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Research Article

Evaluating a Portable Method and Two Irrigation Drippers for Field Application of Entomopathogenic Nematodes

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Abstract: Entomopathogenic nematodes (EPNs) can be applied using drip irrigation systems. However, the choice of driplines and types of drippers significantly impacts the efficacy of field applications. This study investigated the performance of EPN applications using two common dripper types (katif and cylindrical drippers) under both pot and field conditions. The primary objective of the study was to optimize EPN applications and create a modular system in which driplines and drippers can be selected based on the target pest or plant. In our modular system, driplines were connected to a battery-powered backpack sprayer rather than an irrigation system. The efficacy of EPN applications was assessed on *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae at a commercial dose of approximately 50 IJs cm^{-2}. The results revealed that only 60% of the nematodes were discharged from the cylindrical drippers, with 40% becoming trapped in the irrigation system. In contrast, over 90% of the nematodes were successfully discharged from the katif dripper. As a result, the katif dripper exhibited significantly higher larval mortality compared to all other application methods. These findings emphasize the substantial impact of the dripper type on EPN discharge, while also highlighting the applicability of the modular method for EPN applications.

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1. Introduction

The use of biopesticides is increasing due to the adverse effects of chemical pesticides on the environment (Glare et al., 2012; Olson, 2015). Biocontrol agents, such as entomopathogenic nematodes (EPNs), contribute significantly to the biopesticide market. EPNs, soil-dwelling microscopic roundworms, are among the successfully employed organisms in biological control. They are obligate endoparasites that require an insect host to complete their life cycle (Kaya and Gaugler, 1993). Although there are other insect-parasitic species, *Heterorhabditis* and *Steinernema* are the foremost genera. Specialized free-living third-stage juveniles of EPNs, known as infective juveniles (IJs), are the only stage outside the host that can seek and infect a suitable host in the soil. As IJs enter the host, they release their symbiotic bacteria, which kill the host and turn it into a cadaver in a short period. After several

generations in the host, IJs emerge from the cadaver and seek new hosts in the soil (Gaugler, 2002). EPNs are effectively used against a broad host range of pests (Askar et al., 2023; Dede et al., 2023). They can be mass-produced in fermenters and on solid media, and they are potent alternatives to chemicals used for below ground pests, where pesticides mostly fail (Shapiro-Ilan et al., 2012; Devi, 2018; Şahin et al., 2018; Ulu and Susurluk, 2021).

As with other biological control agents, EPNs have some disadvantages. Mass production of EPNs requires high initial expenses; their shelf life is approximately 40 days (E-nema GmbH product info), they need a cold chain during transport, and they need to be applied as fresh products (Grewal, 2000; Guy et al., 2017; Kagimu and Malan, 2019). They also show inconsistent virulence in field applications (Jaffuel et al., 2019; Oliveira-Hofman et al., 2019). EPN products are generally not preferred because of their high prices and inconsistent field efficacy, which cannot compete with chemical products. For this reason, there is a tendency to optimize production in liquid culture medium, improve formulations, develop superior characteristics by genetic selection, increase resistance to adverse conditions, and apply using different techniques (Mukuka et al., 2010; Singh and Upadhyay, 2018; Nxitywa and Malan, 2021).

Many methods can be used in the field applications of EPNs, depending on the target pest or plant. These application methods include spray and fertilization equipment, irrigation systems, and specific application techniques, such as cadaver application (Wright et al., 2005; Garcia et al., 2008; Raja et al., 2015). New robotic techniques are also optimized for EPN applications (Erdoğan et al., 2021, 2023). Although EPNs can be applied with all these techniques in theory, the most preferred application method is drip irrigation because EPNs live belowground and need moisture to move through the soil. However, there are some obstacles in the application of EPN using drip irrigation. For instance, a heterogeneous EPN distribution was observed throughout driplines (Garcia et al., 2008; Campos-Herrera, 2015). The filters used in irrigation systems prevent the exit of nematodes (Łaczyński et al., 2007). There are many types of drippers, and their physical structures differ in such a way that they affect the application of EPNs (Erdoğan et al., 2020). For instance, a wider flow path or higher flow rate allows the EPNs to pass more easily. To overcome these obstacles, the drip irrigation system should also be adapted to EPN application, using suitable dripper types, irrigation pressure, filters, or formulation. However, it is unlikely to replace drip irrigation systems already installed in the field. In other words, if the irrigation system is not suitable for EPN applications, it is necessary to use external application equipment. Thus, EPNs can be applied more efficiently using a more modular irrigation technique.

Improper application of EPNs not only results in waste of commercial products and effort but also tarnishes the reputation of EPNs as effective biocontrol agents. To ensure successful field applications, it is essential to have a practical and adaptable technique. Therefore, we integrated driplines with a battery-powered backpack sprayer to create a modified portable irrigation system for the EPN application. Our goal was to develop a system that could be tailored to various conditions and offer a more controlled, precise, and practical method for applying EPNs in a wide range of agricultural settings, including crops, fields, and greenhouses.

2. Material and Methods

2.1. Entomopathogenic nematodes

The study involved three nematode species: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae*. Commercial products for these EPN species were provided by a local distributor (Bioglobal A.Ş.). The selection of these different species was based on variations in their biology, host-seeking strategies (cruiser, ambusher, and intermediate, respectively), and physical characteristics such as body size (Poinar and Grewal, 2012). Specifically, *S. carpocapsae* and *H. bacteriophora* have relatively shorter body length (L) and diameter (D) (L: <600 µm, D: <25 µm), whereas *S. feltiae* is larger (L: >850 µm, D: >25 µm). To obtain populations of these EPNs, an *in vivo* method was employed, using the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), as the host. Each larva was inoculated with 100 IJs in a mixture of silver sand and then kept in incubation at 24 °C ± 1. Subsequently, around 250 000 fresh IJs were harvested from the White trap and placed

into a 50 ml falcon tube containing tap water. One-week-old IJs and nematode batches with 99% viability were used in the experiments.

2.2. Modified application method

A 16-liter battery-powered backpack sprayer was utilized as both the container and the application device (Figure 1). The battery powers the pump, creating pressure to facilitate the spraying process. The backpack sprayer's outlet was connected to the driplines using a hose clamp. Two distinct types of drippers were selected for the study: the on-line katif dripper (Rivulis Eurodrip, India) positioned above the dripline, and the in-line cylindrical dripper (MGF Irrigation, Turkey) positioned inside the dripline. The katif dripper is designed to be pressure-compensated (PC) and features a simplified and wide flow path without a labyrinth, as depicted in Figure 2A. Conversely, the cylindrical dripper incorporates a distinctive narrow flow path labyrinth, as illustrated in Figure 2B, but it is non-pressure-compensated (non-PC). These drippers have different flow rates, with the katif dripper having a flow rate of 12 l h^{-1} and the cylindrical dripper having a flow rate of 4 l h^{-1}. Both drippers are equipped with inlet filters that have pore sizes larger than 500 µm, ensuring easy passage for the IJs, as depicted in Figure 2. It's worth noting that both types of drippers have widespread usage on a global scale.

Figure 1. Modular application method. Battery-powered backpack sprayer was combined with different types of driplines. Main body (a), coupled with the dripline (b), coupling part (c) and battery (d).

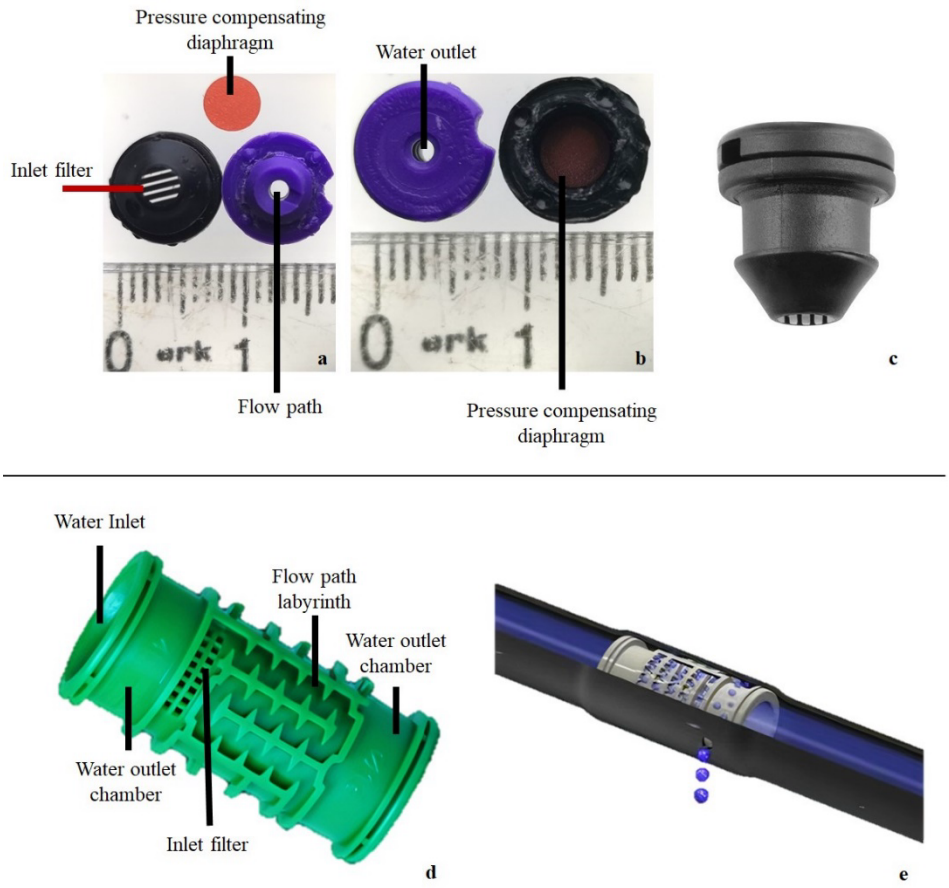

Figure 2. Illustrations of katif (upper part) and cylindrical (lower part) drippers. Parts of the disassembled katif dripper can be seen from the rear (a), above (b), and ready to use the katif dripper (c), the physical structure of the cylindrical dripper (d) and the water flow illustration of the cylindrical dripline (e).

The driplines featured consistent dimensions, with a diameter of 16 mm and a length of 10 meters. They were equipped with a total of 50 drippers, positioned at 20 cm intervals along the entire length of the dripline. Filters were omitted from the system as we relied on pre-filtered tap water for filling the sprayer tank. The backpack sprayer had an adjustable outlet pressure that allowed for pressure settings of up to 2 bars. Nonetheless, all applications were executed under a consistent 1-bar pressure setting, which was ideal for the non-pressure-compensating (non-PC) cylindrical dripper in use. Pressure within the system was measured using a pressure gauge filled with glycerin. Each application was systematically named by combining the initials of the EPN species and the specific dripper type. For example, "Hb Katif" served as the nomenclature for the application of *Heterorhabditis bacteriophora* via a katif-type dripper.

2.3. Evaluation of the drippers

The initial experiment aimed to assess the suitability of drippers for EPN application. The first step involved examining the uniformity of water discharge from the drippers prior to EPN applications. A 10-meter-long dripline was extended between two iron fences, suspended approximately 20 cm above the ground. A total of 300 ml glass containers were positioned beneath each dripper. The process began by filling the driplines with tap water to ensure uniformity. Subsequently, 5 liters of tap water were passed through the dripline, and the water discharge from all drippers was measured and recorded. In the second experiment, a solution of 250 000 IJs in 5 liters of tap water was administered to the glass containers, positioned under every 5th dripper (under the 1st, 5th, 10th, and so forth, up to the 50th dripper). Prior to the EPN application, the driplines were filled with tap water. Following the application, the number of IJs and their mortality rate within each container were quantified, allowing for the

determination of the total discharged IJs per container. This process was duplicated for both the driplines and the specific EPN species under examination. Each experiment was replicated three times to ensure accuracy and reliability.

2.4. Pot application

Plastic seedling pots with a diameter of 9 cm and a height of 12 cm (approximately 750 ml) were employed to evaluate the application's effectiveness. A mixture of sterilized silver sand and sandy soil at a 1:1 ratio was used to fill the pots halfway. Four caged *G. mellonella* larvae were positioned in the center of each pot, and the remaining space was filled with the same soil mixture. The moisture content of the pots was adjusted to 10% (w/w) using tap water after the application. Six pots were prepared and positioned under the 1st, 10th, 20th, 30th, 40th, and 50th drippers (one pot for every 10 drippers). This setup allowed for an assessment of nematode distribution through the dripline. Similar to previous experiments, the driplines were filled with tap water, and a suspension of 250 000 IJs in 5 liters was applied. Subsequently, the pots were placed in a dark climate chamber maintained at 25 °C ± 1, with 70% relative humidity for incubation. Four days post-incubation, dissection of the deceased larvae confirmed EPN infection. As a positive control, the same amount of IJs per pot was administered using a Pasteur pipette. In contrast, tap water was utilized for drip irrigation as a negative control. The application dose closely approximated the commercial dose of 50 IJs cm^{-2}. Each experiment was conducted three times.

2.5. Field application

Field experiments were conducted on a 1000 m^2 research plot. Prior to the application, the soil was tested for EPN abundance. For this purpose, soil samples from the field were analyzed using the insect bait method, and no EPNs were detected. The field received daily irrigation before the application. The same procedure used in the pot experiments was followed for the field applications. Initially, the driplines were filled with tap water, and 250 000 IJs were applied with 5 liters of water. After 24 hours, three different drippers along the dripline were randomly selected, and four caged *G. mellonella* larvae were buried at a 20 cm depth in the soil under each selected dripper. The buried larvae remained in the soil for 5 days without additional irrigation. Subsequently, the mesh cages were removed from the soil, and dead larvae were dissected to confirm EPN infection. The same amount of IJs per dripper was applied with a Pasteur pipette as a positive control, while tap water served as the negative control. Each experiment was replicated four times, and there was a 2 m buffer zone between replicates. An untreated plot was used for each replicate. Field applications were carried out at sunset, and the average soil temperature was 26 °C.

2.6. Data analysis

Statistical analyses were conducted using GraphPad Prism v9.4 software. Prior to analysis, all data underwent a normality check using the Shapiro–Wilk test. One-way ANOVA was performed to evaluate the results. Since positive control groups were included, Dunnett's multiple comparison post hoc test and Bonferroni correction were used to determine the significance between larval mortalities in pot and field trials, respectively. Tukey's HSD post-hoc test was employed to compare the means of the IJ discharge data. Additionally, correlation analysis was performed to evaluate the relation between discharged water volume and IJ numbers. All analyses were conducted with a significance level set at $p<0.05$.

3. Results

3.1. Evaluation of drippers

At a consistent pressure of 1 bar during the experiments, the water discharge rates through the drippers remained uniform (Figure 3). Upon evaluating the drippers' performance, it became evident that the katif dripper was notably superior and better suited for EPN application [$F(5, 60) = 18.54$; $p<0.001$]. The cylindrical dripper, on the other hand, consistently impeded IJ discharge for all EPN species (Figure 4). Despite the declining trends and variations in IJ discharge from the drippers located

further along the line (Figure 5), the distribution of IJs throughout the dripline had no impact on larval mortality. The IJ population's mortality remained below 1% both before and after application, signifying that the drippers did not adversely affect IJ survival.

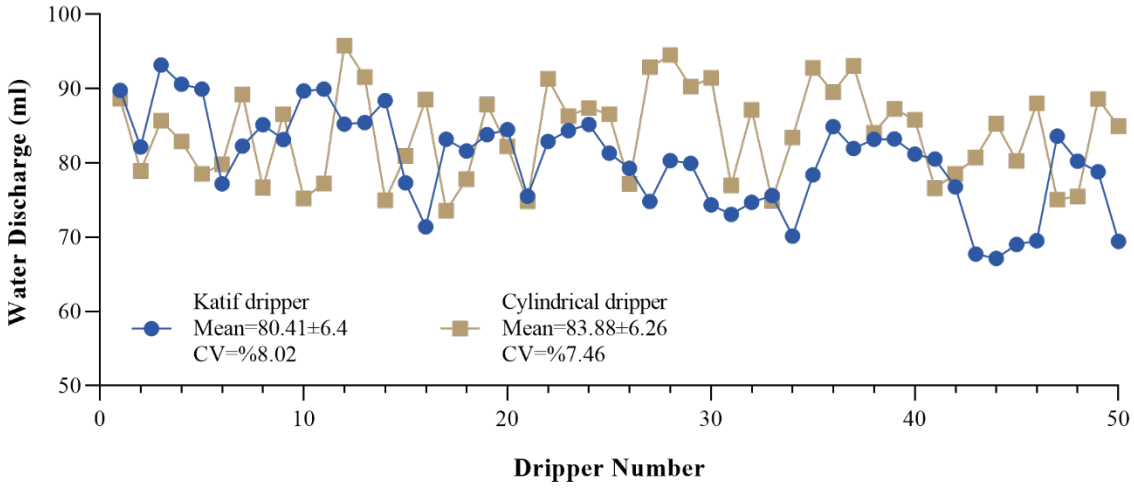

Figure 3. Water discharge of katif and cylindrical drippers from all 50 drippers throughout the driplines.

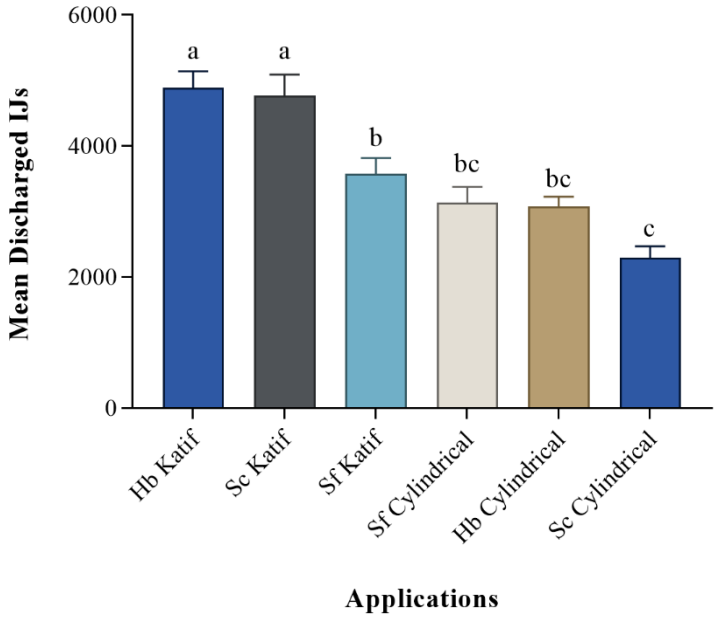

Figure 4. Comparison of mean discharged IJs of different species and dripper types. *H. bacteriophopra* (Hb) and *S. carpocapsae* (Sc) Katif dripper applications significantly differ from other applications. Different letters indicate significant differences according to Tukey's HSD test ($p<0.05$). Error bars represent standard error mean.

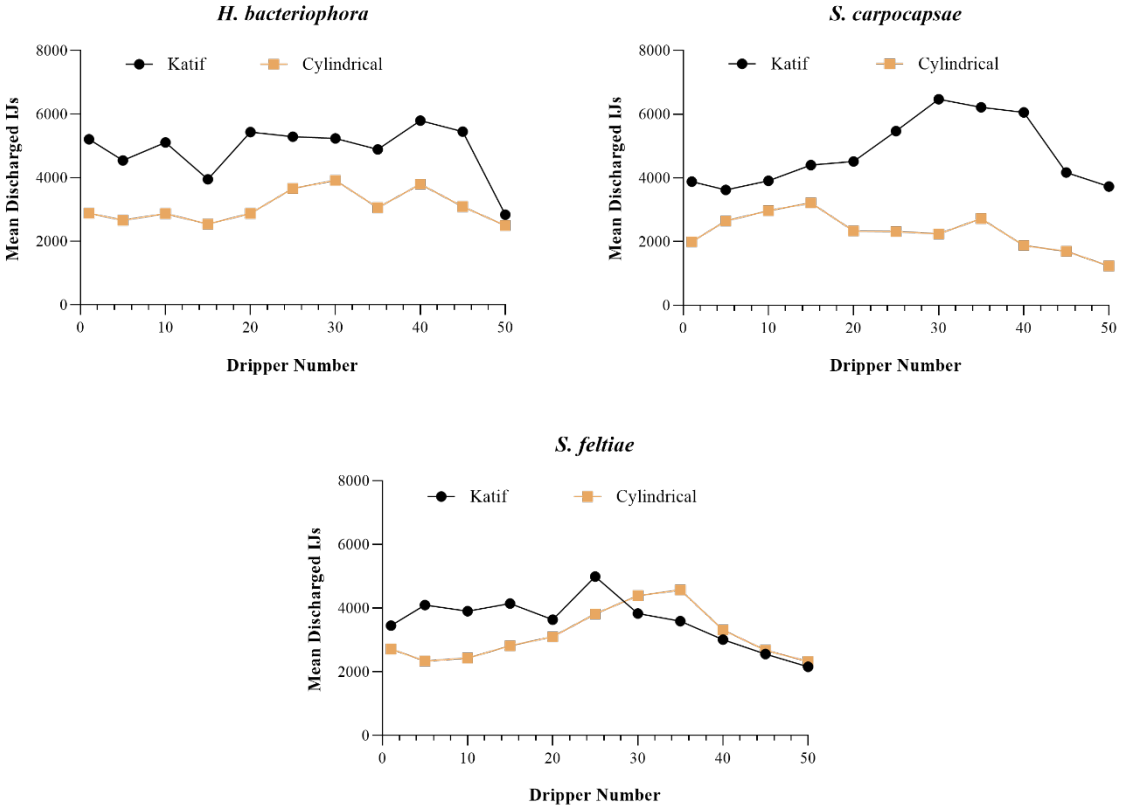

Figure 5. Discharged IJs throughout the driplines.

The correlation analysis revealed that the volume of water and IJ discharge from the drippers were unrelated. Correlation coefficients exhibited low values in nearly all applications and were determined to be statistically insignificant (Table 1). The sole exception was the Sc Katif application, which displayed a strong correlation.

Table 1. Correlation coefficient (r) and p-values (p) between water discharge (X) and IJ discharge of all applications (Y)

Water Discharge ***X***	**Hb Katif** ***Y1***	**Hb Cylindrical** ***Y2***	**Sc Katif** ***Y3***	**Sc Cylindrical** ***Y4***	**Sf Katif** ***Y5***	**Sf Cylindrical** ***Y6***
r	0.085	-0.072	0.810	0.419	-0.144	-0.270
p	0.804	0.834	0.0025	0.199	0.673	0.423

3.2. Pot applications

In all experiments, whether through dripper or positive control applications, all larvae within the pots were dead (Figure 6). The application dosage per pot was approximately 5000 IJs, slightly above the commercial dose (50 IJs cm^{-2}). A few larvae in the negative control group died due to excessive water. Notably, all applications exhibited significantly greater efficacy than the negative control, and there were no discernible distinctions between the applications and the positive control [$F(7, 40) = 3088.41$; $p<0.001$].

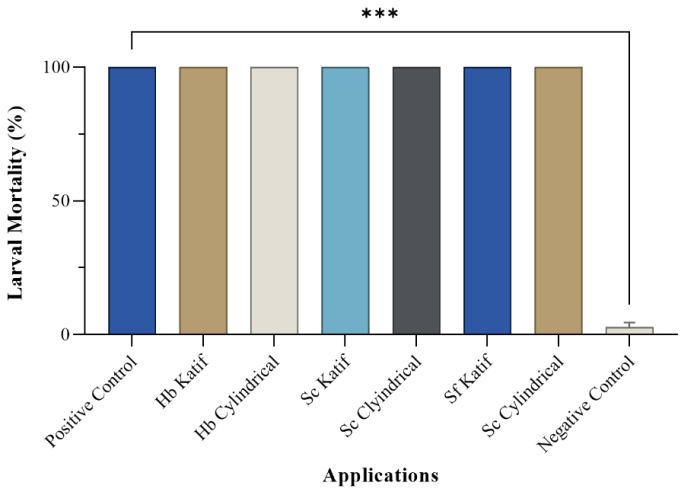

Figure 6. Larval mortality among pot applications. All applications except negative control remain significantly the same according to Dunnet's multiple comparisons post hoc test (p<0.05) (*** indicates p < 0.001). The error bar represents the standard error mean.

3.3. Field Applications

Field application results exhibited a notable reduction in larval mortality in contrast to pot applications, a result that was anticipated. However, some applications could be regarded as less effective. When compared to the positive control, all katif applications displayed notably similar levels of larval mortality (Figure 7). In contrast, larval mortality in all cylindrical applications remained below 20% [F(7, 16) = 34.26; p<0.001].

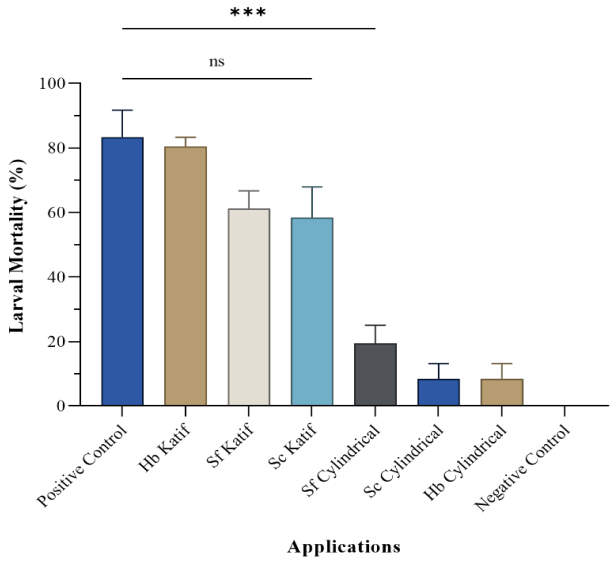

Figure 7. Comparison of the larval mortality in field applications. All katif applications showed no significance compared to positive control according to Bonferroni multiple comparisons post hoc test (p<0.05) (*** indicates p<0.001, ns: not significant). Error bars represent standard error mean.

4. Discussion

In this study, a portable method was used to perform the application of EPNs under field conditions. Instead of using an irrigation system, the driplines were connected to a battery-powered backpack sprayer. The aim was to provide a more precise, controlled, and modular way to apply EPNs.

The results of this study are promising and demonstrate that this portable technique can be used in EPN applications. We also determined that the two drippers with different physical structures had a significant impact on the EPN application. In field conditions, the larval mortality rate was higher in the katif dripper, while it remained low in the cylindrical dripper. Many studies have been conducted on the application of EPNs in drip irrigation (Curran and Patel, 1988; Reding et al., 2004; Arrington et al., 2016). However, few studies have examined the effects of drippers on EPN application (Erdoğan et al., 2020). In fact, we believe that in some studies comparing EPN efficacy with chemical pesticides, EPNs failed due to unsuitable dripper selection (Arrington et al., 2016). Our results indicate that the dripper type should be considered in EPN applications.

Three common EPNs species were used in this study. We aimed to determine whether the body size and foraging strategies of these species affect larval mortality. First, powder formulations were used in the initial tests to make the results of the study more realistic, but we had to produce IJs ourselves because of the difficulty in supplying commercial products. Wax moth larvae were used to evaluate the pathogenicity as they are easy to produce and susceptible to EPNs (Moreira et al., 2013). Similarly, we used wax moth larvae to determine the efficacy of the applications (Brusselman et al., 2012a; Dito et al., 2016). There were no differences between pot experiments (Figure 6). All larvae died because of the small pot volume, despite applying the recommended field dose (~50 IJ cm^{-2}). However, the results of the field trials have varied. The first notable result was that *S. feltiae* had a low discharge rate, even in the katif dripper (Figure 4). *S. feltiae* had the longest body length (~850µm) among the EPNs used (*S. carpocapsae* and *H. bacteriophora* had less than 600µm). Although the katif dripper does not have any flow labyrinth, we believe that the IJs of *S. feltiae* get stuck while passing through the dripper because of its long body (unpublished data). As expected, the field efficacies were in harmony with the discharged IJ amount (Figure 7).

EPNs have been applied in many spray and fertilizing equipment and irrigation systems, such as soil injectors, boom sprayers, hand sprayers, and spinning discs (Mason et al., 1998; Lara et al., 2008; Morton and García del Pino, 2008; Raja et al., 2015). Although nematodes can be applied to the soil using these methods, there are problems, such as the inability to adjust the nematode dose, lack of homogeneous distribution, and low application efficacy. The Dosatron injector is another popular technique for EPN applications. However, the EPNs tend to settle in the water; therefore, they must be mixed continuously in the tank (where Dosatron pulls up the EPN suspension) and during the application, which requires an additional power source. A Dosatron also contains moving parts that can damage EPNs. These problems mentioned above showed that drip irrigation (or fertigation) is the most suitable method and is recommended by commercial EPN producers (Wennemann et al., 2003; Wang et al., 2009; Arrington et al., 2016; Erdoğan et al., 2020). Although drip irrigation is the most preferred method, it also has problems in EPN application, as mentioned above (Conner et al., 1998).

Consequently, it is known that fewer EPNs emerge from further drippers in long driplines (Cabanillas and Raulston, 1996; Wennemann et al., 2003). EPNs are distributed more homogeneously throughout the dripline in high-flow drip irrigation systems. However, at the same time, these EPNs must also leave the system and reach the target for a successful application. We used katif and cylindrical drippers, which have completely different physical structures (Figure 2). While the katif dripper drips water in the dripline without hindrance, the cylindrical dripper drips water by passing it through a long flow labyrinth. According to the study results, the katif dripper statistically outperformed the cylindrical dripper in terms of both nematode discharge and larval mortality. Although the first thing that comes to mind is that the nematodes were trapped in the cylindrical dripper (inside the flow labyrinth), however, the pipes were split in half after experiments, and nematodes were also found inside the dripline. That means the flow rate of the dripper also affects the nematode discharge. EPNs are carried by water when they exit the irrigation system. For successful application, drippers must allow IJs to pass through the flow path and exit from the irrigation system. Although the water and IJ discharge from the drippers are closely related, it cannot simply be said that they are related to each other. As a matter of fact, correlation analysis between water and IJ discharge showed that there is no significant relation. Pressure-compensated drippers mostly have a uniformly distributed water discharge. However, non-PC drippers may have fluctuating data. Because we carried out all the experiments at constant pressure, the water discharge between the drippers did not vary significantly (Figure 3). However, using non-PC drippers may lead to an uneven distribution of EPN throughout the field. In addition to the physical properties of the dripper, pressure compensation increases the homogeneity and success of its application.

Many methods have been used to increase the success of EPNs. While EPNs are only applied against belowground pests, they can also be applied to aboveground parts owing to various supplementary chemicals and new-generation formulations (Shapiro-Ilan et al., 2012; Platt et al., 2018, 2020). The efficiency of EPNs can be increased by using spreader-adhesive chemicals during their application (Portman et al., 2016). It is also possible to increase its efficacy by changing the EPN behavior in the soil. For example, in a study conducted by Oliveira-Hofman et al. (2019), it was observed that the behavior of EPNs was changed by using pheromones. Although *S. carpocapsae* is an ambusher, its dispersal increases after pheromone application (Kaplan et al., 2020). Customized applications of EPNs can be developed to replace conventional systems. For instance, Erdoğan et al. (2021) designed a robotic system to apply IJs to the desired location. In their work, a customized mixing method was developed for EPNs, and a new peristaltic pump was used that did not damage EPNs during application. In another set of studies, pressure and nozzle types suitable for EPN application were researched, and the appropriate nozzle types were determined (Brusselman et al., 2011, 2012b). Furthermore, Erdoğan et al. (2020) investigated suitable dripper types for EPNs and found a significant difference in application success among different drippers, in parallel with this study. These studies showed that the efficacy of EPNs can be increased during and after application. Improvements at many stages, from production to field application of EPNs, will make significant contributions to environmental pest control in the future.

Although the use of pesticides is inevitable in agricultural production, the use of biological products is increasing (Çelik et al., 2023). EPNs are natural and safe alternatives to chemical control. The most critical disadvantage of EPNs is that their commercial formulations are expensive (Dunn et al., 2021). Other factors also reduce the effectiveness of EPNs under field conditions. Many microorganisms, natural enemies, stimulants, and odors in the soil affect the behavior of EPNs and reduce their success. As with other biological control agents, EPNs must be able to compete with pesticides. To make EPNs more attractive, products must be cheaper, more effective, and easier to apply. In addition to reducing production costs, studies are also carried out to increase the efficiency in field conditions and to increase the success of EPNs with new application methods (Wright et al., 2005; Beck et al., 2013; Kapranas et al., 2017; Dunn et al., 2020).

In summary, the study's objective was to assess a portable method applicable to various scales and product patterns. The modular approach allows adaptation to longer dripline configurations, different dripper types, microjets, or nozzles for specific target pests or crops. It was highlighted that the use of appropriate drippers is crucial for successful field applications. Additional research is essential to refine the effective application of EPNs in field conditions. The aspiration is that refined application methods will enhance the future efficacy of EPNs.

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References

Arrington, A. E., Kennedy, G. G., & Abney, M. R. (2016). Applying insecticides through drip irrigation to reduce wireworm (Coleoptera: Elateridae) feeding damage in sweet potato. *Pest Management Science*, *72*(6), 1133–1140. https://doi.org/10.1002/ps.4089

Askar, A. G., Yüksel, E., Bozbuğa, R., Öcal, A., Kütük, H., Dinçer, D., Canhilal, R., Dababat, A. A., & İmren, M. (2023). Evaluation of Entomopathogenic Nematodes against Common Wireworm Species in Potato Cultivation. *Pathogens*, *12*(2), Article 2. https://doi.org/10.3390/pathogens12020288

Beck, B., Brusselman, E., Nuyttens, D., Moens, M., Pollet, S., Temmerman, F., & Spanoghe, P. (2013). Improving foliar applications of entomopathogenic nematodes by selecting adjuvants and spray nozzles. *Biocontrol Science and Technology*, *23*(5), 507–520. https://doi.org/10.1080/09583157.2013.777692

Brusselman, E., Beck, B., Pollet, S., Temmerman, F., Spanoghe, P., Moens, M., & Nuyttens, D. (2012a). Effect of spray volume on the deposition, viability and infectivity of entomopathogenic nematodes in a foliar spray on vegetables. *Pest Management Science*, *68*(10), 1413–1418. https://doi.org/10.1002/ps.3325

Brusselman, E., Beck, B., Pollet, S., Temmerman, F., Spanoghe, P., Moens, M., & Nuyttens, D. (2012b). Effect of the spray application technique on the deposition of entomopathogenic nematodes in vegetables. *Pest Management Science*, *68*(3), 444–453. https://doi.org/10.1002/ps.2290

Brusselman, E., Beck, B., Temmerman, F., Pollet, S., Steurbaut, W., Moens, M., & Nuyttens, D. (2011). Distribution of entomopathogenic nematodes in a biopesticide spray. *Transactions of the ASABE*, *54*(6), 1981–1989. https://doi.org/10.13031/2013.40646

Cabanillas, H. E., & Raulston, J. R. (1996). Effects of furrow irrigation on the distribution and infectivity of *Steinernema riobravis* against corn earworm in corn. *Fundamental and Applied Nematology*, *19*(3), 273–281.

Campos-Herrera, R. (2015). Nematode pathogenesis of insects and other pests: Ecology and applied technologies for sustainable plant and crop protection. In R. Campos-Herrera (Ed.), *Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection* (pp. 231–254). Springer International Publishing. https://doi.org/10.1007/978-3-319-18266-7

Conner, J. M., McSorley, R., Stansly, P. A., & Pitts, D. J. (1998). Delivery of *Steinernema riobravis* through a drip irrigation system. *Nematropica*, *28*(1), 95–100.

Curran, J., & Patel, V. (1988). Use of a trickle irrigation system to distribute entomopathogenic nematodes (Nematoda: Heterorhabditidae) for the control of weevil pests (Coleoptera: Curculionidae) of strawberries. *Australian Journal of Experimental Agriculture*, *28*(5), 639–643. https://doi.org/10.1071/EA9880639

Çelik, S., Tozlu, G., & Kotan, R. (2023). The Investigation of Effect of Bacteria in Biological Control of Red Spider Mite (Tetranychus spp.) and Plant Yield Parameter in Cotton (Gossypium hirsutum L.). *Yuzuncu Yil University Journal of Agricultural Sciences*, *33*(4), 689–699. https://doi.org/10.29133/yyutbd.1319995

Dede, E., Bütüner, A. K., & Susurluk, A. (2023). Biocontrol potential of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain against the beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae). *Turkish Journal of Entomology*, *46*(4), Article 4. https://doi.org/10.16970/entoted.1162125

Devi, G. (2018). Mass production of entomopathogenic nematodes—A review. *International Journal of Environment, Agriculture and Biotechnology*, *3*(3), 1032–1043. https://doi.org/10.22161/ijeab/3.3.41

Dito, D. F., Shapiro-Ilan, D. I., Dunlap, C. A., Behle, R. W., & Lewis, E. E. (2016). Enhanced biological control potential of the entomopathogenic nematode, *Steinernema carpocapsae*, applied with a protective gel formulation. *Biocontrol Science and Technology*, *26*(6), 835–848. https://doi.org/10.1080/09583157.2016.1159659

Dunn, M. D., Belur, P. D., & Malan, A. P. (2020). In vitro liquid culture and optimization of *Steinernema jeffreyense* using shake flasks. *BioControl*, *65*(2), 223–233. https://doi.org/10.1007/s10526-019-09977-7

Dunn, M. D., Belur, P. D., & Malan, A. P. (2021). A review of the in vitro liquid mass culture of entomopathogenic nematodes. *Biocontrol Science and Technology*, *31*(1), 1–21. https://doi.org/10.1080/09583157.2020.1837072

Erdoğan, H., Ulu, T. C., & Kuşçu, H. (2020). The effect of different dripper properties on entomopathogenic nematode application in drip irrigation. *KSU Journal of Agriculture and Nature*, *23*(1), 230–236. https://doi.org/10.18016/ksutarimdoga.vi.533723

Erdoğan, H., Ünal, H., & Lewis, E. E. (2021). Entomopathogenic nematode dispensing robot: NEMABOT. *Expert Systems with Applications*, *172*(February), 114661. https://doi.org/10.1016/j.eswa.2021.114661

Erdoğan, H., Ünal, H., Susurluk, İ. A., & Lewis, E. E. (2023). Precision application of the entomopathogenic nematode *Heterorhabditis bacteriophora* as a biological control agent through the Nemabot. *Crop Protection*, *174*, 106429. https://doi.org/10.1016/j.cropro.2023.106429

Garcia, L. C., Raetano, C. G., & Leite, L. G. (2008). Application technology for the entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* sp. (Rhabditida: Heterorhabditidae and Steinernematidae) to control *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in corn. *Neotropical Entomology*, *37*(3), 305–311. https://doi.org/10.1590/S1519-566X2008000300010

Gaugler, R. (2002). Entomopathogenic nematology. In R. Gaugler (Ed.), *Entomopathogenic nematology*. CABI. https://doi.org/10.1079/9780851995670.0000

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Köhl, J., Marrone, P., Morin, L., & Stewart, A. (2012). Have biopesticides come of age? *Trends in Biotechnology*, *30*(5), 250–258. https://doi.org/10.1016/j.tibtech.2012.01.003

Grewal, P. S. (2000). Enhanced ambient storage stability of an entomopathogenic nematode through anhydrobiosis. *Pest Management Science*, *56*(5), 401–406. https://doi.org/10.1002/(SICI)1526-4998(200005)56:5<401::AID-PS137>3.0.CO;2-4

Guy, A., Gaffney, M., Kapranas, A., & Griffin, C. T. (2017). Conditioning the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis megidis* by pre-application storage improves efficacy against black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) at low and moderate temperatures. *Biological Control*, *108*, 40–46. https://doi.org/10.1016/j.biocontrol.2017.02.005

Jaffuel, G., Imperiali, N., Shelby, K., Campos-Herrera, R., Geisert, R., Maurhofer, M., Loper, J., Keel, C., Turlings, T. C. J., & Hibbard, B. E. (2019). Protecting maize from rootworm damage with the combined application of arbuscular mycorrhizal fungi, *Pseudomonas* bacteria and entomopathogenic nematodes. *Scientific Reports*, *9*(1), 3127. https://doi.org/10.1038/s41598-019-39753-7

Kagimu, N., & Malan, A. P. (2019). Formulation of South African entomopathogenic nematodes using alginate beads and diatomaceous earth. *BioControl*, *64*(4), 413–422. https://doi.org/10.1007/s10526-019-09945-1

Kaplan, F., Perret-Gentil, A., Giurintano, J., Stevens, G., Erdogan, H., Schiller, K. C., Mirti, A., Sampson, E., Torres, C., Sun, J., Lewis, E. E., & Shapiro-Ilan, D. (2020). Conspecific and heterospecific pheromones stimulate dispersal of entomopathogenic nematodes during quiescence. *Scientific Reports*, *10*(1), 5738. https://doi.org/10.1038/s41598-020-62817-y

Kapranas, A., Malone, B., Quinn, S., O'Tuama, P., Peters, A., & Griffin, C. T. (2017). Optimizing the application method of entomopathogenic nematode suspension for biological control of large pine weevil *Hylobius abietis*. *BioControl*, *62*(5), 659–667. https://doi.org/10.1007/s10526-017-9824-x

Kaya, H. K., & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology*, *38*(1), 181–206. https://doi.org/10.1146/annurev.en.38.010193.001145

Łaczyński, A., Dierickx, W., & De Moor, A. (2007). The effect of agitation system, temperature of the spray liquid, nematode concentration, and air injection on the viability of *Heterorhabditis bacteriophora*. *Biocontrol Science and Technology*, *17*(8), 841–851. https://doi.org/10.1080/09583150701527474

Lara, J. C., Dolinski, C., De Sousa, E. F., & Daher, R. F. (2008). Effect of mini-sprinkler irrigation system on *Heterorhabditis baujardi* LPP7 (Nematoda: Heterorhabditidae) infective juvenile. *Scientia Agricola*, *65*(4), 433–437. https://doi.org/10.1590/S0103-90162008000400017

Mason, J. M., Matthews, G. A., & Wright, D. J. (1998). Appraisal of spinning disc technology for the application of entomopathogenic nematodes. *Crop Protection*, *17*(5), 453–461. https://doi.org/10.1016/S0261-2194(98)00042-8

Moreira, G. F., Batista, E. S. de P., Campos, H. B. N., Lemos, R. E., & Ferreira, M. da C. (2013). Spray nozzles, pressures, additives and stirring time on viability and pathogenicity of entomopathogenic nematodes (Nematoda: Rhabditida) for greenhouses. *PLoS ONE*, *8*(6), e65759. https://doi.org/10.1371/journal.pone.0065759

Morton, A., & García del Pino, F. (2008). Field efficacy of the entomopathogenic nematode *Steinernema feltiae* against the Mediterranean flat-headed rootborer *Capnodis tenebrionis*. *Journal of Applied Entomology*, *132*(8), 632–637. https://doi.org/10.1111/j.1439-0418.2008.01300.x

Mukuka, J., Strauch, O., Hoppe, C., & Ehlers, R. U. (2010). Improvement of heat and desiccation tolerance in *Heterorhabditis bacteriophora* through cross-breeding of tolerant strains and

successive genetic selection. *BioControl*, *55*(4), 511–521. https://doi.org/10.1007/s10526-010-9271-4

Nxitywa, A., & Malan, A. P. (2021). Formulation of entomopathogenic nematodes for the control of key pests of grapevine: A review. *South African Journal of Enology and Viticulture*, *42*(2), 123–135. https://doi.org/10.21548/42-2-4479

Oliveira-Hofman, C., Kaplan, F., Stevens, G., Lewis, E., Wu, S., Alborn, H. T., Perret-Gentil, A., & Shapiro-Ilan, D. I. (2019). Pheromone extracts act as boosters for entomopathogenic nematodes efficacy. *Journal of Invertebrate Pathology*, *164*, 38–42. https://doi.org/10.1016/j.jip.2019.04.008

Olson, S. (2015). An analysis of the biopesticide market now and where it is going. *Outlooks on Pest Management*, *26*(5), 203–206. https://doi.org/10.1564/v26_oct_04

Platt, T., Stokwe, N. F., & Malan, A. P. (2018). Potential of local entomopathogenic nematodes for control of the vine mealybug, *Planococcus ficus*. *South African Journal of Enology and Viticulture*, *39*(2), 208–215. https://doi.org/10.21548/39-2-3158

Platt, T., Stokwe, N. F., & Malan, A. P. (2020). A review of the potential use of entomopathogenic nematodes to control above-ground insect pests in South Africa. *South African Journal of Enology and Viticulture*, *41*(1), 1–16. https://doi.org/10.21548/41-1-2424

Poinar, G. O., & Grewal, P. (2012). History of entomopathogenic nematology. *Journal of Nematology*, *44*(2), 153–161.

Portman, S. L., Krishnankutty, S. M., & Reddy, G. V. P. (2016). Entomopathogenic nematodes combined with adjuvants presents a new potential biological control method for managing the wheat stem sawfly, *Cephus cinctus* (Hymenoptera: Cephidae). *PLoS ONE*, *11*(12), e0169022. https://doi.org/10.1371/journal.pone.0169022

Raja, R. K., Hazir, C., Gümüş, A., Asan, C., Karagöz, M., & Hazir, S. (2015). Efficacy of the entomopathogenic nematode *Heterorhabditis bacteriophora* using different application methods in the presence or absence of a natural enemy. *Turkish Journal of Agriculture and Forestry*, *39*(2), 277–285. https://doi.org/10.3906/tar-1410-33

Reding, M. E., Zhu, H., & Derksen, R. (2004). Drip irrigation as a delivery system for imidacloprid and nematodes for control of scarab grubs in nursery crops. In *USDA-Agricultural Research Service* (p. 44691).

Şahin, Y. S., Bouhari, A., Ulu, T. C., Sadıç, B., & Susurluk, İ. A. (2018). New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH strain against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae). *Turkish Journal of Entomology*, *42*(4), 305–312. https://doi.org/10.16970/ENTOTED.471095

Shapiro-Ilan, D. I., Han, R., & Dolinksi, C. (2012). Entomopathogenic nematode production and application technology. *Journal of Nematology*, *44*(2), 206–217.

Singh, A., & Upadhyay, V. (2018). A review on entomopathogenic nematodes: *Heterorhabditis* and *Steinernema*. *Advances in Bioresearch*, *9*(2), 214–222. https://doi.org/10.15515/abr.0976-4585.9.2.214222

Ulu, T. C., & Susurluk, A. (2021). Optimization of in vitro solid culture of Heterorhabditis bacteriophora Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain. *Turkish Journal of Entomology*, *45*(4), 441–449. https://doi.org/10.16970/entoted.1004749

Wang, X., Zhu, H., Reding, M. E., Locke, J. C., Leland, J. E., Derksen, R. C., Spongberg, A. L., & Krause, C. R. (2009). Delivery of chemical and microbial pesticides through drip irrigation systems. *Applied Engineering in Agriculture*, *25*(6), 883–893. https://doi.org/10.13031/2013.29242

Wennemann, L., Cone, W. W., Wright, L. C., Perez, J., & Conant, M. M. (2003). Distribution patterns of entomopathogenic nematodes applied through drip irrigation systems. *Journal of Economic Entomology*, *96*(2), 287–291. https://doi.org/10.1093/jee/96.2.287

Wright, D. J., Peters, A., Schroer, S., & Fife, J. (2005). Application technology. In P. S. Grewal, R. U. Ehlers, & D. I. Shapiro-Ilan (Eds.), *Nematodes as Biocontrol Agents* (pp. 91–106). CABI. https://doi.org/10.1079/9780851990170.0091