nematode infectivity and survival, while others have reported synergistic behavior that enhances pest control [12,39]. The susceptibility of infective juveniles (IJs) of EPNs to these chemical agents can vary widely depending on several factors, including the species and strain of the nematode, the application method and dose of the pesticide, timing of application, and environmental conditions [13]. For instance, Koppenhöfer et al. (2002) [24] demonstrated that certain chemical insecticides could be combined with EPNs without compromising their effectiveness, while other studies highlighted the adverse impacts of some pesticides on EPN survival and performance [13,28,38]. These varying outcomes underscore the importance of understanding specific interactions between EPNs and pesticides to optimize their combined use.

Incorporating EPNs into integrated pest management (IPM) programs allows for more sustainable and environmentally friendly pest control strategies. This combined approach can reduce the application rates of chemical insecticides, thereby mitigating environmental contamination risks and minimizing the development of insecticide resistance [12,34]. Furthermore, the use of EPNs supports the principles of IPM by promoting biological control methods and reducing the ecological footprint of pest management practices [26,27]. By leveraging the synergistic potential of EPNs and chemical insecticides, farmers can achieve effective pest control while supporting sustainable agricultural practices.

This study aims to investigate the compatibility of two EPN species, *Steinernema feltiae* and *Heterorhabditis bacteriophora*, with four commonly used insecticides: Deltamethrin, Flubendiamide, Spinetoram, and Betacyfluthrin. By assessing the impact of these insecticides on EPN mortality, infectivity, and reproduction, this research seeks to identify combinations that maximize pest control efficacy while minimizing adverse effects on non-target organisms and the environment. The findings will contribute to the development of more effective and sustainable IPM strategies for managing MCB infestations in maize crops.

2. Materials and Methods

2.1. Entomopathogenic Nematodes

Steinernema feltiae KV6 and *Heterorhabditis bacteriophora* EO7 isolates were used in the study (Table 1).

Table 1. Entomopathogenic nematodes used in the study.

Isolate	Accession number	Reference
<i>Steinernema feltiae</i> KV6	MN853593	Özdemir et al. 2020
<i>Heterorhabditis bacteriophora</i> EO7	MN853594	Özdemir et al. 2021

Nematodes were propagated in last instar larvae of the greater wax moth, *G. mellonella* at 25 °C as described by Woodring and Kaya (1988) [40]. The emerged infective juveniles (IJs) collected with modified White’s traps were stored in Ringer solution in culture flasks at 10–12 °C for maximum a week at a density of 3000 IJ/ml. Before the experiments, EPN viability was checked and only the stocks which have >95% viability were used (Laznik and Trdan 2014).

2.2. Collection and Identification of of MCB Larvae

MCB larvae were collected from infested corn fields in Şanlıurfa, Turkey. Collected stems were carefully dissected and larvae were removed and put into plastic boxes with perforated covers to permit airflow. Fresh corn stems were provided as food and the boxes were immediately transported to the laboratory. Following transportation, the healthy larvae were fed with fresh stems at room temperature under a 12:12 photoperiod for 2 days to select the healthy ones for bioassays.

To extract genomic DNA, the GeneMATRIX tissue and bacterial DNA purification kit (EURx) was employed. The extracted DNA was stored at -20°C until required for amplification. The PCR primers used were LCO1490 (5-GGTCAACAAATCATAAAGATATTGG-3) and HCO2198(5-AAACTTCAGGGTGACCAAAAAATCA-3) [41]. The PCR amplification protocol consisted of an initial denaturation step at 95 °C for 10 min followed by 40 cycles of comprising denaturation at 94 °C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 5 min. The PCR products underwent verification through electrophoresis and were subsequently sent for sequencing to Macrogen, Inc. (South Korea). The resulting sequences were then deposited in GenBank under Accession numbers: PP504953 and PP504954.

2.3. Pesticides Used in Compatibility Bioassays

The pesticides used in this study are listed in Table 2. These pesticides were selected based on their common usage in cornfields across Turkey, as confirmed through personal communications with representatives from both the Ministry of Agriculture and Forestry of Turkey and private-sector companies.

Table 2. Insecticides used in this study.

Active ingredient	Commercial name	Formulation*	Field dose (ml l ⁻¹)	Chemical group	Mode of action
Spinetoram 250 WG	Delegate®	WG	0.2	Spinosyns	Nicotinic acetylcholine receptor (nAChR) allosteric modulators
Deltamethrin	Decis®	SC	20 g/l	Pyrethroids	Sodium channel modulators

Flubendiamide	Tunga®	SC	25 g/l	Diamides	Ryanodine receptor modulators
Betacyfluthrin	Betagard®	EC	0.5	Pyrethroids	Sodium channel modulators

* Stock solutions were prepared using commercial formulations for all pesticides as recommended field dose (FD), double dose (2FD), and half dose (FD/2) according to labels of the products.

2.4. Effect of Pesticides on Survival

Pesticide dilutions were prepared using distilled water in 50 ml beakers. For each pesticide solution, 2 ml was added to 35 mm diameter Petri dishes, achieving final concentrations corresponding to the field dose (FD), half the field dose (FD/2), and double the field dose (2FD). A total of 100 infective juveniles (IJs) of each nematode species, suspended in 10 µl of distilled water, were added to the dishes and exposed to the pesticides. Controls were conducted using only distilled water. The pesticide-treated IJs were incubated in a shaking incubator at 150 rpm in the dark at 25 ± 2 °C for 24- and 48-hour periods. After incubation, dead and live nematodes were counted under a stereomicroscope (Olympus SZ61). Nematodes were considered dead if they did not respond to probing. The experiment included ten biological replicates and two technical replicates for each nematode species.

2.5. Effect of Pesticides on Infectivity

The last instar larvae of MCB were used as hosts to determine the infectivity ability of IJs treated with pesticides. A 10 ml field dose of pesticide solution was prepared in 50 ml flasks. After adding EPNs, the flasks were kept in an orbital shaker at 26 °C (Ulu et al. 2016). After 48 hours of exposure, nematodes were washed using a 20-micron sieve. Subsequently, EPNs were placed in Falcon tubes, and distilled water was added. After 30 minutes, the supernatant was discarded. This process was repeated three times to ensure the complete removal of pesticides. After the final rinsing, 150 IJs/100 µl were applied to a petri dish (35 mm diameter) containing filter paper. A last instar MCB larva was introduced to each petri dish and incubated at 25 ± 1 °C. After 24 and 48 hours, insect cadavers were counted and dissected to verify the presence of nematodes. The experiment was conducted with twelve biological replicates and three technical replicates.

2.6. Effect of Pesticides on Reproduction Capacity

The nematode reproduction capacity for each species was assessed using the previously mentioned method for evaluating the effect of pesticides on infectivity. MCB larvae were transferred to White’s traps immediately after death was observed. They were then incubated at 25 ± 1 °C in dark conditions for 10 days. The total number of emerged IJs from each larva was counted. The experiment was conducted with six biological replicates and two technical replicates.

2.7. Statistical Analysis

The results were first converted into percent mortality and then subjected to arcsine ($n' = \arcsin(\sqrt{n})$) transformation and ANOVA. Variance analysis was conducted with the transformed data, and differences among treatments were analyzed using Tukey’s multiple comparison tests ($P < 0.05$). All statistical analyses were performed with the MINITAB® Release 21 software package. The data from the survival assay were analyzed using one-way ANOVAs. Mean separation was performed using Tukey’s procedure with $\alpha = 0.05$. The analysis of the infectivity assay was done using two-way ANOVA, while the reproduction capacity assay was analyzed using one-way ANOVA, with mean separations performed using Tukey’s procedure with $\alpha = 0.05$.

3. Results

3.1. Effect of Insecticides on Survival

The ANOVA table shows significant effects of the main factors (insecticide, EPN, time, and dose) and their interactions on nematode mortality rates ($P < 0.0001$). This indicates that the type of insecticide, EPN, the duration of exposure, and the dose all significantly influence the mortality rates of the nematodes (Table 3).

Table 3. ANOVA results for the mortality rates of entomopathogenic nematodes (EPN) This is a table.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Insecticide (I)	3	4918,3	1639,43	354,93	0,000*
EPN (N)	1	2136,9	2136,95	462,64	0,000*
Time (T)	1	3905,1	3905,07	845,44	0,000*
Dose (D)	2	2080,1	1040,03	225,16	0,000*
I*N	3	3020,8	1006,95	218,00	0,000*
I*T	3	4765,0	1588,34	343,87	0,000*
I*D	6	2353,5	392,24	84,92	0,000*
N*T	1	1660,3	1660,32	359,45	0,000*
N*D	2	725,3	362,63	78,51	0,000*
T*Do	2	1135,4	567,71	122,91	0,000*
I*N*T	3	3303,4	1101,15	238,40	0,000*
I*N*D	6	1871,1	311,85	67,51	0,000*
I*T*D	6	2155,2	359,20	77,77	0,000*
N*T*D	2	716,8	358,38	77,59	0,000*
I* N*T*D	6	2032,1	338,69	73,32	0,000*
Error	1104	5099,4	4,62		
Total	1151	41878,7			

Table 4 presents the mortality rates of EPNs treated with various pesticides at two time points: 24 and 48 hours after exposure. The data includes different doses of pesticides: field dose (FD), half field dose (FD/2), and double field dose (2FD).

Table 4. Mortality rates (%) of EPNs treated (n=100 IJs) with pesticides at 24 and 48h after exposure.

EPN strain	Dose	Deltamethrin		Flubendiamide		Spinetoram		Betacyfluthrin	
		24H	48H	24H	48H	24H	48H	24H	48H
H.b	FD/2 ¹	0,17±0,38Ca ²	0,42±0,58Ca	0,13±0,34Bb	0,67±0,64Ba	0,58±0,65Aa	0,58±0,72Ba	0,38±0,50Bb	2,17±1,24Ca
EO7	FD	0,63±0,71Ba	0,95±1,08Ba	0,38±0,78ABb	1,83±0,92Aa	0,63±0,71Ab	2,25±1,07Aa	0,67±0,64Bb	3,29±1,23Ba
	2FD	1,29±1,04Ab	1,96±1,20Aa	0,83±0,92Ab	2,5±1,32Aa	0,96±0,86Ab	2,67±1,71Aa	1,63±1,25Ab	4,83±1,83Aa
S.f	FD/2	0,88±1,40Ab	2,33±1,34Ba	0,50±0,66Bb	1,33±1,00Ba	0,50±0,59Bb	1,50±0,98Ba	0,50±0,66Bb	7,25±2,21Ca
KV6	FD	1,30±1,12Ab	4,63±2,93Aa	0,83±0,81ABb	2,33±1,20Aa	1,04±1,00Bb	2,20±1,56ABa	0,88±1,00ABb	14,17±6,00Ba
	2FD	1,70±1,33Ab	6,41±3,59Aa	1,08±0,83Ab	2,67±1,50Aa	1,75±1,26Ab	2,50±1,29Aa	1,42±1,39Ab	38,04±10,51Aa

¹ FD= Field dose. ²Capital letters among doses, lowercase letters among time in each insecticide are indicated statistically different from each other ($P < 0.05$, Tukey test).

Deltamethrin shows relatively low mortality rates across all doses and time points, indicating lower toxicity to EPNs compared to other insecticides. A statistically significant difference in mortality rates is observed between the doses at 24 hours, with the highest mortality at 2FD (0.67±0.64%). At 48 hours, the mortality rate significantly increases for FD (2.25±1.07%) and FD/2 (2.17±1.24%), indicating time-dependent toxicity. Flubendiamide and spinetoram exhibit higher mortality rates, particularly at 48 hours and higher doses, suggesting greater toxicity. At 24 hours, the mortality rate caused by flubendiamide is relatively low across all doses, but at 48 hours, there is

a significant increase, especially for FD (14.17±6.00%) and FD/2 (7.25±2.21%). This indicates a delayed toxicity effect of flubendiamide on EPNs. Spinetoram shows significantly higher mortality rates at 48 hours for FD (38.04±10.51%), indicating high toxicity with increasing time. The mortality rate for 2FD (2.67±1.50%) is also notable, suggesting that higher doses amplify the pesticide’s effect. Betacyfluthrin exhibits lower toxicity at 24 hours but shows increased mortality at 48 hours, especially for FD (2.67±1.20%). The mortality rates across all doses at 48 hours are significantly higher than at 24 hours, indicating time-dependent effects.

Among the pesticides, Spinetoram at FD shows the highest mortality rate at 48 hours (38.04±10.51%), indicating it is the most toxic to EPNs over time. Flubendiamide also shows a significant increase in mortality at 48 hours, particularly at FD. Deltamethrin and betacyfluthrin show lower overall toxicity compared to spinetoram and flubendiamide but still present a time-dependent increase in mortality.

3.2. Effect of Insecticides on Infectivity

Data analyses on the pooled results are given in Table 5. Time and insecticide treatment significantly influence the mortality rates of Mediterranean Corn Borer (MCB) larvae infected with pesticide-treated EPNs.

Table 5. ANOVA results for the mortality rates of MCB infected with insecticides-treated EPNs.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time (T)	1	21,852	21,8522	90,23	0,000
Insecticide (I)	4	48,639	12,1598	50,21	0,000
EPN (N)	1	0,164	0,1636	0,68	0,412
T*I	4	4,580	1,1449	4,73	0,001
T*N	1	0,500	0,5000	2,06	0,152
I*N	4	10,182	2,5456	10,51	0,000
T*I*N	4	1,428	0,3571	1,47	0,211
Error	220	53,279	0,2422		

The infectivity assays showed that the infection rates of both species are increasing over time. Infectivity of insecticide-treated *H. bacteriophora* and *S. feltiae* on MCB larvae was higher ($P < 0.05$) than control groups after being exposed to deltamethrin in both time interval (Table 6). Deltamethrin and flubendiamide show the highest mortality rates for both EPN strains, particularly at 48 hours. The mortality of MCB larvae was highest (100%) ($P < 0.05$) at 48 h after exposure to deltamethrin treated *S.feltiae*. Spinetoram also exhibits high mortality rates, but slightly lower compared to feltamethrin and flubendiamide. Betacyfluthrin shows variability, with significant differences in mortality rates between 24 and 48 hours, particularly for *H.bacteriophora*.

Table 6. Mortality rates (%) of MCB larvae with pesticide treated EPN strains at 24 and 48 h after exposure (n=100 IJs).

EPN strain	Deltamethrin		Flubendiamide		Spinetoram		Betacyfluthrin	
	Time treated	untreated	treated	untreated	treated	untreated	treated	untreated
<i>H.b</i>	24 h	90,00±4,92Ba*	95,83±6,69Aa	85,83±5,43Ba	91,67±9,38Aa	64,17±9,96Bb	95,83±6,69Aa	71,67±11,15Bb
	48 h	96,67±9,53Aa	99,17±2,89Aa	99,17±2,89Aa	95,83±6,69Aa	77,50±9,65Ab	99,17±2,89Aa	87,50±7,54Ab
<i>EO7</i>	24 h	94,17±5,15 Ba	80,00±9,53Bb	82,50±12,88Ba	80,00±9,53Ba	61,67±11,15Bb	80,00±9,53Ba	85,00±5,22Ba
	48 h	100,00±0,00Aa	90,83±9,00Ab	94,17±9,96Aa	90,83±9,00Aa	84,17±9,00Aa	90,83±9,00Aa	96,67±4,92Aa

3.3. Effect of Pesticides on Reproduction Capacity

The progeny production capacity of *H. bacteriophora* and *S. feltiae* varies significantly depending on the pesticide exposure. There are significant differences between the EPNs, with *H. bacteriophora* shows higher progeny production compared to *S. feltiae*, particularly in the presence of deltamethrin and the control group ($P < 0.05$) (Table 7). However, *S. feltiae* shows relatively higher progeny production when exposed to spinetoram and betacyfluthrin.

Table 7. The progeny production (Mean $\pm$ SE) capacity of *Heterorhabditis bacteriophora* and *Steinernema feltiae* exposed with field dose of six pesticides after 10 days.

	<i>H.b</i> EO7	<i>S.f</i> KV6
Deltamethrin	87900 $\pm$ 21655 Aa*	53317 $\pm$ 34032 Ba
Flubendiamide	60292 $\pm$ 26772 Aa	73500 $\pm$ 54173 Aa
Spinetoram	30500 $\pm$ 27649 Ab	53500 $\pm$ 43847 Aa
Betacyfluthrin	31875 $\pm$ 19649 Ab	43583 $\pm$ 32636 Aa
Control	74862 $\pm$ 45688 Aa	37965 $\pm$ 29245 Ba

4. Discussion

This study provides valuable insights into the interactions between entomopathogenic nematodes (EPNs) and various chemical insecticides in managing the Mediterranean corn borer (*Sesamia nonagrioides*). The findings suggest that EPNs can be as effective as chemical insecticides under optimal conditions [42,43], and their combined use in integrated pest management (IPM) programs can be efficient in terms of time, effort, and cost [44]. The results indicate the potential for incorporating EPNs with insecticides in IPM strategies to optimize pest control and reduce environmental impacts.

The type of insecticide used significantly influences EPN mortality rates. Among the tested insecticides, deltamethrin showed the lowest toxicity to EPNs, with mortality rates of 0.67% at double the field dose (2FD) after 24 hours and 2.25% at the field dose (FD) after 48 hours. This observation is in line with recent studies that highlight the lower toxicity of pyrethroids, such as deltamethrin, on EPNs due to their specific action on insect nervous systems [30,34,45]. Deltamethrin, a pyrethroid, acts on sodium channels in nerve cells, leading to pest paralysis and death. Several authors have reported the non-toxic nature of various pyrethroids to EPNs [24,30,46,47]. Although Head et al. (2000) [48] noted that pyrethroids strongly influence EPN infectivity but not viability, Nermuť and Mráček (2010) [49] found minimal impact of pyrethroids on both mortality and infectivity of *Steinernema feltiae*, *S. arenarium*, and *S. kraussei*. Specifically, deltamethrin did not reduce EPN survival [34,45]. Conversely, flubendiamide and spinetoram exhibited higher toxicity, particularly after 48 hours and at higher doses. The delayed toxicity effect of flubendiamide may be related with EPN resilience to immediate calcium disruption caused by ryanodine receptor modulators but noted adverse effects over prolonged exposure. El Roby et al. (2023) [50] evaluated the compatibility of EPNs *Heterorhabditis bacteriophora* (HP88) and *Steinernema carpocapsae* (AT4) with lambda-cyhalothrin and flubendiamide against *Spodoptera frugiperda* larvae, finding synergistic effects. Spinetoram, a spinosyn targeting nicotinic acetylcholine receptors, causing continuous activation and eventual paralysis, exhibited significantly higher mortality rates at 48 hours, corroborating findings by De Nardo and Grewal (2003) [39] on the adverse impacts of certain insecticides on EPN performance. Betacyfluthrin, another pyrethroid acting on sodium channels, showed variability in toxicity, suggesting different EPN species have varying resistance levels. De Nardo and Grewal (2003) [39] and Özdemir et al. (2020) [13] highlighted the synergistic use of imidacloprid with EPNs to enhance pest control efficacy without significantly affecting nematode viability.

The infectivity assays demonstrated that deltamethrin had minimal impact on nematode infectivity, preserving their ability to infect the host. This aligns with recent studies suggesting pyrethroids do not significantly hinder EPN infectivity [13,46,47,51]. Flubendiamide and spinetoram significantly reduced infectivity, likely due to their action on nematode physiology and behavior.

Conversely, some chlorantraniliprole formulations were reported to have no adverse effects on EPN survival or infectivity [52] (Yan et al., 2012). Guo et al. (2016) [53] found synergistic or additive interactions when combining *H. bacteriophora* with chlorantraniliprole against *Holotrichia oblita* larvae, leading to faster larval mortality than EPNs or insecticides alone. El-Ashry et al. (2020) observed additive effects when combining flubendiamide with various EPNs for controlling *Helicoverpa armigera*. Özdemir et al. (2021) [38] reported no adverse effects of chlorantraniliprole on the survival and infectivity of *S. feltiae* KV6 Turkish isolate used against *Leptinotarsa decemlineata* larvae. However, Khan et al. (2021) [54] noted high larval mortality when combining Flubendiamide with *H. indica*. Incompatibility of some EPNs with certain insecticides was also reported in various studies, suggesting careful selection is needed [55–57].

The progeny production capacity varied significantly between *H. bacteriophora* and *S. feltiae* depending on insecticide exposure. *H. bacteriophora* showed higher progeny production in the presence of deltamethrin and the control group, whereas *S. feltiae* exhibited higher progeny production when exposed to spinetoram and betacyfluthrin. This species-specific response underscores the importance of selecting appropriate EPN species for use with particular insecticides to maximize biocontrol efficacy. Rovesti and Deseö (1990) [32] and Özdemir et al. (2020) [13] reported varying effects of insecticides on EPN reproductive capacity, consistent with our results. Ishibashi and Takii (1993)[29] reported that long-term exposure to sub-lethal doses of insecticides could adversely affect EPN reproductive capabilities, aligning with our findings that betacyfluthrin and spinetoram significantly influenced progeny production. Ozdemir et al. (2020) [13] showed that sub-lethal effects on EPN reproduction vary widely depending on nematode species and the specific chemicals used, supporting the need for tailored pest management strategies.

The integration of EPNs with chemical insecticides offers a promising approach to reducing reliance on chemical controls alone, mitigating environmental contamination, and minimizing insecticide resistance development. Previous studies have highlighted the compatibility of EPNs with various agricultural practices and their minimal impact on non-target organisms. The findings of this study support the potential for EPNs to enhance the efficacy of chemical insecticides to control MCB, particularly when used strategically.

The varying responses of EPNs to different insecticides emphasize the need for careful selection and optimization of pest control strategies. For instance, the lower toxicity of deltamethrin to EPNs suggests it could be effectively combined with EPNs without significantly impacting their survival and reproductive capabilities. Conversely, the higher toxicity of spinetoram and flubendiamide necessitates more cautious application to avoid detrimental effects on EPN populations.

5. Conclusions

This study provides valuable insights into the interactions between EPNs and chemical insecticides, highlighting the potential for their integrated use in IPM programs. The findings indicate that Deltamethrin is the most compatible insecticide, not adversely affecting the survival, infectivity, and reproduction of EPNs. Future research should explore the field application of these findings and investigate the physiological mechanisms underlying EPNs' resistance to certain insecticides. By leveraging the synergistic potential of EPNs and insecticides, sustainable and effective pest management solutions can be developed, contributing to the long-term health and productivity of agricultural systems.

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