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Field application of encapsulated entomopathogenic nematodes using a precision planter

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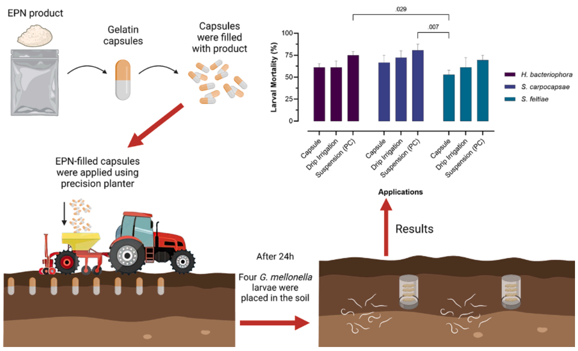
HIGHLIGHTS

- A novel entomopathogenic nematode application method has been investigated.
- Gelatin capsules were used as a container for powdered formulation.
- Encapsulated biological agents are released to the field with a precision planter.
- Delivery via capsule or drip irrigation is equally effective in field trials

GRAPHICAL ABSTRACT



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ABSTRACT

The use of entomopathogenic nematodes (EPNs) as a biological control agent in agriculture has shown efficacy against various soil-dwelling pests. Despite its potential, high production costs and inconsistent field efficiency remain significant challenges. Although EPNs can be applied using irrigation systems and spraying equipment, optimized applications are required. This study aimed to evaluate the feasibility of applying EPNs in gelatin capsules and planting with a precision planter. It was hypothesized that this method would lead to more controlled and uniform EPN application. The effects of EPN encapsulation on dispersal and field persistence in the soil were also investigated. Larval mortality for capsule applications was between 53 and 67% under field conditions, with no statistical difference compared to the drip irrigation applications. Dispersal trials were carried out using custom steel olfactometers, and capsule application did not have any adverse effects on the dispersal of infective juveniles for 24, 48 and 72 h. Persistence trials revealed no significant differences between the capsule and control groups, with a maximum persistence of 50 days. The results suggest that the capsule technique could be a promising option for large-scale EPN applications, and further optimization may lead to improved results.

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1. Introduction

Entomopathogenic nematodes (EPNs) are roundworms that kill insects and are used for biological control in pest management. Infective juveniles (IJs) are the only stage of EPNs outside the host, and can penetrate the host through the mouth, anus, and spiracles (Koppenhöfer et al., 2020). IJs kill the host with the help of symbiotic bacteria in the gut (Ciche et al., 2006). The bacterium converts insects into a food source for IJs using the enzymes it secretes as it reproduces. IJs consume the host for several generations and then migrate to soil to find new hosts. EPNs can be mass-produced in large-scale fermenters, and are effective against several important agricultural pests (Devi, 2018; Shapiro-Ilan et al., 2012). EPNs can be used in integrated pest control programs because they have minimal effects on nontarget organisms and are environmentally safe (Abate et al., 2017; De Nardo et al., 2006).

Several methods are available for the application of EPNs. They can resist high pressure and shear stress; thus, they can be applied using standard spraying equipment (Anifantis et al., 2020; Fife et al., 2003; Moreira et al., 2013). The easiest and most reliable spray methods are the backpack and boom sprayers (Gan-Mor and Matthews, 2003; Garcia et al., 2008). Another method for applying EPNs is via irrigation. Although furrow irrigation and mini-sprinkles are used, the most common method is drip irrigation (Campos-Herrera et al., 2022; Conner et al., 1998; Lara et al., 2008). They can be applied using drip irrigation alone or in combination with chemicals, similarly to fertigation or chemigation (Reding et al., 2008). EPNs can also be applied in a granular form or with various fertilization equipment (Cortés-Martínez et al., 2017; Mason et al., 1998). Successful applications have also been made using syringe, serum bottle or pre-infected larvae (Gumus et al., 2015; Raja et al., 2015). Although EPNs have long been applied only to belowground pests, they can also be applied above soil using new formulation techniques and additive chemicals. With the help of spreaders, adhesives, and UV protectors, successful applications against foliar pests has been reported (Mazurkiewicz et al., 2021; Platt et al., 2020; Van Niekerk and Malan, 2015). Recently, the efficacy of EPNs has increased with the development of alginate and gel formulations (Ebrahimi, 2022; Fallet et al., 2022; NanGong et al., 2021). Lastly, drone technologies have been introduced in EPN applications (Berner and Chojnacki, 2017).

Although EPNs can be applied using many methods described above, they are limited in practice. The homogeneous field application of EPNs remains a major obstacle. The major problem with drip irrigation is the risk of an uneven distribution along the dripline and the loss of IJs in driplines (Wang et al., 2009; Wennemann et al., 2003). In addition, dripper type significantly affects the discharge of EPNs from the irrigation system (Erdoğan et al., 2020). Due to the variety of dripper types available, it is not always possible to find the optimal conditions for EPNs (Wright et al., 2005). Moreover, the water in the dripline can heat up and cause EPNs to die in low-flow irrigation systems (Raja et al., 2015). Sprinkler systems can be used as alternatives to drip irrigation; however, spray applications cannot be used in every field, and additional irrigation is required before and after the application. Moreover, spraying nozzles and application technique need to be compatible with EPN application (Beck et al., 2013; Brusselman et al., 2012; Moreira et al., 2013). EPNs are applied using watering cans in gardens and greenhouses, however, this method is not suitable large fields. The lack of optimization in current methods available for EPN applications poses a risk of inefficient product utilization and increased application costs, which can result in economic losses and reduced efficacy in controlling insect pests. EPNs have inconsistent efficacy under field conditions, which strongly correlates with their application method. The application of EPNs has become increasingly problematic in larger fields. Therefore, alternative methods are required for EPN applications.

This study examined the field applications of EPNs in gelatin capsules using a precision planter. Capsules containing the commercial EPN powder formulation were planted in the soil. The potential of our method was tested and compared with common drip irrigation application. The effects of capsule application on EPN dispersal and field persistence were also examined. We aimed to focus on an alternative approach to the application of EPNs in larger fields. The most significant advantage of our method is that the capsules have a fixed dose of EPNs, which leads to a homogeneous, consistent and controlled application throughout the field. Thus, less product can be used in a larger area than in conventional application methods. We also hypothesized that gelatin capsules can protect IJs against soil scavengers, and help IJs to skip initial penetration process.

2. Materials and methods

2.1. Studied organisms

The present study utilized commercial strains of three entomopathogenic nematodes, *Heterorhabditis bacteriophora* (Nematop®), *Steinernema carpocapsae* (Nemastar®), and *S. feltiae* (Nemaplus®), which possess varying foraging methods and body sizes. Commercial EPN products of E-nema GmbH (Raisdorf, Germany) were purchased from a local distributor (Bioglobal Bio-Solutions). It is important to note that these products have a shelf life of about 40 days and must be kept within the recommended temperature range (E-nema product info). Fresh products were obtained immediately before the trials and kept at 4 °C until application. The mortality rate of the nematodes in the products was evaluated prior to their application (<1%). The larvae of the great wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), was used as a test insect for laboratory and field efficacy trials. These larvae were reared in the laboratory according to the method described by Kaya and Stock (1997).

2.2. Field preparation

Field trials were conducted during the summer of 2021. A rectangular land of approximately 30 m × 60 m was located at 40°13′49.8″ N 28°51′37.1″ E. Before the trials, the land was plowed with a turn plow and tilled over with a disc harrow. Due to rainy days in the summer, weeds were present in the field. To avoid any impact from herbicide application on the trials, the area was plowed with a rotary cultivator one week prior to the start of the trials, effectively removing the weeds and ensuring an even soil surface. The trials were conducted during mid-summer, and the soil was watered intermittently for one week to maintain a suitable temperature and moisture level. During the trials, the soil temperature was recorded as 26 °C. For each experiment, 3 m × 60 m plots were created. Each plot was counted as a replicate and a 1-meter buffer zone was left between the plots. Soil samples were collected from the field prior to the experiments, and no presence of EPN was detected.

2.3. Encapsulation of nematodes

In preliminary studies, the most suitable capsule size and volume were determined to be 4. The capsules were 14.3 mm in length and 5 mm in diameter. The fill weight of the capsule varied according to the density of the substance used but was approximately 200 mg. Theoretically, a capsule can be filled with a maximum of 20,000 IJ using the powdered product. The capsule size and dose were chosen based on the commercial doses used in field applications. Many capsule filler kits are available for purchase, but we were unable to find a supplier and had to create our own kit. A filler kit was designed and printed using a 3D printer to accelerate the capsule-filling process. Our kit consists of two trays, one for the body and one for the cap of the capsules, each with a capacity of 225 capsule parts arranged in a 15 × 15 hole configuration. The kit operates on the same principles as those found in the market. The capsules were filled immediately before the experiments and freshly applied (Fig. 1). The capsules were made of gelatin and were easily

Fig. 1. Empty (A) and ready-to-apply EPN-filled (B) gelatin capsules.

dissolved in water for less than an hour. Prior applications showed that the capsules dissolved within a few hours in moist soils.

2.4. Pot trials

The trials were carried out in 1 L plastic pots measuring 10 × 10 × 14 cm. The trials were set up using a completely randomized design. The pot trials consisted of four different applications: (1) capsule, (2) drip irrigation, (3) suspension (positive control), and (4) tap water (negative control). Three pots were used for each application, and the trials were set up with three replicates. The pots were initially filled with dry, sterile sandy soil. Three larvae were placed in the middle of each pot in a steel wire mesh cage. Approximately 2,500 nematodes per pot were used for all EPN applications (Fallet et al., 2022; Jaffuel et al., 2020; Kapranas et al., 2020). In capsule application, one capsule was filled with 25 mg of powder product and placed 2 cm deep into the pot soil. In the drip irrigation application, the dripline was connected to a knapsack sprayer, stretched between two stakes to stabilize the dripline and keep it parallel to the ground, and the pots were placed under the dripline. All drip irrigation applications were performed at a pressure of 1 bar. In suspension application, 25 mg of the powder formulation was dissolved in tap water 30 min before the experiments, and the EPN suspension was applied to the pot with a pipette. Before applications, the moisture content of all the pot soils was adjusted to 10% using tap water. Identical trials were conducted for three EPN species.

2.5. Field trials

Applications for field trials were arranged according to a randomized block design. The experimental plan included: (1) capsule, (2) drip irrigation, (3) suspension (positive control), and (4) tap water (negative control) applications. Each of four 60-meter-long rows were divided into four equal parts, and all applications were randomly distributed among the four rows (Fig. 2). Each application was performed 75 times (spots) per row (every 20 cm), and 300 times per plot. In capsule application, the capsules were filled with 200 mg of powder formulation and applied using a precision planter. Our planter was an axe type 4-row pneumatic precision planter (Koymak Agricultural Machines). In suspension application, 200 mg of the powder formulation was dissolved in tap water 30 min before the experiments, and the EPN suspension was applied directly to each spot using a pipette. In drip irrigation application, a common inline drip irrigation line (cylindrical dripper, 8 l/h flow rate) was connected to a battery-powered knapsack sprayer, and nematodes were applied with tap water. All drip irrigation applications were operated under 1 bar pressure. The average dose of all applications was 50 IJs/cm^2. Identical trials were conducted for three EPN species.

Before capsule application, the precision planter was adjusted to 70 cm row spacing, 20 cm in-row spacing, and 15 cm planting depth. The tractor moved from the beginning of the plot, and only the related seedbed was filled when it came to the capsule row (Fig. 3). Thus, all capsule applications in one plot were performed simultaneously. The remaining applications were carried out once the capsules had been planted. Finally, the entire field was irrigated equally using a drip irrigation system. The trials were repeated three times on different dates. Fresh EPN products and new driplines were used in each replicate.

One day after the field applications, a 15 cm deep well was drilled at randomly selected spots in each application, and four *G. mellonella* larvae were buried in a steel mesh cage (Bal and Grewal, 2015). The larvae were then covered with soil and kept in the soil for four days. Three cages were used for each treatment (application). Irrigation was not performed after larvae were buried. A red ribbon was tied and marked with stakes to locate the cages in the soil. Four days later, the cages were removed and all larvae were dissected to confirm nematode infection.

2.6. Persistence in field

Field trials have also been used to calculate persistence in the field. To evaluate the persistence of EPNs, wax moth larvae were periodically buried (every 15 days) at randomly selected spots. Similar to the field

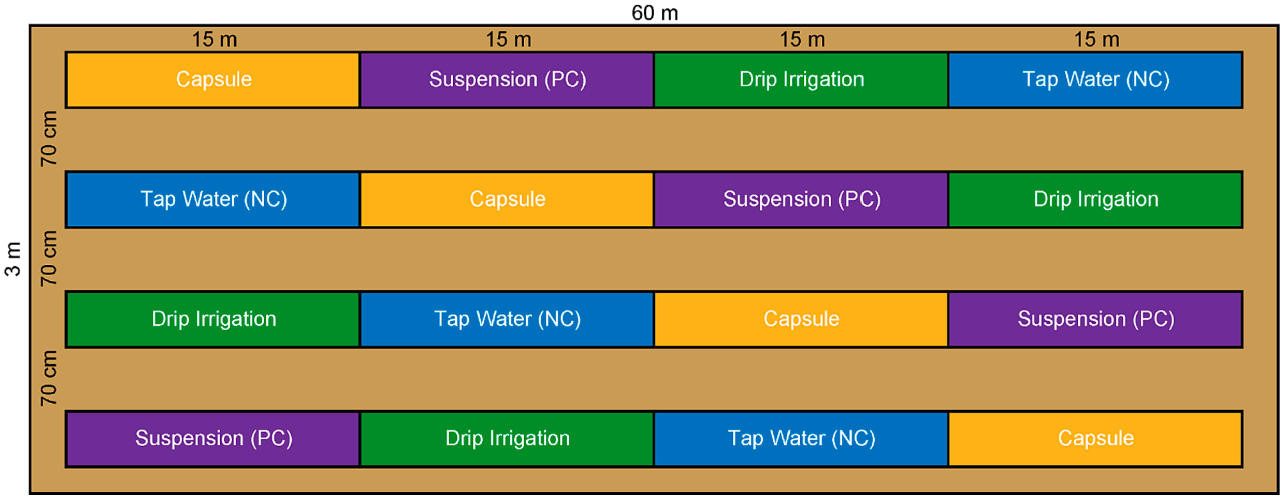

Fig. 2. Experimental design for one plot. Each plot consisted of four rows. All applications distributed randomly along rows. PC: Positive control, NC: Negative control.

Fig. 3. Sowing EPN capsules with a precision planter (A, B) and capsules on the seed plate (C, blue arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trials, four larvae were placed in a steel mesh cage at a depth of 15 cm. The cages were removed after four days, and the larvae were checked for infection. Three different sets of larvae (three mesh cages and 12 larvae) were used for each day and EPN species. New and fresh application spots were chosen for each day post application (DPA). This process was continued until no further infection was observed. While persistence was calculated in days, the mortality rate of larvae was recorded at each DPA.

2.7. *Dispersal in olfactometers*

Custom steel two-way olfactometers were used in the dispersal trials. The distance between each arm of the olfactometer and the center was approximately 10 cm, and the diameter of the arms was approximately 2.5 cm. The olfactometers were filled with sterile silica sand, and the moisture content of the sand was set to 10% using sterile DI water. Dispersal trials included (1) capsule, (2) powder formulation without capsule, and (3) suspension applications (positive control). Approximately 20,000 IJs were used for each application. In capsule application, a capsule was filled with 200 mg of the product and placed in the center of the olfactometer. In the powder formulation application, 200 mg of the powder product was placed at the center of the olfactometer. In the suspension application, 200 mg of the powder product was dissolved in 500 μL of tap water 30 min before the experiments and applied to the center of the olfactometer with a pipette. After 24 h, the olfactometers were disassembled and the sand on the arms was washed in separate beakers. After filtration, the nematodes in the two arms and at the center were counted. Dispersal ratio was calculated by dividing the number of IJs in the arms by the total number of nematodes. Identical experiments were conducted for 48 and 72 h. Three olfactometers were used for each application and each olfactometer counted as a replicate. All the olfactometer trials were performed at 25 °C.

2.8. *Statistical analysis*

All datasets were checked for normality using the Shapiro-Wilk's test, and data from the negative control applications in pot, field and persistence trials, where there was no larval mortality or where the mortality rate was less than 5%, were excluded from the statistical analysis. Despite transformation, the data from pot trials did not meet the assumptions for parametric tests, and therefore, a nonparametric Kruskal-Wallis test followed by Dunn's multiple comparison test was conducted. For the evaluation of larval mortalities in the field trials, a two-way ANOVA was conducted with the application method and EPN species used as factors, followed by the LSD post-hoc test. Dispersal results were analyzed using a two-way ANOVA with the application method and EPN species as factors, followed by the Tukey's HSD post-hoc test. Two-way repeated measures ANOVA was performed for persistence trials, where application (EPN species × Application method combined) and duration were used as factors. Means of the larval mortalities in persistence trials were compared using Bonferroni's multiple comparisons post-hoc test. Differences were considered significant if $p \leq 0.05$. All analyses and graphs were generated using GraphPad Prism v9.4.

3. Results

3.1. *Efficacy in pots*

No significant differences were observed in the larval mortality percentages between the methods tested in the pot experiments ($H = 2.167$; $df = 2$; $p = 0.338$ for *H. bacteriophora*; $H = 0.473$; $df = 2$; $p = 0.789$ for *S. carpocapsae*; $H = 4.572$; $df = 2$; $p = 0.102$ for *S. feltiae*). There was no larval mortality in the negative control of *H. bacteriophora* and *S. carpocapsae*, while it was less than 5% in *S. feltiae* (data was excluded from the analysis). The lowest larval mortality among all applications was encapsulated *S. feltiae* (85.2%). All larvae were dead in drip irrigation application of *H. bacteriophora*, and suspension application of *S. feltiae* (Fig. 4).

3.2. *Efficacy in field*

We found that the larval mortalities were not significantly different among all applications except for the suspension applications of *H. bacteriophora* and *S. carpocapsae*, which showed significantly higher mortality rates compared to the capsule application of *S. feltiae* ($F_{(2,\ 72)} = 3.466$; $p = 0.037$). The highest efficiency was observed in the suspension application of *S. carpocapsae* (80.6%), and the lowest efficiency was observed in the capsule application of *S. feltiae* (52.8%) (Fig. 5). There was no larval mortality in the negative control applications (data was excluded from the analysis). The interaction effect on larval mortality was not significant ($F_{(4,\ 72)} = 0.103$; $p = 0.981$). There was also no significant effect of EPN species on larval mortality ($F_{(2,\ 72)} = 2.224$; $p = 0.116$).

3.3. *Persistency in the soil*

Persistence trials included data on larval mortality for five different DPA up to two months following field application (Fig. 6). Larval mortalities significantly decreased over time ($F_{(4,\ 48)} = 77.0$; $p < 0.001$), but no significant differences were found between applications ($F_{(5,\ 12)} = 1.99$; $p = 0.153$). No successful infection was observed with any application at 65 DPA. In general, although larval mortalities decreased at higher DPA, there was an increase at 20 DPA in *S. feltiae* (PC) compared

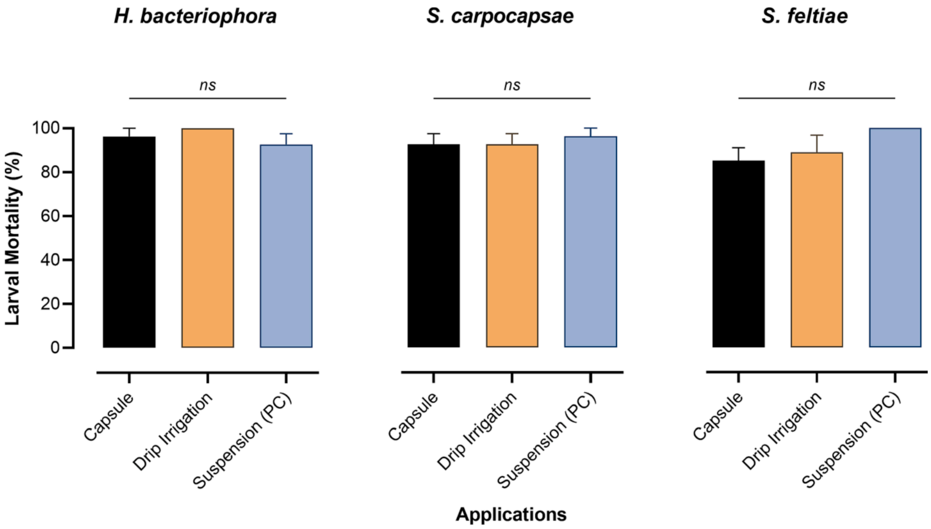

Fig. 4. Effects of three application methods applications on larval mortality in the pots. The bars represent the standard error of the mean. Applications were compared individually for each EPN species. PC: Positive control.

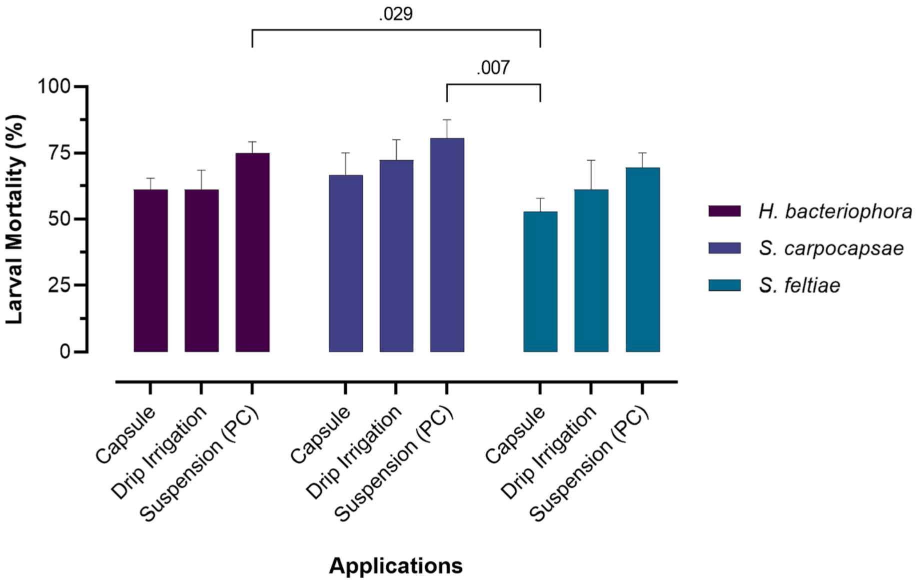

Fig. 5. Effects of three application methods applications on larval mortality in the field. The bars represent the standard error of the mean. PC: Positive control.

to 5 DPA. The last successful infection was detected in *S. carpocapsae* (PC) at 35 DPA, while the last infection of other applications was 50 DPA.

3.4. *Effect of capsule application on dispersal*

As expected, dispersal increased with time, but there were no statistically significant differences between the applications on dispersal of IJs for each duration (24, 48, and 72 h) ($F_{(2,\ 18)} = 0.386$; $p = 0.685$ for 24 h; $F_{(2,\ 18)} = 0.293$; $p = 0.749$ for 48 h; $F_{(2,\ 18)} = 0.381$; $p = 0.689$ for 72 h). In the 24 h trials, the dispersal ratio in all three species was approximately 5%. Compared to 24 h, the dispersal ratio increased over 10% in 48 h trials, but no further increase was detected in 72 h (Fig. 7). EPN species showed significant effect on dispersal for 24 and 48 h, but there was no significant effect for 72 h ($F_{(2,\ 18)} = 4.28$; $p = 0.03$ for 24 h; $F_{(2,\ 18)} = 7.60$; $p = 0.004$ for 48 h; $F_{(2,\ 18)} = 2.37$; $p = 0.122$ for 72 h). This effect was found only in powder applications. Additionally, there was no significant interaction effect on dispersal ($F_{(4,\ 18)} = 2.11$; $p = 0.121$ for 24 h; $F_{(4,\ 18)} = 0.283$; $p = 0.885$ for 48 h; $F_{(4,\ 18)} = 0.364$; $p = 0.831$ for 72 h).

4. Discussion

The application of entomopathogenic nematodes in the field can be achieved through various methods such as irrigation systems, fertilizing machinery, and spraying equipment. However, there is no globally accepted method as each approach presents its own benefits and limitations. Drip irrigation is one of the most common methods used for EPN application. However, because drip irrigation systems are rare in large-scale fields, alternative methods are required for EPN application. This study investigated a novel approach for the application of EPNs in large fields by using a precision planter to sow gelatin capsules containing EPNs. The results indicate that the field efficiency of the capsule method was comparable to that of both drip irrigation and the positive control. Furthermore, the persistence and dispersal of infective juveniles were not impacted by the capsule technique.

The efficacy trials were conducted in pots and field. The dose was calculated based on the standard recommendation for both applications.

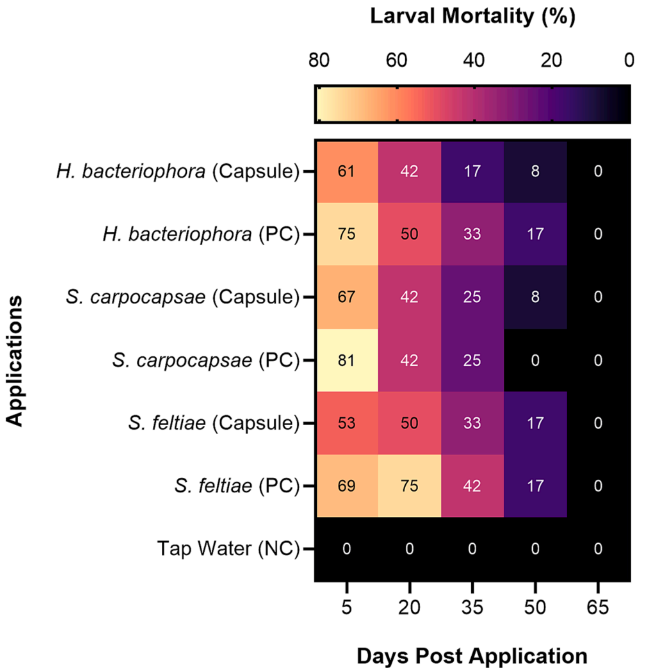

Fig. 6. Persistence and larval mortality percentages for each application. The x-axis indicates persistence by days, and the numbers inside squares indicate larval mortality on each day of application. PC: Positive control, NC: Negative control.

We applied approximately 2,500 IJ per pot (25 IJs/cm^2) and 20.000 IJ (50 IJs/cm^2) to each spot in the field. The larval mortalities in the pot trials were higher than expected, which were above 95% in all applications. High efficiency is an expected result in pot trials because they were performed in a controlled environment. According to Fallet et al., (2022), the gel formulation of EPNs was 100% effective against caterpillars in pots. Similarly, Jaffuel et al., (2020) found that the application of EPN + bead and EPN suspension was more than 90% effective against *D. balteata* larvae in pots. NanGong et al., (2021) demonstrated similar results, showing that the alginate capsule and suspension treatments were almost 100% effective against *A. ipsilon* in pots. In contrast to pot trials, efficacy decreases under field conditions. EPNs have inconsistent field activities, and numerous investigations have achieved varying field efficacies (de Waal et al., 2018; Dlamini et al., 2020; Odendaal et al., 2016; Radová and Trnková, 2010). Raja et al., (2015) compared the efficacy of different application techniques, and larval mortality was determined between 56 and 68%. Brusselman et al. (2012) examined the effect of spraying volume on EPN infection capacity and determined that the infection rate ranged from 76 to 19% in field applications on *G. mellonella*. In one of the few studies that used gelatin capsules, Valle et al., (2009) reported that six different cadaver coverings, including the use of gelatin capsules, were >90% effective against *G. mellonella*, and gelatin capsule application was not significantly different from the control treatment (no coverings). The larval mortality data obtained from the evaluation of new formulations and application techniques typically demonstrated similar or improved outcomes when compared to conventional methods or control treatments (Fallet et al., 2022; Kim et al., 2021). Likewise, the results of our capsule application method were found to be comparable to those of the drip irrigation and positive control applications. This suggests that there is potential and need for optimization of the capsule technique. Nonetheless, the focus of this study was to emphasize the potential of capsule application as opposed to optimizing its efficiency, which will be our next step.

Since the persistence of EPNs in the field varies depending on various factors, periodic (monthly) reapplication is recommended for successful pest management. Although we also encountered variable larval infections over time, gelatin capsules did not affect the field persistence of EPNs. Average larval mortality decreased to below 30% at 35 DPA and 15% at 50 DPA. Our findings concur with those of existing research. For example, Steyn et al., (2019) found that the field activity of EPNs against *Thaumatotibia leucotreta* decreased below 10% after 28 days. Since we used susceptible *G. mellonella*, larval mortality in our study was expected to be higher than their results. Similarly, Malan and Moore, (2016) determined that EPN activity against false codling moth larvae decreased to below 20% 35 days after application. It was expected that capsules would not affect persistence because gelatin capsules dissolve in the soil within a few hours. However, the initial contact of EPNs with the soil surface can affect the efficacy. Moistening and cooling the soil before and after EPN application is recommended for optimal IJ conditions. (Koppenhöfer and Fuzy, 2007; Toledo et al., 2014). We believe that this risk is greatly reduced with the capsule method because the precision planter places the EPNs deep into the soil. In comparison, alginate formulations may be more favorable since increase EPN effectiveness while extending field persistence and shelf life, and they offer more benefits than gelatin capsules (Ebrahimi, 2022; Kapranas et al., 2020; NanGong et al., 2021). However, alginate optimization and production processes are more complicated, and the use of gelatin capsules is much simpler.

The dispersal of IJ is influenced by numerous field variables such as humidity, temperature, salinity, gravity, and pH (Finnegan et al., 1999; Fu et al., 2021; Ramos-Rodríguez et al., 2007; Ruan et al., 2018; Wu et al., 2018). In addition, nematode pheromones (ascarosides) and prenol can also increase dispersal of EPNs (Kin et al., 2019; Wang et al., 2022). However, since our aim was to examine the effect of the capsule itself, dispersal trials were performed using olfactometers without any cues or stimulants. There was a significant difference between species in powder application but, the dispersal of IJs was unaffected by gelatin capsules. This may be because gelatin dissolves quickly in moistened environments. The structure of alginates affects the storage duration, viability, escape ratio, and effectiveness of EPNs. For instance, more IJs can escape from low-concentration alginates prepared with $CaCl_2 \cdot 2H_2O$ (Kim et al., 2021). Post-treatment of alginate capsules with Ca^{2+} enhances the EPN escape ratio (Kim et al., 2015). Regardless of the alginate composition, the type of EPN is known to affect the escape ratio from capsules (Kagimu and Malan, 2019). The chemical and physical properties of alginate capsules can be altered; thus, the optimum parameters must be determined during production. Unlike the complex structure of alginates, gelatin capsules generally have a standard structure and dissolve quickly when in contact with water. In recent years, isolated pheromones have been shown to alter the behavior of EPNs (Oliveira-Hofman et al., 2019). For instance, an ambusher EPN species displays cruiser behavior after pheromone treatment. Moreover, pheromones can boost the field efficacy of EPNs (Kaplan et al., 2020). Gelatin capsules can be used to apply EPNs together with ascarosides. EPNs can either be maintained in the application area with stimulants or rapidly dispersed after application, depending on the goal.

Encapsulation typically refers to the alginate capsules or beads used in EPN research. Brown algae-derived alginates are polysaccharides that polymerize into spheres when in contact with Ca^{2+} (Hiltpold et al., 2012). EPNs can be formulated by trapping them in alginate beads. This was the goal of earlier EPN investigations using alginates to preserve nematodes for extended periods and to enhance their use in the field (Kaya et al., 1987; Kaya and Nelsen, 1985). Alginate and similar dissolvable polymers also allow EPN application in many above-ground insects. For instance, the formulation of calcium alginate gel was improved by Navon et al., (2002), allowing the effective use of EPNs against *Spodoptera littoralis* and *Helicoverpa armigera*. Similarly, Hiltpold et al., (2012) found that using EPNs against *Diabrotica virgifera* in alginate capsules was more effective than the traditional techniques. There is numerous research on alginate encapsulation but only some on gelatin capsules. Hui and Webster, (2000) conducted the first EPN study by using gelatin capsules to test the host-finding capability of *S. feltiae*.

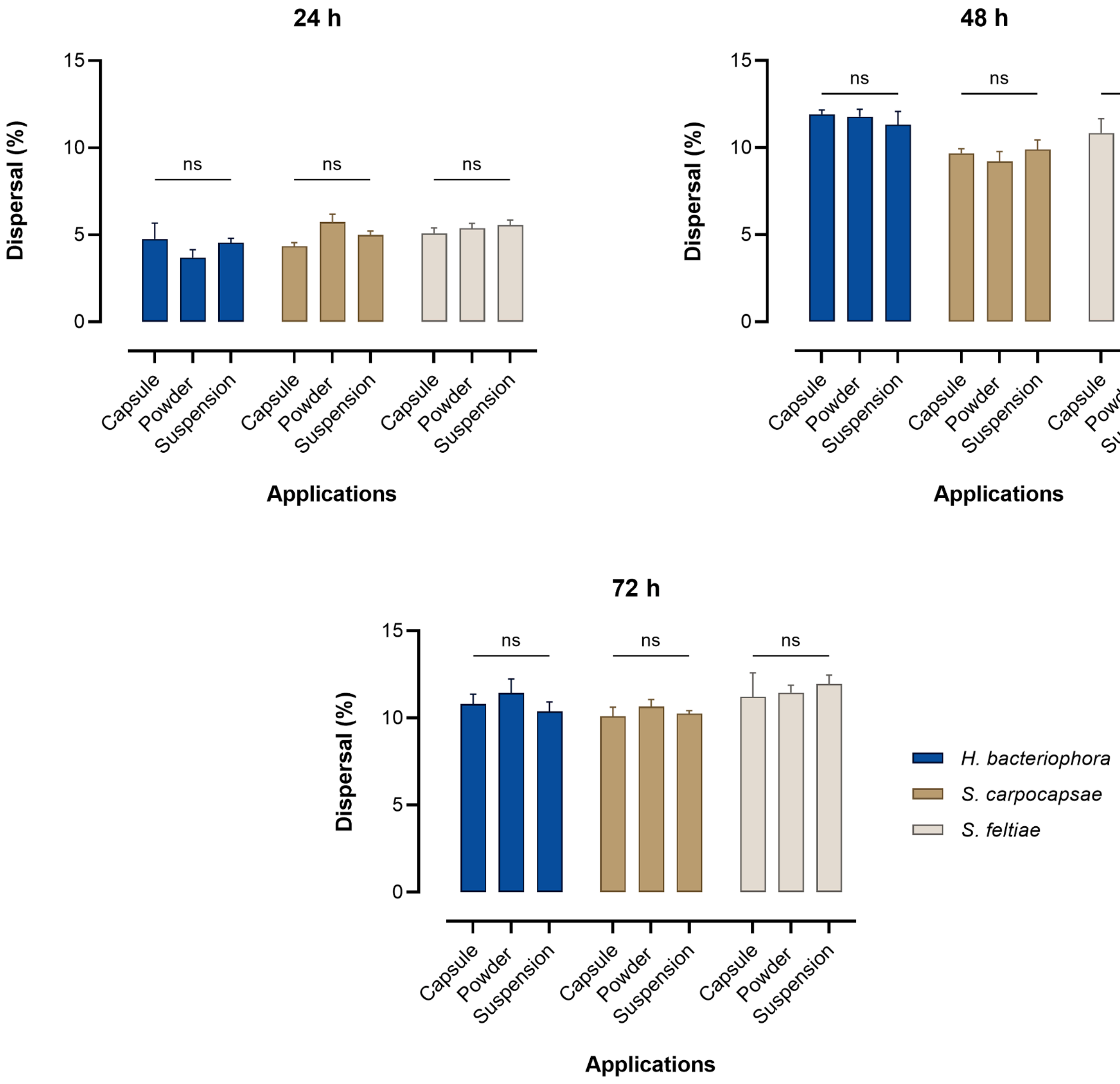

Fig. 7. Percentage (mean ± SEM) of dispersal in olfactometers. The results indicate the ratio of IJs moved to the arms. Only the comparison of applications in the same species is shown. Ns: not significant.

They constrained the movement of the host larvae by placing them in size 2 capsules. However, their study did not investigate the effect of the capsule on the application of EPNs. Another study focused on enhancing the infected larva approach. Scavengers either consume the larvae or carry them away, which is a significant drawback of this strategy (Ulug et al., 2014). To prevent this, Valle et al., (2009) placed the larvae in size 000 capsules to protect infected larvae from scavengers. They found that gelatin capsules did not affect IJ emergence and successfully protected it from ants. Despite their structural differences, the objective of encapsulation studies is to improve the effectiveness of EPNs in field applications and to effectively use the product (Kapranas et al., 2020).

Although the capsule technique appears feasible, we encountered some problems before and during its application. The biggest problem with capsule preparation was that the capsules started to soften because the powdered EPN formulation was not completely dry. Although maintaining the filled capsules at low temperatures delays softening, it is best to fill the capsules immediately before application. Another problem during preparation was that the number of nematodes in the capsules was not precisely equal, owing to the inhomogeneity of the formulation. There was a 10%deviation in the number of nematodes in the capsules. Although this difference may seem significant, it is still a more even application than conventional methods. For example, in some applications with drip irrigation, more than 50% loss is experienced towards the end of the drip line (Wang et al., 2009). One of the problems encountered during application is that the seedling machine can sometimes plant more than one capsule (known as multiples), as the capsules are not as rigid as a seed. This problem can be avoided if the capsules are filled more densely using a capsule filler machine or a planter with a double eliminator. Since filling capsules by hand is very time-consuming, we developed a simple capsule filling kit using a 3D printer. With this kit, we were able to fill 225 capsules in approximately 20 min, which is still slow for large-scale applications. Commercial capsule filling machines can fill thousands of capsules in minutes; however, the cost of the machine must be taken into consideration. Although the capsule itself (~$2 per 1,000 capsules) and the capsule filling process incur extra costs, we believe that these costs can be compensated for by the efficient and effective use of EPNs.

In conclusion, entomopathogenic nematodes play an important role as an environmentally friendly alternative to pesticides and are crucial for sustainable agriculture. Despite the importance of EPNs, their effective application remains a challenge, and practical application methods are of great interest. In this study, EPN formulations in powder form were filled into gelatin capsules at specific doses and sown using a precision planter. Our results demonstrate that encapsulating EPNs in gelatin does not have any adverse effect on their field efficiency, persistence, and dispersal. The capsule technique can also be used to apply the equal or less product to a larger area. Moreover, this method may provide an alternative approach for the large-scale application of EPNs. Although the capsule technique presents certain challenges, we believe that with optimization, it could serve as a valuable method for large-scale EPN application. Further studies are needed to investigate realistic improvements to this method.

CRediT authorship contribution statement

Tufan Can Ulu: Conceptualization, Methodology, Project administration, Writing – review & editing. **Hilal Erdoğan:** Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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