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Original article (Orijinal araştırma)

Effect of selected pesticides on the orientation of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae)

Bazı pestisitlerin entomopatojen nematodların (Rhabditida: Heterorhabditidae ve Steinernematidae) yönelimleri üzerine etkisi

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Abstract

Entomopathogenic nematodes (EPNs) play a crucial role in biological control, but they can be also applied together with pesticides. Therefore, the compatibility of pesticides with EPNs and the influence on their behavior significantly affect field success. This study investigated how selected pesticides (Deltamethrin, Imidacloprid, Pendimethalin, 2,4-D, and Boscalid + Pyraclostrobin) affect the orientation behavior of three commercial EPN species. Trials were conducted using steel olfactometers followed by the assessment of EPN dispersal ratios after 24 hours. The study was conducted in the Laboratory of the Plant Protection Department, Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University between 2022 and 2023. According to the results, while nearly all pesticides exhibited a significant impact on the dispersal behavior of EPNs, the most notable effects were observed in the trials involving 2,4-D and Imidacloprid. These two pesticides demonstrated both repellent and attractive effects on different EPN species. The impact of other pesticides was comparatively negligible. All EPN species exhibited higher orientation towards larvae than the control application. The orientation behavior displayed variations depending on the pesticide type and the EPN species involved. It is expected that this study will contribute to our understanding of the relationship between EPNs and pesticides, and ultimately enhancing the efficacy of EPNs.

Keywords: Behavior, *Heterorhabditis*, pesticide, *Steinernema*, orientation

Öz

Entomopatojen nematodlar (EPN'ler) önemli biyolojik mücadele ajanı olmalarına karşın pestisitler ile birlikte de uygulanabilmektedir. Bu nedenle, pestisitlerin EPN'ler ile uyumluluğu ve EPN davranışı üzerindeki etkisi arazi başarısını etkilemektedir. Bu çalışmada, beş pestisitin (Deltamethrin, Imidacloprid, Pendimetalin, 2,4-D ve Boscalid + Pyraclostrobin) üç ticari EPN türünün yönelim davranışını nasıl etkilediği araştırılmıştır. Denemeler, çelik olfaktometrelerde gerçekleştirilmiş ve 24 saatlik inkübasyon sonrasında EPN yayılma oranları belirlenmiştir. Çalışma 2022-2023 yılları arasında Bilecik Şeyh Edebali Üniversitesi Ziraat ve Doğa Bilimleri Fakültesi Bitki Koruma Bölümü Laboratuvarında gerçekleştirilmiştir. Neredeyse tüm pestisitler EPN yayılımı üzerinde önemli etki göstermiş, en farklı sonuçlar 2,4-D ve Imidacloprid denemelerinde tespit edilmiştir. Her iki pestisit de EPN türüne göre hem itici hem de çekici etkide bulunmuştur. Diğer pestisitlerin etkileri daha sınırlı kalmıştır. Tüm EPN türleri, kontrol uygulamasına kıyasla larvalara daha yüksek yönelim göstermiştir. Sonuçlar, EPN türlerine ve pestisitlere bağlı olarak yayılma oranlarında farklılıklar olduğunu göstermiştir. Bu çalışmanın EPN'ler ve pestisitler arasındaki ilişkiyi anlamaya ve EPN uygulama etkinliğini artırmaya katkı sağlayacağı beklenmektedir.

Anahtar sözcükler: Davranış, *Heterorhabditis*, pestisit, *Steinernema*, yönelim

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Introduction

Entomopathogenic nematodes (EPNs) from the genera *Heterorhabditis* and *Steinernema* are obligate endoparasites on insects and they have found extensive use in biologically controlling agricultural pests. EPNs undergo a distinctive stationary phase known as the infective juvenile (IJ), which represents the sole free-living stage in their life cycle. Upon encountering a host, the IJs penetrate the host and release symbiotic bacterium into the host hemolymph. The bacterium proliferates within the host while simultaneously inducing host mortality through the secretion of an array of toxins and enzymes. The bacterium converts the host into a food source that EPNs feed on, facilitating their continued development. After undergoing 2-3 generations within the host, the available food becomes depleted, signaling the IJs to exit the cadaver and search for new hosts (Kaya & Gaugler, 1993).

Over the course of several years, EPNs have demonstrated remarkable efficacy in controlling agricultural pests (Koppenhöfer et al., 2020; Mokrini et al., 2020; Taşkesen et al., 2021). Their increasing adoption can be attributed to their ability for cost-effective mass production in bioreactors and their easy application through conventional spray equipment and drip irrigation systems (Wright et al., 2005; Susurluk & Ehlers, 2008; Shapiro-Ilan et al., 2012; Peters, 2013). Thanks to innovative formulation techniques and application methodologies, their efficacy extends not only below-ground but also in targeting insect pests above the soil (Şahin et al., 2018; Platt et al., 2020; Fallet et al., 2022). Despite the notable achievements in pest management, field applications of EPNs frequently yield inconsistent outcomes (Jaffuel et al., 2020). Furthermore, if not administered by professionals, the probability of success decreases due to possible inaccuracies in application. As a result, EPNs are often applied concurrently with conventional agricultural pesticides. An important benefit is the compatibility of EPNs with various pesticides, and in certain combinations, even synergistic effects have been observed (Amizadeh et al., 2019; Yüksel et al., 2019). Furthermore, simultaneous application also saves time and reduces application costs (Laznik & Trdan, 2017). Notably, this cooperative strategy of applying pesticides together with EPNs is particularly advantageous compared to predator insects, which are often susceptible to insecticides and thus incompatible for simultaneous application.

The interaction between EPNs and pesticides has long intrigued scientists. Determining whether a pesticide can be combined in a tank mixture or applied simultaneously with another pesticide carries significant importance. Early investigations explored the impacts of organophosphorus and carbamate compounds, which were commonly used pesticides during that period, on the development and viability of EPNs. These studies underscored the lack of compatibility between these pesticides and nematode species (Hara & Kaya, 1982, 1983). As the utilization of EPNs in agriculture continues to grow, numerous studies have examined the compatibility between various EPN species and pesticides. These investigations have unveiled a wide range of pesticides that are now recognized for their compatibility with EPNs, allowing for their simultaneous application (Ulu et al., 2016; Bajc et al., 2017; Laznik & Trdan, 2017; Aioub et al., 2021). While the majority of studies focused on insecticides, investigations into the effects of fungicides, acaricides, and herbicides have also been conducted (Koppenhöfer et al., 2003; Bajc et al., 2017; Laznik & Trdan, 2017; Sabino et al., 2019; Özdemir et al., 2020). It is important to emphasize that the compatibility is influenced by the specific pesticide and the EPN species (Laznik & Trdan, 2014). Moreover, prolonged exposure to pesticides intensifies their harmful effects.

While the majority of compatibility studies have mainly focused on EPN efficacy and viability, only a limited number of them have explored the changes in EPN behavior due to pesticide exposure. Numerous pesticides are recognized to induce paralysis or diminish mobility in EPNs, even potentially leading to their death (Hara & Kaya, 1983). Furthermore, certain pesticides have been documented to disrupt the locomotion patterns of EPNs and reduce the host-detection capabilities of infective juveniles (Gaugler & Campbell, 1991). Additionally, specific organic phosphorus and carbamate compounds trigger a behavior known as nictation in some EPN species, simultaneously reducing their rate of movement (Ishibashi & Takii, 1993). Because of the scarcity of studies investigating the impact of pesticides on EPN behaviors, several aspects of this relationship remain unexplored. Given the frequent coexistence of pesticides and EPNs in nature, the influence of pesticides on EPN behavior holds significance for their overall effectiveness.

This study investigated the orientation behavior of three commonly found EPN species when exposed to selected pesticides, chosen for their common usage in Turkey and their high likelihood of encountering nematodes in their natural habitats. The study involved controlled trials using steel olfactometers to evaluate how these EPNs responded to different concentrations of pesticides. The objective was to uncover the reactions of EPNs in the presence of pesticides, a departure from previous research that primarily focused on compatibility and effectiveness evaluations of EPNs with pesticides. While significant insights have been gained into nematode behavior, certain aspects still remain unclear. Hence, this study aims to provide a deeper understanding of the interaction between pesticides and EPNs, which is crucial given their concurrent application.

Materials and Methods

Entomopathogenic nematodes and pesticides

The study involved three prevalent EPN species: *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema feltiae* (Filipjev, 1934), and *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae). These species are distributed globally and exhibit distinct host-seeking strategies (Susurluk et al., 2003). Additionally, all three species are recognized as registered biocontrol products in Turkey. They are labeled for managing many major pests, including mushroom gnats, western flower thrips, white grubs, and flathead woodborers. The commercial EPN products were supplied by Bioglobal A.Ş (Antalya, Türkiye), while the strains themselves were commercial strains obtained from e-nema GmbH (Schwentinental, Germany).

There were two insecticides, two herbicides, and one fungicide in the study. The selection of pesticides was based on active substances that are recognized to be compatible with EPNs (Chavan et al., 2018; Özdemir et al., 2021), suitable for application to soil or near-soil areas (or systemic), and likely to interact with EPNs in their natural environment. Further information regarding the pesticides employed in the study is presented in the Table 1.

Table 1. Details of the pesticides used in the study

Active Compound	Product Name (Formulation)	Pesticide Group	Chemical Class	Mode of Action
Imidacloprid	Insector® (SC)	Insecticide	Neonicotinoids	Acetylcholine mimic
Deltamethrin	Jetsis® (EC)	Insecticide	Pyrethroids	Sodium Channel Modulator
2,4-D	Ester H® (EC)	Herbicide	Phenoxy-carboxylates	Auxin mimics
Pendimethalin	Giant® (EC)	Herbicide	Dinitroanilines	Inhibition of Microtubule Assembly
Boscalid + Pyraclostrobin	Bellis® (WG)	Fungicide	Pyridine-carboxamides + Methoxy-carbamates	Succinate dehydrogenase inhibitors + Quinone outside Inhibitors

Olfactometer setup

The study utilized olfactometers that were originally produced for behavioral studies of EPNs, by the author. The stainless steel olfactometer comprises two primary components: the arm and the central part. While the design allows for up to three arms to be attached to each olfactometer (Ulu & Erdoğan, 2023; Ulu et al., 2023), this study employed a single arm configuration. The remaining two arm sockets were closed with a steel hex plug. The olfactometer has an approximate diameter of 2.5 cm and a length of about 10 cm from the central application hole to the arm's end. The Figure 1 illustrates the olfactometer's design and dimensions.

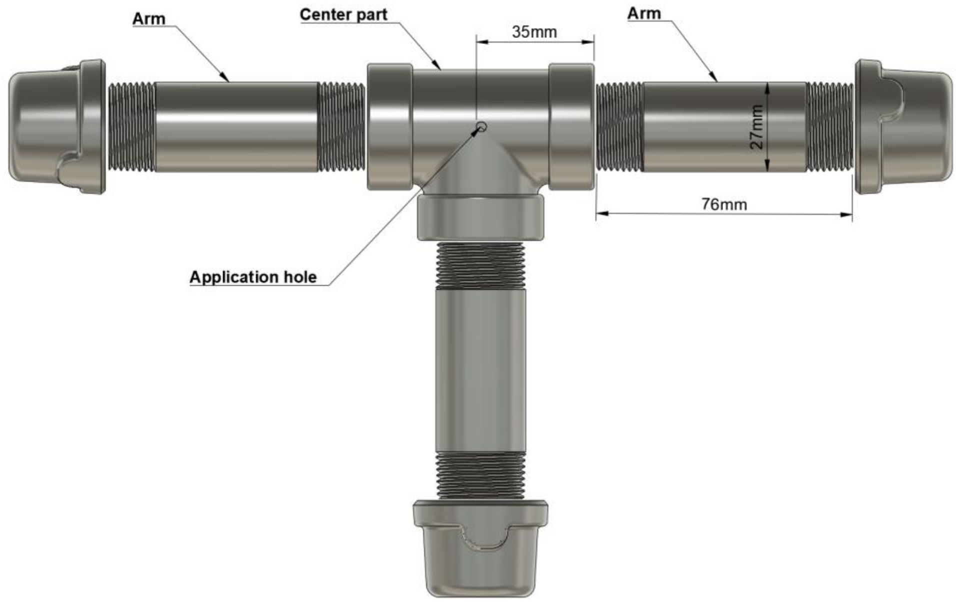

Figure 1. Illustration and dimensions of the olfactometer.

Experimental design

The study took place in the Laboratory of the Plant Protection Department, Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University between 2022 and 2023. The olfactometers was filled with dust-free silica sand, a chemically inert substrate characterized by a particle diameter approximately 250 microns. Prior to filling, the sand in the center part was moistened with deionized water (DI), while the arm was moistened with pesticide solutions. The highest recommended field dose (X), half dose (0.5X), and double dose (2X) of each pesticide were prepared in glass beakers. The resulting pesticide solution was used to moisten the sand. The moisture content of the sand was adjusted to 10% for both parts. Subsequently, 5000 IJs were applied through the application hole with a pipette. The nematodes were applied using 50 microliters of DI water. The nematode cultures were no older than 1 week, and their mortality ratios were below 1%. Once the application was complete, the hole was sealed with parafilm, and the olfactometers were incubated at a controlled temperature of 24°C for 24 hours. The olfactometers are not airtight, but they are tight enough to preserve inner moisture for more than 24 h (<1% moisture loss during experiments), while allowing air exchange with the surrounding environment. The olfactometers were set up with the left arm, lower arm, and right arm interchangeably to mitigate directional effects and bias. Following the incubation period, the center part of the olfactometer was disassembled from its arm, and the sand within was gently washed in a container. The nematode suspension obtained was then filtered through a 25-micron mesh, and nematodes were collected in a beaker. The nematodes present in both the arm and center part were counted separately, and dispersal ratio was calculated according to the formula: Total number of infective juveniles (IJs) in the arm / Total number of IJs in the olfactometer. Each treatment was replicated using 5 olfactometers. Additionally, olfactometer setups were established at three distinct time points, resulting in a cumulative total of 2 + 2 + 1 olfactometers. These setups were performed using fresh populations of nematodes, comprising 5 technical replicates and 3 biological replicates in total. The negative control treatment consisted solely of DI water, while *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae were employed as the positive control treatment. The larva was placed at the end of the arm in a 3D printed mesh cage. It's worth noting that the larval treatment was not compared with pesticides, as its purpose was to observe the enhanced dispersal of IJs in the presence of an attractive cue. Mortality ratios of IJs were assessed during the counting procedure and were observed to be below 5% across all treatments.

Statistical analysis

All data were subjected to a normality test using the Shapiro-Wilk's method, and the homogeneity of variances was assessed using the Brown-Forsythe test. As all assumptions were met, a one-way ANOVA was employed to analyze the dispersal ratio of the treatments. Since each experiment had its corresponding control treatment, the Dunnett's comparison test was used to compare various pesticide doses with the negative control treatment. An unpaired t-test was applied to compare control and larva applications in the positive control. All analyses were conducted at a significance level of $p < 0.05$, and the graphs were generated using GraphPad v9.5.

Results and Discussion

Orientation of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)

In the positive control scenario, the IJs displayed statistically significant orientation towards the larval arm when compared to the control group (t (8)= 7.08, $p < 0.001$). In the control application, the proportion of IJs in the arm was 25.4%, which then increased to 43.4% in the presence of larvae. The dispersal ratio of *H. bacteriophora* varied based on the pesticide. No statistical change was observed in the orientation in Deltamethrin (F (3, 16) = 1.71, $p = 0.204$) and Boscalid + Pyraclostrobin (F (3, 16) = 0.64, $p = 0.598$) treatments. Pendimethalin, however, showed an increased orientation at higher doses. Although no statistical difference was noted in the 0.5X dose, an increased dispersal was observed in X and 2X doses compared to the control (F (3, 16) = 12.4, $p < 0.001$). The initial dispersal ratio in the control group was 23.2%, which then increased to 29.7% at the X dose. On the other hand, Imidacloprid displayed repellency at 0.5X and X doses, which diminished at 2X doses (F (3, 16) = 10.7, $p < 0.001$). Notably, among the pesticides tested, 2,4-D exhibited the most prominent effect, significantly reducing dispersal of IJs (F (3, 16) = 42.6, $p < 0.001$). A strong repellency was observed even at the 0.5X dose. The dispersal ratio, initially at 23.4% in the control group, declined to 8.7% at the 2X dose (Figure 2).

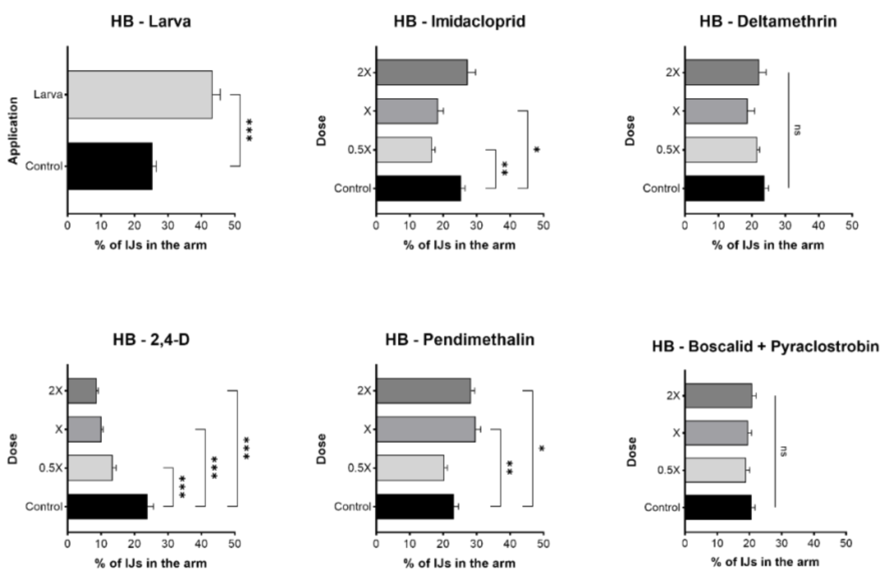

Figure 2. The dispersal ratios (X axis) of *Heterorhabditis bacteriophora* in the presence of a larva (first graph) and after a 24-hour exposure to selected pesticides. Error bars indicate standard error mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate statistically significant differences compared to the corresponding control (represented by black bars), as determined by Dunnet's comparison test ($p < 0.05$) for pesticides, and unpaired t test ($p < 0.05$) for the larva application. Ns: not significant ($p > 0.05$).

Orientation of *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae)

In the positive control, *S. feltiae* exhibited a lesser orientation towards the larva in comparison to *H. bacteriophora*, yet a significant dispersal was observed when compared to the control application (t (8)= 3.56, p = 0.007). With a dispersal ratio of 17.2% in the control group, the presence of larva led to an increase to 24.5%. The dispersal of S. *feltiae* IJs was generally less influenced by pesticides in comparison to *H. bacteriophora*. Although there was a numerical decrease in the dispersal especially at 2X doses, no significant effect was observed for Imidacloprid (F (3, 16) = 1.21, p = 0.339), Deltamethrin (F (3, 16) = 2.50, p = 0.097), Pendimethalin (F (3, 16) = 3.05, p = 0.059), and Boscalid + Pyraclostrobin (F (3, 16) = 3.35, p = 0.045), compared to control. In contrast, unlike *H. bacteriophora*, the orientation of *S. feltiae* was increased at 0.5X and X doses of 2,4-D; however, this effect was not observed at the 2X dose (F (3, 16) = 6.20, p = 0.005). Starting at 19.3% in the control group, the dispersal ratio increased to 28.4% at the X dose (Figure 3).

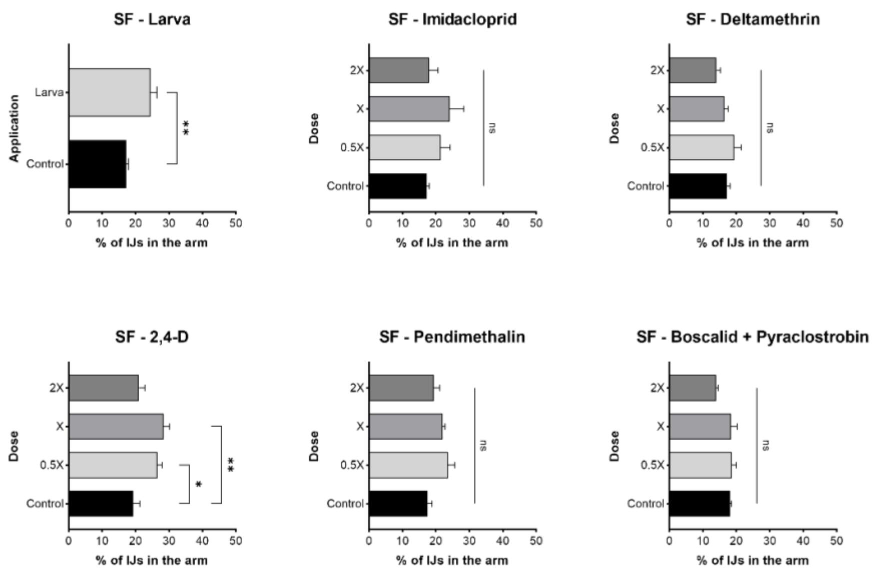

Figure 3. The dispersal ratios (X axis) of *Steinernema feltiae* in the presence of a larva (first graph) and after a 24-hour exposure to selected pesticides. Error bars indicate standard error mean. * p < 0.05, ** p < 0.01, *** p < 0.001 indicate statistically significant differences compared to the corresponding control (represented by black bars), as determined by Dunnet's comparison test (p < 0.05) for pesticides, and unpaired t test (p < 0.05) for the larva application. Ns: not significant (p > 0.05).

Orientation of *Steinernema carpocapsae (*Weiser, 1955) (Rhabditida: Steinernematidae)

As observed with the preceding two species, the orientation of *S. carpocapsae* towards the larvae displayed a significant enhancement when compared to the control group (t (8)= 7.24, p < 0.001). The dispersal ratio, which initially stood at 15.8% in the control group, increased to 25.4% in the larva application. Interestingly, while Deltamethrin had no impact on the dispersal of the other two species, it adversely affected *S. carpocapsae*, significantly reducing the dispersal ratio at the highest dosage (F (3, 16) = 7.61, p = 0.002). Specifically, the dispersal ratio, which was at 16.6% in the control group, reduced to 9.8% at the 2X dose. Similarly, there was a decline in dispersal ratios at higher doses in the case of Boscalid + Pyraclostrobin application (F (3, 16) = 5.35, p = 0.010). In contrast, Imidacloprid exhibited a notable increase in the orientation of *S. carpocapsae (F* (3, 16) = 9.69, p < 0.001). The dispersal ratio, initially measured at 15.7% in the control group, nearly doubled to 28.8% at the 2X dose. On the other hand, the application of 2,4-D (F (3, 16) = 2.22, p = 0.125) and Pendimethalin (F (3, 16) = 2.44, p = 0.102) showed no observable effect on the orientation of IJs (Figure 4).

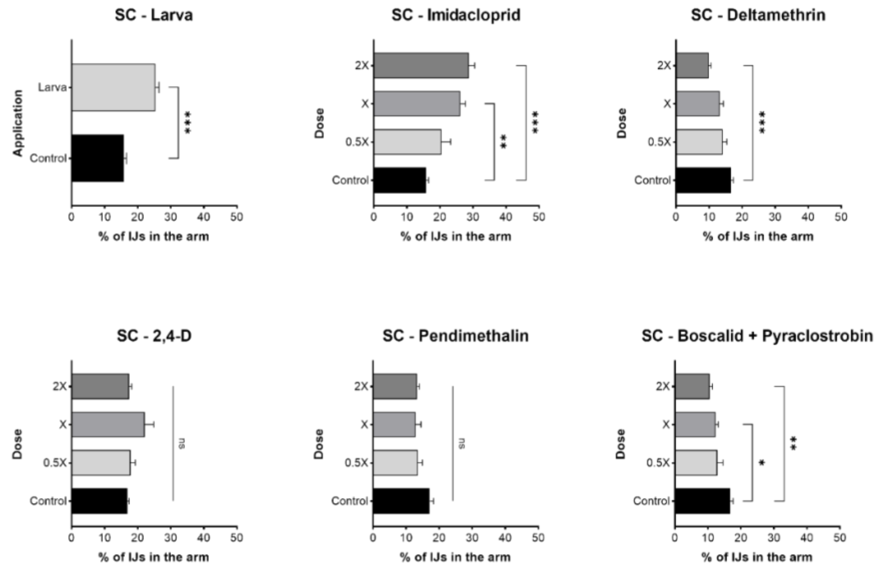

Figure 4. The dispersal ratios of *Steinernema carpocapsae* in the presence of a larva (first graph) and after a 24-hour exposure to selected pesticides. Error bars indicate standard error mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate statistically significant differences compared to the corresponding control (represented by black bars), as determined by Dunnet's comparison test ($p < 0.05$) for pesticides, and unpaired t test ($p < 0.05$) for the larva application. Ns: not significant ($p > 0.05$).

The highest dispersal ratio among all pesticide applications was 29.4% at the 2X dose of *H. bacteriophora* Pendimethalin application. When considering the increase rate compared to the related control groups, the highest increase was observed with 1.82 times in the 2X dose of *S. carpocapsae* Imidacloprid application. The application of *H. bacteriophora* with larvae achieved the highest dispersal ratio among all applications, reaching 43.4%. On the other hand, the lowest dispersal ratio of 8.71% was determined at the 2X dose of *H. bacteriophora* 2,4-D application. In comparison to the control group, the dispersal decreased by a factor of 0.64.

The practice of applying EPNs alongside pesticides has become increasingly common in recent years. Thus, the present study focused on examining the effects of specific pesticides, which are commonly used and likely to intersect with EPNs in natural environments, on the orientation behavior of EPNs. The findings were varied and dependent on the EPN species and the pesticides, and the results were aligned with similar studies (García del Pino & Jové, 2005; Laznik & Trdan, 2017). For instance, *H. bacteriophora* demonstrated a repellent response to Imidacloprid and 2,4-D, while showing attraction towards Pendimethalin. On the other hand, *S. feltiae* exhibited attraction solely to 2,4-D, with no significant impact from other pesticides. Finally, *S. carpocapsae* was attracted by Imidacloprid and repelled by Deltamethrin and Boscalid + Pyraclostrobin. Among all EPN strains, there was a noteworthy orientation towards larvae in the positive control groups.

Previous reports have indicated the compatibility of Imidacloprid with many EPN species (Koppenhöfer et al., 2002; Atwa et al., 2013; Laznik & Trdan, 2014; Kwizera & Susurluk, 2017; Özdemir et al., 2020). The findings of this study revealed that *S. carpocapsae* demonstrated attraction towards Imidacloprid, which could contribute to their compatibility. On the other hand, it was observed that *H. bacteriophora* exhibited a certain level of repellency against Imidacloprid. Given that Imidacloprid is commonly used in drip irrigation for pest control, it becomes imperative to investigate the potential movement of *H. bacteriophora* away from the root zone in fields where Imidacloprid is applied. A similar concern can be said for Deltamethrin. While previous studies generally indicated Deltamethrin's compatibility with EPNs (Negrisoli et al., 2010), this study revealed its repellent effect on *S. carpocapsae*. Given the widespread and excessive usage of Deltamethrin, investigating its interaction under field conditions becomes vital. Despite the limited number of studies focusing on 2,4-D, previous research findings have indicated both compatibility and incompatibility (Laznik & Trdan, 2017; Chavan et al., 2018). The current study aligns with this existing literature, as 2,4-D exhibited a strong repellent effect on *H. bacteriophora*, while it displayed a degree of attraction for *S. feltiae*. Unfortunately, arriving at a conclusive interpretation is challenging, as the remaining EPN-pesticide combinations exhibit minimal to no impact. However, even though it is hard to explain differential results (Radová, 2011), the inconsistent impact of pesticides and their doses on EPNs could be attributed to factors such as the specific active substances, formulation additives, mode of actions, nematode host-seeking strategies, or their interpretation of different stimuli.

Extensive research has explored the compatibility between EPNs and pesticides, revealing that numerous pesticides can be simultaneously applied well with EPNs (Radová, 2011; Bajc et al., 2017; Chavan et al., 2018; Özdemir et al., 2021). Nevertheless, investigations into how pesticides influence diverse behavioral traits of EPNs remain limited. Only a few studies have investigated the influence of pesticides on the behavior of EPNs. For instance, the impact of Oxamyl on the motility and host-seeking abilities of *H. bacteriophora* and *S. carpocapsae* has been observed, indicating a reduction in these capacities (Gaugler & Campbell, 1991). At elevated doses, paralysis entirely halts the movement of IJs. Conversely, Acephate, belonging to the same chemical group as Oxamyl, and Permethrin, a synthetic pyrethroid, have been indicated to enhance the nictation behavior of *S. carpocapsae*, and are considered suitable for combined application (Ishibashi & Takii, 1993). Furthermore, investigations have established that Fipronil does not adversely impact *H. bacteriophora* and *S. carpocapsae*, and even though the movement of *S. arenarium* was reduced, its efficacy remained unaffected (García del Pino & Jové, 2005). As evident from these studies, the absence of adverse effects of pesticides on the mortality or infectivity of EPNs does not necessarily imply the absence of changes in their behavior. Similar concerns have been brought up in the past as well (Zimmerman & Cranshaw, 1990). In addition, it has been indicated that insecticides targeting the nervous system can disrupt the sensory organs of nematodes, leading to a reduction in their host-finding capability (Patel & Wright, 1996). However, compatibility tests carried out in controlled laboratory conditions suggest that EPN efficiency remains high due to the ideal host encounter environment. This suggests that the high compatibility and efficacy results achieved under laboratory conditions are less likely to be reproduced in the field. Similarly, in this study, the post-application mortality ratio of EPNs remained below 5% based on the counts. From this viewpoint, it can be stated that the pesticides used in this study are compatible with EPNs, a conclusion supported by numerous studies (Rovesti et al., 1990; Negrisoli et al., 2010; Chavan et al., 2018; Özdemir et al., 2021). However, when exploring the effects of these chemicals on behavior, it becomes apparent that they can elicit both repellent and attractive responses. Given the extensive use of pesticides in agriculture, it's highly conceivable that potent chemicals in soil and plants can influence nematode behavior below ground. These responses could substantially shape the field effectiveness of EPNs within their natural environment. Hence, it is anticipated that studies conducted under field conditions will offer a more realistic perspective compared to efficacy tests conducted in ideal conditions within a limited area.

It's important to recognize that field efficiency may not directly mirror results obtained in controlled laboratory conditions. Controlled environments involve only a few stimuli, whereas the natural conditions involve the interaction of numerous stimuli. Our understanding of nematode orientation and dispersal highlights various factors that enhance or diminish these behaviors (Shapiro-Ilan et al., 2019). Attention to even the smallest details is crucial to enhance the success of biological control efforts. Understanding the behavior of EPNs and the idea of manipulating the behavior to increase efficiency is expected to be further explored in the future (Andaló et al., 2012). For instance, recent research indicates that pheromones contribute to enhanced nematode dispersion and host-seeking capacity, offering the potential to bolster field efficacy (Oliveira-Hofman et al., 2019; Kaplan et al., 2020). Certainly, the outcomes achieved in controlled laboratory settings should be duplicated and validated within real-world field conditions. In addition, the evaluation of the behavioral effects of chemicals should not be conducted in isolation; rather, it should be considered in conjunction with their impact on the behavior, viability, and infectivity of EPNs.

In conclusion, the impact of chemical pesticides on the orientation behavior and dispersal of EPNs varies based on the specific pesticides and EPN species. Some pesticides are found to strongly repel certain species, while in other cases, they attract IJs. Given the limited number of studies on the impact of chemical pesticides on EPN behavior, generalizing the findings from this study is not straightforward. However, research on the compatibility of EPNs with pesticides has demonstrated varying outcomes depending on the specific chemicals and EPN types involved. Consequently, the results of this study suggest that the effects of chemicals on behavior are likely to exhibit similar variability. Simultaneously applying EPNs and chemicals proves to be a time and cost-effective approach. Considering the frequent coexistence of pesticides and EPNs in the natural environment, understanding their interactions becomes crucial for the effective implementation of biological control. Besides assessing how pesticides affect EPN efficacy, examining shifts in their behavior holds equal significance for effective pest management. Due to the varying results, it's advisable to continue conducting compatibility trials and consistently explore interaction between new EPN strains and chemical compounds. Thus, conducting a comprehensive investigation into the connection between pesticides and EPNs emerges as a pivotal step for future research.

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