Impact of entomopathogenic nematodes on *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) life stages in the laboratory and under semi-field conditions

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ABSTRACT

The biocontrol potential of entomopathogenic nematodes (EPNs) against soil-dwelling life stages of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) was evaluated under laboratory and semi-field conditions. The pathogenicity of native isolate of *Steinernema feltiae* (T1) and three commercial strains, *S. feltiae*, *S. carpocapsae* (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* (Rhabditidae: Heterorhabditidae) were tested at concentrations of 400 and 1000 infective juveniles (IJs)/cm^2 against the second instar larvae (L2), prepupae and pupae of the thrips. This was followed with trials compared efficacy of *S. carpocapsae* on the thrips fed on different host plants, i.e. onion shoots, *Allium cepa* (L.), pods of green beans, *Phaseolus vulgaris* (L.) and cucumber fruits, *Cucumis sativus* (L.). In the semi-field experiments, effects of two EPNs, *S. carpocapsae* and *H. bacteriophora*, at concentrations of 10^4 or 2×10^4 IJs/ml against the thrips larval stage were tested. Our results showed that the highest onion thrips corrected mortality (CM) was recorded for prepupae (92%) and pupae (92.59%) at 1000 IJs/cm^2 by *S. feltiae* (T1) and *S. carpocapsae*, respectively and the mortality caused by the commercial strain of *S. feltiae* against thrips larval stage at 400 IJs/cm^2 was the lowest rate (CM= 3.7%). Among different host plants, green bean feeding induced the highest CM by *S. carpocapsae* at 1000 IJs/cm^2 against the onion thrips larvae. In the semi-field experiments, application of two concentrations of EPNs had no significant effect on the larval mortality, compared to the control treatment.

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INTRODUCTION

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) has become a global pest of growing concern during the past two decades (Diaz-Montano *et al.,* 2011).This pest attacks a wide range of host plants including field and greenhouse crops (Moritz, 1997). Onion thrips can cause yield reduction more than 50% but it can be more problematic by transmitting some plant viruses like Tomato spotted wilt virus and Iris yellow spot virus (Diaz-Montano *et al.,* 2011; Jones, 2005). Onion thrips has a short life cycle (Diaz-Montano *et al.,* 2011). Its post-embryonic developmental stages involve first and second larval instars (L1 and L2), prepupa, pupa, and adult (Diaz-Montano *et al.,* 2011; Lall and Sinch, 1986). *T. tabaci* mean developmental time on onion at 25ºC were 5.93±1.00, 1.96±0.50, and 3.56±0.50 days for larvae, prepupae, and pupae, respectively (Moritz, 1997). Biological control of this pest has been considered for many years due to the rapid development of the thrips resistance to insecticides and the weak spray coverage on the inner leaves where the insects are found (Diaz-Montano *et al.,* 2011; MacIntyre Allen *et al.,* 2005).The most considered biological control agents are generalist predators like *Amblyseius* spp. (Acaridae: Phytoseiidae) and *Orius* spp. (Hemiptera: Anthocoridae) (Loomans *et al.,* 1997). Since the thrips predators and parasitoides are effective only when thrips comes out of its refuge (Diaz-Montano *et al.,* 2011) and their application is only partly successful in some crops (Loomans *et al.,* 1997), the option of using pathogens is worth attention. Entomopathogenic nematodes (EPNs) of Steinernematidae and Heterorhabditidae have pathogenicity effect against a variety of soil-inhabiting insects (Kaya and Gaugler, 1993). The life cycle of EPNs consists of an egg, four juvenile stages, and an adult (Boemare *et al.*,1996). Infective

juvenile (IJ) (third juvenile stage) has a symbiotic relationship with bacteria in the genera *Photorhabdus* and *Xenorhabdus* (Karimi *et al.,* 2010). IJ releases the bacteria after host penetration (Dowds and Peters, 2003). The bacteria cause the septicemia and kill the host usually within 24-48 h (Adams and Nguyen, 2002).

Among different thrips species, efficacy of EPNs on two more important pests, western flower thrips (WFT) *Frankliniella occidentalis* (Tomalak *et al.,* 2005) and *Thrips palmi* have been studied more. The pathogenicity of EPN species/strains against soil-dwelling stages of WFT showed that heterorhabditid nematodes tend to be more infectious than the steinernematids against prepupal and pupal stages of WFT (Tomalak *et al.,* 2005). Yet, generally high concentrations even for the most efficient EPN species/strains are needed to achieve satisfactory control levels (Ebssa *et al.,* 2003). Moreover, high costs required for the application of EPNs, so the combination of EPNs with other pest management strategies is necessary.

Many studies demonstrated the effects of arthropod resistant plants in improving the efficiency of arthropod pathogens by decreasing the vigor and physiological state of the insect pests (Smith, 2005). Inter- and intraspecific differences in plant chemistry (allelochemicals) and structure can change the physiology and growth of the insect host, affecting its susceptibility to the pathogenic infection (Cory and Hoover, 2006).

The objectives of this research were: 1) to assess the efficacy of four native and exotic species/strains of EPNs on the soil-dwelling life stages (L2, prepupa, and pupa) of the onion thrips, 2) to compare the virulence of *S. carpocapsae,* to onion thrips fed on different plants, and 3) to evaluate the effects of two EPNs, *S. carpocapsae* and *H. bacteriophora* on the thrips under semi-field condition.

MATERIALS AND METHODS

Nematodes and thrips culture

EPNs used in this study were *Steinernema feltiae* (Tabriz 1) obtained from the Tabriz University, Iran, originally collected from soil in agriculture fields, and *Heterorhabditis bacteriophora* (Larvanem®), *Steinernema carpocapsae* (Capsanem®) and *Steinernema feltiae* (Entonem®) supplied by Koppert B. V. (Berkelen Rodenrijs, The Netherlands). EPN species/strains were reared at 25±2ºC in greater wax moth larvae *Galleria mellonella* (Lepidoptera: Pyralidae) (Ehlers and Shapiro-Ilan, 2005). Infective juveniles (IJs) were stored in tap water at 6ºC no longer than one month before using in the tests. The nematodes were allowed to acclimatize for at least 5 hours at room temperature before they were used. Different concentrations from EPNs were prepared following the nematode quantification method (Kaya and Stock, 1997).

The onion thrips colony was began with naturally infested onion shoots collected from onion field that had not been exposed to pesticides. Using the modified protocol described by Loomans and Murai (1997), onion thrips was reared on onion shoots *Allium cepa* L. (Amaryllidaceae) in an incubator (25±1ºC, 60-70% relative humidity (RH), and 18:6h L:D photoperiod). To obtain synchronized insects for the tests, cages contained about 100 *T. tabaci* adults on their host were made. Every 24h, the old shoots were replaced by the new ones. The old shoots were placed in a plastic jar (0.5 liter). Under this condition, the late second instar larvae (L2), prepupae and pupae were collected from old shoots 8, 10 and 11 days after replacement, respectively.

EPNs on thrips life stages in the laboratory

Assays were done based on previous work on WFT (Buitenhuis and Shipp, 2005) under laboratory conditions. For the experiments, sterile plastic containers (diameter 4.5, and height 3 cm) were used. For ventilation, a small hole (diameter 1 cm) was drilled in the center of container lid and a cloth tissue (100µm pore size) was glued on it. Insect glue was painted on the inner part of the lid (sticky trap), except the hole, to trap adults. The container bottom was covered by five layers of filter paper. The EPNs tested concentrations were selected 400 and 1000 IJs/cm^2 in one ml of distilled water (based on Ebssa *et al.,* 2001, 2003). The control treatment was pipetted only with distilled water. Synchronized L2, prepupae and pupae (8, 10, and 11 days after shoots replaced in the culture cages, respectively, as described above) were first examined under the stereomicroscope and then ten individuals of larvae, prepupae, or pupae were introduced to the container. The container was sealed by parafilm and kept in an incubator (25±1ºC, 60-70% RH, and 18:6h L:D photoperiod**)**. After 24 and 48 hrs, dead insects and emerged adults trapped on the sticky traps (for

pupal tests) were counted. Insects were considered dead according to cadaver color change and their no response to mechanical stimulus. In all treatments, a sub-sample of dead insects was dissected to detect the presence of nematodes. Each experiment was replicated three or four times and whole treatments were repeated twice over time, giving a total of six to eight replications per treatment, respectively.

Tri-trophic experiments: host plant on the efficacy of EPNs against onion thrips

To evaluate the effect of host plant, the onion thrips was reared on its two other hosts, pods of green beans *Phaseolus vulgaris* L. (Fabaceae) and cucumber fruits *Cucumis sativus* L. (Cucurbitaceae) (Loomans and Murai, 1997; Tommasini and Maini, 1995) in an incubator (25±1ºC, 60-70% RH, and 18:6 h L:D photoperiod) at least for four generations. The most pathogenic EPN species/strain against L2 was used to assess the host plant effect using the assay arena described above.

Effect of EPNs under semi-field conditions

Green beans *P. vulgaris* was grown in 1.5 L plastic pots with a top diameter of 10 cm containing 70% peat and 30% per liter in a controlled room condition (25±2ºC, 40±10 RH, and 16:8 h L:D photoperiod). At the two-leaf stage, one hundred L2 were released on individual potted common bean plant.

To avoid larval scape, each plant surrounded by talcous cylinder (diameters of 10, and a height of 6 cm) that was embedded into the soil of each pot. The open end at the top of the talcous cylinder was closed with cloth tissue (100 µm pore size). For additional ventilation, six holes (diameter 5 cm) were drilled in the cylinder and covered with the same type of described cloth tissue. These pots were kept in a controlled room condition (25±2ºC, 40±10 RH, and 16:8 h L:D photoperiod).

One hour after the thrips were introduced on the plants, the plants were sprayed up to run off with water, *S. carpocapsae* or *H. bacteriophora* at concentrations of 10^4 or 2×10^4 IJs ml^{-1} by using a handheld sprayer (Buitenhuis and Shipp, 2005). Each solution contained 0.01% Tween 80 as a wetting agent for increasing EPN survival by decreasing desiccation (Qiu*et al.,* 2008).

After one day, all the plants were cut at the base of the stems, individually shacked on a black paper, and the number of thrips recaptured per plant was recorded. Then, the open end of the cylinder was covered with yellow sticky cards to be as a sticky trap for the emerging adults. The number of the onion thrips offspring for a period of 15 days (from the day of introduction), and the number of recaptured larvae were considered to calculate mean mortality. Each experiment was replicated three times and whole treatments were repeated twice over time, giving a total of six replications per treatment.

Statistical analysis

First, corrected mortality (CM) was calculated using Abbot's formula (Abbott, 1925). Then the data were arcsine transformed before subjected to statistical analysis. The analysis was carried out in a completely randomized design. In the experiments determining effect of EPN species/strains on thrips stages in the laboratory, the analysis was done in a factorial experiment including EPN species/strain, concentration and onion thrips stage factors. Main or interaction effects of factors were analyzed using the PROC ANOVA procedure in SAS program version 9.2. Significant differences of means were evaluated using Fisher's LSD comparison test at 95% level (SASInstitute, 2008).

RESULTS

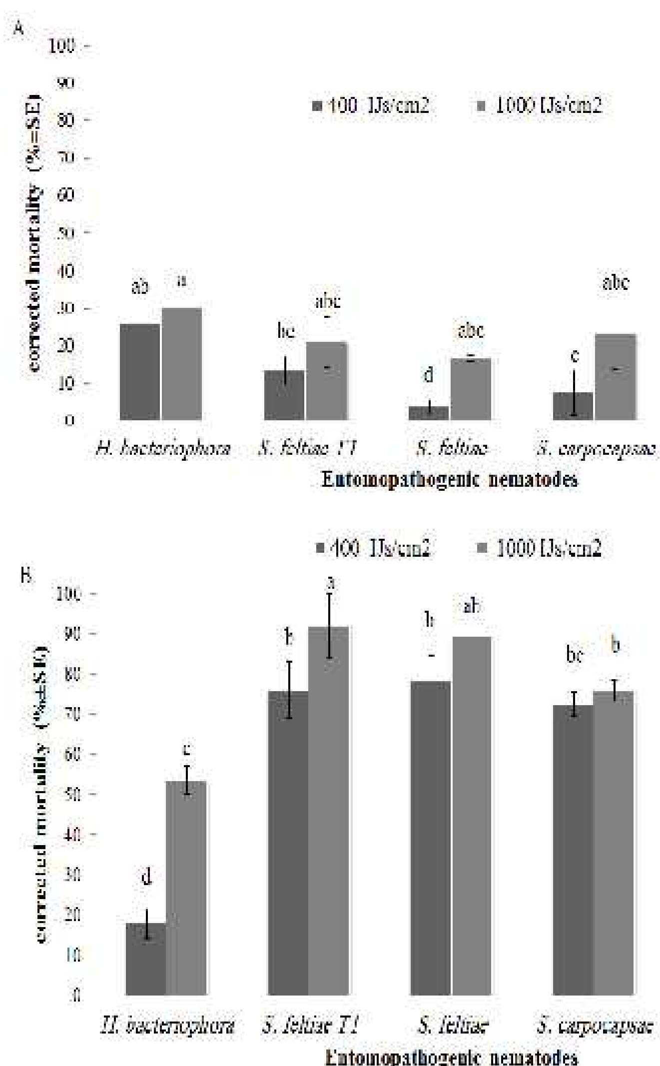
Efficacy of EPNs in the laboratory

Analysis of data indicated that thrips stages, EPN species and concentrations caused significant differences in terms of efficacy against the onion thrips. For all experiments, mean mortality values in control treatments were about 17.5% after 24 hrs. After 48 hrs, mean mortalities in control treatments were high, so that results for 48 hrs (from all treatments) were supposed not reliable and they were omitted. It has been reported that EPNs can kill their host within 24-48h and WFT prepupae and pupae were infected and killed by *S. feltiae* within only 2-4h after infection (Tomalak *et al.,* 2005). Applying EPNs to different thrips developmental stages resulted in significant differences between them (Table 1).

Table 1. Summary of ANOVA results for corrected mortalities of different onion thrips life stages caused by different concentrations (400 and 1000 IJs cm^{-2}) of entomopathogenic nematode species/strains (*Steinernema carpocapsae*, *S. feltiae*, *S. feltiae* (T1), and *Heterorhabditid bacteriophora*).

Source	*df*	*F*	P
EPN species	3	12.70	0.0001**
EPN concentration	1	40.09	0.0001**
Thrips stage	2	130.93	0.0001**
Species×concentration	3	0.09	0.9631
Species×stage	6	14.25	0.0001**
Concentration×stage	2	0.95	0.3930
Species×concentration×stage	6	0.07	0.6381

The prepupae and pupae showed higher susceptibility to EPNs than L2 (Figure 1). Significant variation also resulted between different EPN species/strains (Table 1). CM values for the larval stage ranged between 3.7±2% for *S. feltiae* and 30±2.1% for *H. bacteriophora* (Figure 1A).



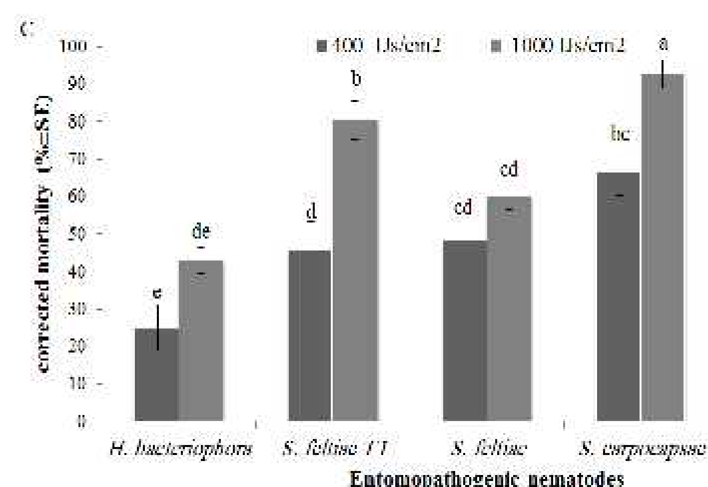


Fig 1. Mean corrected mortality (CM) (%) (± SE) caused by different entomopathogenic nematodes (EPNs) at 400 and 1000 IJs cm^{-2} against second instar larvae (L2) (A), prepupae (B) and pupae (C) of *T. tabaci*. Bars with different letters indicate statistical significances (= 0.05, Lsd test).

Native and commercial *S. feltiae* strains showed the highest CM in the prepupal stage (Figure 1B) while, *S. carpocapsae* was the best one in the pupal stage (Figure1C). In contrast, *H. bacteriophora* showed no significant differences in mortalities on the different developmental stages at 400 IJs cm^{-2} (F= 21.84, df= 2, P=0.415) and had little variation at 1000 IJs cm^{-2} (F= 1.02, df= 2, P= 0.0018).
Interaction effect between EPN species/strains and thrips life stages was significant (Table 1), indicating that EPN species/strains differed in their efficacy on *T. tabaci* depending on thrips life stages. EPNs concentration caused significant differences on the thrips mortality (Table 1). Highest CM values were obtained at 1000 IJs cm^{-2} for *S. feltiae* at larval stage (Figure 1A), *S. feltiae*T1 and *H. bacteriophora* at prepupal stage (Figure 1B), and *S. feltiae* T1and *S. carpocapsae* at pupal stage (Figure 1C).

Host plant studies

Since *S. carpocapsae* induced the highest mortality on the thrips larvae, this EPN species was used to assess host plant effect. CM values ranged between 3.57±0.2 and 41.63±0.9%, depending on the EPN concentration and host plant. *S. carpocapsae* caused the highest significant mortality against the onion thrips that had been reared on green bean at 400 IJs cm^{-2} (F= 8.8, df= 2, P= 0.0164). Mortality caused by different nematode concentrations varied

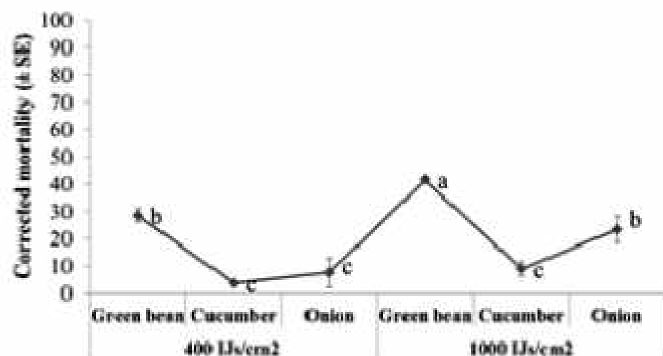


Fig 2. Mean corrected mortality (CM) (%) (±SE) caused by *Steinernema carpocapsae* at 400 and 1000 IJs cm^{-2} against L2 of the onion thrips reared on different host plants (green bean, onion and cucumber). Bars with different letters indicates statistical significances at each concentration (=0.05, Lsd test).

significantly when the thrips had been fed on green bean or onion (F= 21.45, df= 1, P= 0.0098) (Figure 2).

Effect of EPNs under semi-field conditions

Data analysis indicated that no significant differences were found between different treatments and concentrations in the semi-field experiments (F= 0.62, df= 5, P= 0.687) (Figure 3).

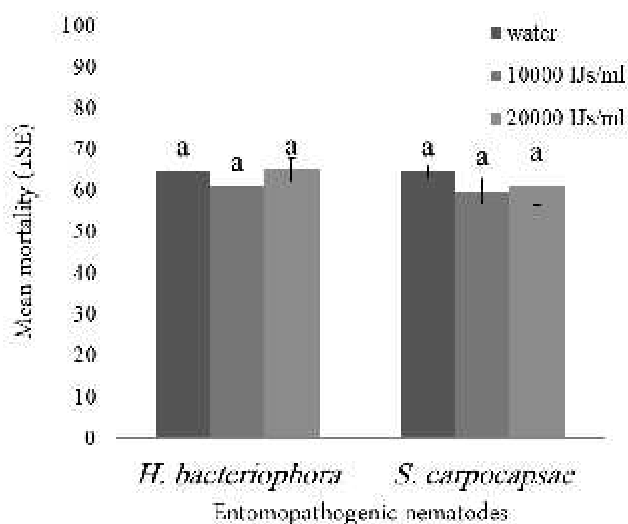


Fig 3. Mean mortality (%) (± SE) caused by *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* against second instar larvae (L2) on common bean at two concentrations (10^4 and 2×10^4 IJs ml^{-1}). Bars with different letters indicate statistical significances (= 0.05, Lsd test).

Discussion

Results of the laboratory tests indicated that EPNs have high efficiency for controlling soil-dwelling life stages of the onion thrips. However, pathogenicity of EPNs against the onion thrips largely differed among EPN species/strains, concentrations, thrips developmental stages and thrips host plants. All species/strains had some degree of effect to kill soil dwelling stages of the onion thrips. In this study, we examined the efficacy of both native and commercial *S. feltiae* that in many cases it was showed no significant variation in the infectivity between them. Other studies showed that EPN origin can influence differently the nematode pathogenicity against insect pests. Hay and Richardson (1995) studied the efficacy of 16 EPN isolates against mushroom sciarid fly *Lycoriella solani* and showed variation in the pathogenicity of EPNs with different geographical origins. Grewal *et al.,* (2002) compared the susceptibility of the white grubs to 16 EPN species/strains isolated from within or outside the studied geographic ranges. Their results revealed that nematode species/strains from different areas did not vary in term of pathogenicity toward the tested scarab species. McGraw and Koppenhöfer (2008) showed that commercial and endemic EPN strains did not vary in virulence against *Listronotus maculicollis* (Coleoptera: Curculionidae) stages. *Heterorhabditis bacteriophora*, *S. feltiae* (both strains), and *S. carpocapsae* induced the highest mortality in the larval, prepupal and pupal stages, respectively. The lowest thrips prepupal and pupal mortalities were induced by *H. bacteriophora*. Differences in EPNs pathogenicity can be due to factors such as differences in host recognition and penetration, dissimilar ability to overcome host immune system, and different host finding behavior including ambushing, crusing, and intermediate strategy (Grewal*et al.,* 2005). *H. bacteriophora* is a cruiser forager that actively looks for its prey (Ciche, 2007), *S. carpocapsae* has an ambushing strategy that waits for potential prey, and *S. feltiae* is known to have an intermediate strategy (Lewis, 2002).

Among different developmental stages of the thrips, prepupal and pupal stages are more sensitive to EPNs than larvae. One explanation for this is low mobility of prepupae and pupae that facilitates nematode attachment to them (Koppenhofer *et al.,* 2002). Prepupae and pupae are quiescent, non-feeding, and inactive instars (Moritz, 1997) that

move only when disturbed. Other pests controlled by EPNs, such as the sugar beet beetle pupa (Saleh *et al.,* 2009), leafminer larvae (Williams and Walters, 2000), larvae of *Tuta absoluta* (Garcia-del-Pino *et al.,* 2013) and immature whitefly (Cuthbertson *et al.,* 2003) are also immobile. There are also some reports that *Steinernema* and *Heterorhabditis* spp. were more effective against prepupal and pupal stages of WFT than the late L2 (Buitenhuis and Shipp, 2005; Premachandra *et al.*,2003; Tomalak *et al.,* 2005).

In our study, increasing the dose rate from 400 to 1000 IJs cm^{-2} lead to an increased onion thrips mortality as it already was revealed in previous studies for WFT (Ebssa *et al.,* 2003).

Since, using EPNs against thrips pest needs high concentrations and high costs, the option of combining EPNs with other pest management strategies is worth considering. Numerous abiotic and biotic factors influence infection of pathogens (Barbercheck, 1993). Many phytochemicals, especially allelochemicals and nutrients, can modify the physiology and growth of the insect host, that they can change the susceptibility to pathogens and/or the behavior of the insect host (Cory and Hoover, 2006). Our results indicated that thrips larvae that had been fed on pods of green bean were more susceptible to the tested EPN. It may be resulted from the antixenosis effect of green bean, *P. vulgaris* to *T. tabaci*. Frei*et al.* (2003, 2004) investigated antixenosis, antibiosis and tolerance as a mechanism of resistance to *Thrips palmi* in some genotypes of common beans. Antibiosis property of common bean against the onion thrips may increase the thrips susceptibility to EPNs, so that the thrips mortality rate can increase.

In the semi-field condition, foliar application of EPNs did not induce considerable mortality in the thrips larval stage that was similar with results of Buitenhuis and Shipp (2005). It may be due to thrips escaping behavior and habitat concealment (Buitenhuis and Shipp, 2005). Soil application of EPNs can be useful in the integrated management programs of the onion thrips.

In summary our results clearly indicate the potential of EPNs as promising component in future biological control strategies against the onion thrips. Considering that generally high concentrations, even for the most efficient EPN species/strains were required for the sufficient control of *T. tabaci*, resistant plants could be used as an integrated approach. More emphasis should be given to the study of new and more effective strains of EPNs and testing them in the greenhouse and field conditions.

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