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Combination of entomopathogenic nematodes and chemical insecticides for controlling the onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae) in the laboratory condition

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Abstract

Entomopathogenic nematode (EPN) combinations with other control agents are applied to obtain more desirable control of a pest by additive or synergistic effects on the pest mortality. In this study, the potential of EPNs (*Steinernema carpocapsae*, and *Heterorhabditis bacteriophora*) alone, chemical insecticides (imidacloprid, deltamethrin, and abamectin) alone, and EPN-insecticide combinations, against the onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae) was investigated. In the first experiments, the effects of different concentrations of insecticides and EPNs on the thrips larvae were tested and the LC_{10} and LC_{30} values of insecticides were calculated. Then using a pre-treatment bioassay, the thrips larvae were treated with the EPNs at two concentrations, 400 or 1000 infective juveniles (IJs) per cm^2, after they had been exposed to LC_{30} of abamectin (5.16 mg per litre (l), LC_{10} of imidacloprid (50.16 mg/l), or LC_{10} of deltamethrin (10.28 mg/l). Mortality percentages were recorded at different time intervals, 24, 48 and 72 hours after the EPNs utilization. Results showed that the sole application of EPNs caused less than 30% thrips mortality. The combination of *H. bacteriophora* and all insecticides interacted additively on the thrips mortality whereas imidacloprid treatment showed adverse effects on the efficacy of *H. bacteriophora* at 1000 IJs/cm^2. Additive and synergistic interactions resulted in combining insecticides with *S. carpocapsae*. These combinations gave mostly higher thrips mortality than with EPN alone and synergistic interactions were observed in *S. carpocapsae* application at 400 IJs/cm2 with imidacloprid and deltamethrin and also at 1000 IJs/cm^2 with abamectin.

Key words: Abamectin, *Heterorhabditis bacteriophora*, Imidacloprid, Integrated pest management (IPM), *Steinernema carpocapsae*.

تلفیق نماتدهای بیماری‌زای حشرات و حشره‌کش‌های شیمیایی در کنترل تریپس پیاز *Thrips tabaci* (Thysanoptera: Thripidae) در شرایط آزمایشگاه

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چکیده

تلفیق نماتدهای بیماری‌زای حشرات و دیگر عوامل کنترلی با ایجاد اثرات افزایشی یا سینرژیستی در مرگ و میر آفت، برای دستیابی به کنترل مطلوب‌تر یک آفت استفاده شده است. در این مطالعه پتانسیل نماتدهای بیماری‌زای حشرات (*Steinernema carpocapsae* و *Heterorhabditis bacteriophora*) به تنهایی، حشره‌کش‌های شیمیایی (ایمیداکلوپرید، دلتامترین و آبامکتین) به تنهایی و تلفیق نماتدها و حشره‌کش‌ها روی تریپس پیاز *Thrips tabaci* (Thysanoptera: Thripidae) مورد ارزیابی قرار گرفته است. در آزمایش‌های اولیه، اثر غلظت‌های مختلف حشره‌کش‌ها و نماتدها روی لارو تریپس آزمایش و مقادیر LC_{10} و LC_{30} حشره‌کش‌ها اندازه‌گیری گردید. سپس با انجام آزمایش‌های پیش‌تیمار، لارو تریپس ابتدا در معرض LC_{30} آبامکتین (۵/۱۶ میلی‌گرم/لیتر)، LC_{10} ایمیداکلوپرید (۵۰/۱۶ میلی‌گرم/لیتر) یا LC_{10} دلتامترین (۱۰/۲۸ میلی‌گرم/لیتر) قرار گرفته و سپس با دو غلظت ۴۰۰ یا ۱۰۰۰ نماتد جوان بیماری‌زا/سانتی‌مترمربع تیمار شد. درصد مرگ و میر در بازه های زمانی مختلف (۲۴، ۴۸ و ۷۲ ساعت پس از استفاده نماتد) ثبت شد. نتایج نشان داد که استفاده از نماتدها به تنهایی کمتر از ۳۰٪ مرگ و میر تریپس را دنبال داشته است. تلفیق *H. bacteriophora* و همه حشره‌کش‌ها بطور افزایشی بر مرگ و میر تریپس اثر داشت و تیمار ایمیداکلوپراید اثر سوئی بر عملکرد *H. bacteriophora* در غلظت ۱۰۰۰ جوان بیماری‌زا/سانتی‌مترمربع نشان داد. اثرات افزایشی و سینرژیستی در تلفیق حشره‌کش‌ها با *S. carpocapsae* مشاهده شد. این تیمارها در مقایسه با نماتد تنها مرگ و میر بالاتری ایجاد کردند و اثرات سینرژیستی در تلفیق *S. carpocapsae* غلظت ۴۰۰ جوان بیماری‌زا/سانتی‌مترمربع با ایمیداکلوپرید و دلتامترین و همچنین غلظت ۱۰۰۰ جوان بیماری‌زا/سانتی‌مترمربع با آبامکتین مشاهده گردید.

واژه‌های کلیدی: آبامکتین، ایمیداکلوپرید، مدیریت تلفیقی آفت (IPM)، *Heterorhabditis bacteriophora*، *Steinernema carpocapsae*.

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Introduction

Thrips tabaci Lindeman (Thysanoptera: Thripidae) is one of the most serious pest damaging onion (*Allium cepa*), leek (*Allium ampeloprasum*), and chives (*Allium schoenoprasum*) (Elad Pertot & Enkegaard, 2004). It has become a global pest during the past two decades (Diaz-Montano *et al*., 2011). Onion thrips can cause yield reduction of onion more than 50% but it can be more problematic by transmitting plant viruses like Tomato spotted wilt virus and Iris yellow spot virus (Jones, 2005; Diaz-Montano *et al*., 2011). Onion thrips has a short life cycle (Diaz-Montano *et al*., 2011). Onion thrips 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 25°C was 5.93 ± 1.00 (standard error), 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 (Oparaeke, 2006) and the weak spray coverage on the inner leaves where the insect is found (Diaz-Montano *et al*., 2011). The generalist predators like *Amblyseius* spp. (Acari: Phytoseiidae) and *Orius* spp. (Hemiptera: Anthocoridae) are most often considered for biological control of thrips (Loomans and Murai, 1997). Since thrips predators and parasitoides are effective only when thrips leave its refuge (Diaz-Montano *et al.*, 2011) and their application is only partly successful in some crops (Loomans and Murai, 1997), the option of using pathogens is worth attention and consideration.

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae have mutualistic relationship with symbiont bacteria representing an effective (Kaya and Gaugler, 1993) and alternative method for controlling many economically damaging pests. Currently, these biocontrol agents are used against several thrips species because of their pathogenic potential (Smith *et al*., 2005; Tomalak *et al*., 2005; Al-Siyabi *et al*., 2006; Saffari *et al*., 2013; Kashkouli *et al*., 2014). Among different thrips species, much of the work has been concentrated on the western flower thrips (WFT), *Frankliniella occidentalis* Pergande, which is a major pest of many field and greenhouse crops (Tomalak *et al*., 2005; Elimem *et al*., 2014). However, few studies have been conducted to evaluate the susceptibility of other thrips species, such as onion thrips, *T. tabaci*, to EPNs.

Previous studies on the effects of EPNs against WFT (Ebssa *et al*., 2003) and onion thrips (Al-Siyabi *et al*., 2006; Kashkouli *et al*., 2014) showed that high concentrations of EPNs were needed for sufficient control. In addition, EPNs had little effect against the foliar dwelling stages of the thrips such as the first and second instar larvae compared to prepupal and pupal soil dwelling instars (Ebssa *et al*., 2001; Al-Siyabi *et al*., 2006; Kashkouli *et al*., 2014). Therefore, the option of combining EPNs with other pest management agents for improving the control of thrips larval stages is worth considering.

EPNs are combined with other control agents for three major purposes: 1) to control different pest species or stages of one pest, 2) to facilitate nematode application with other control agents in tank-mixed usage, and 3) to achieve better control of a single pest by creating additive or, preferably, synergistic effects on pest mortality (Koppenhofer and Grewal, 2005) that the last one was our purpose in this study.

Imidacloprid, deltamethrin and abamectin are effective insecticides against thrips species (Workman and Martin, 2002; Diaz-Montano *et al*., 2011). These insecticides disrupt normal nervous system function of the insect, resulting in convulsion and paralysis of the insect (Yu, 2008). “This sluggishness facilitates host attachment of infective juvenile of nematodes” (Koppenhofer *et al*., 2000b). Using imidacloprid with simultaneous or delayed application of *S. glaseri* Steiner and *H. bacteriophora* Poinar resulted in synergistic mortality on different species of white grubs (Koppenhofer *et al*., 2000a; 2000b). Moreover, combined application of *S. carpocapsae* Weiser and thiacloprid or spiromesifen resulted in higher *Bemisia tabaci* Gennadius mortality than with the nematode alone (Cuthbertson *et al*., 2008).

The objective of this study was to evaluate the interaction of the EPNs, *S. carpocapsae* and *H. bacteriophora*, with insecticides (imidacloprid, deltamethrin and abamectin) on thrips mortality.

Materials and methods

Insecticides and culture of thrips and nematodes: EPNs used in this study were *H. bacteriophora* (Larvanem®) and *S. carpocapsae* (Capsanem®) supplied by Koppert B. V. (Berkel en Rodenrijs, The Netherlands). These nematodes were reared in the greater wax moth larvae *Galleria mellonella* L. (Lepidoptera: Pyralidae) at 25±2°C (Ehlers and Shapiro-Ilan, 2005). After infective juveniles (IJs) emergence, IJs were maintained in tap water at 6°C less than one month before use in the tests (Kaya and Stock, 1997). The nematodes were allowed to acclimatize for at least 5 hours at room temperature before use in the tests. Different concentrations of EPNs were prepared following the nematode quantification method (Kaya and Stock, 1997).

An onion thrips colony was began with naturally infested onion shoots collected from an onion field. *T. tabaci* was reared on the shoots of *Allium cepa* L. (Amaryllidaceae) (at 25±1°C, and 60-70% relative humidity (r.h.), under L18:D6 photoperiod) (with some pollen for adult rearing) in the laboratory, using the modified protocol described by Loomans and Murai (1997).

Insecticides were imidacloprid (35% SC) (Golsam formulate; Gorgan, Iran), deltamethrin (2.5% EC) (Golsam formulate; Gorgan, Iran), and abamectin (1.8% EC) (Agriman, Germany).

Effects of insecticides, EPNs, and EPN-insecticide combinations on the thrips: 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 the container lid and a cloth tissue (100µm pore size) was glued over it (Ebssa *et al*., 2001). The container bottom was covered by double layer of filter paper and twenty second instar thrips larvae were used for each experiment. The viability of EPNs was also examined before experiments. The control treatments were pipetted only with distilled water.

Effect of insecticides on the thrips: The lethal concentrations of the insecticides (LC_{10} and LC_{30}) were determined using dose-response experiments (Yu 2008). The preliminary doses resulted in 20-80% thrips mortality were selected based on the pre-tests. Twenty thrips larvae were introduced to containers with different concentrations of insecticides (50, 100, 250,400, 500, 800, and 1000 mg/l for imidacloprid; 10, 20, 40, 80, and 120 mg/l for deltamethrin, and 5, 10, 15, 20, and 40 mg/l for abamectin) or water (as the control) had been pipetted into the covered filter paper. After exposure to insecticides for eight hours, thrips were transferred to the same container which had been pipetted with one ml water, for 24 hours.

Effect of EPNs on the thrips: The experiments were designed as mentioned for insecticides. For these experiments, water was used as the pre-treatment (exposure for eight hours) and treatments (exposure to EPNs for 24, 48, and 72 hours) were included: *S. carpocapsae* or *H. bacteriophora* at concentrations of 400 or 1000 IJs/cm^2 in one ml of distilled water. The EPN concentrations were chosen based on previous studies (Ebssa *et al*., 2001; Ebssa *et al*., 2003; Premachandra *et al*., 2003; Kashkouli *et al*., 2014).

Effects of EPN-insecticide combinations on the thrips: The experiments were conducted as mentioned before (part 2.2.1). For these experiments, the pre-treatments (insecticide exposure for eight hours) included: LC_{30} of abamectin (≈5 mg/l), LC_{10} of imidacloprid (≈50 mg/l), or LC_{10} of deltamethrin (≈10 mg/l) and treatments (exposure to EPNs for 24, 48, and 72 hours) included: *S. carpocapsae* or *H. bacteriophora* at concentrations of 400 or 1000 IJs/cm^2 in one ml of distilled water.

After preparing each experiment (parts 2.2.1, 2.2.2 and 2.2.3), the container was closed and maintained in an incubator (25±1°C, 60-70% r.h., and L18:D6 photoperiod). The larval mortality was recorded 24 hours after starting treatment experiments. In all EPN treatments, a sub-sample of dead insects (10% of total dead insects) was dissected to detect the presence of nematodes. Each experiment was replicated three times. All experiments were conducted under the same conditions.

Statistical analysis: The log dosage-probit lines for insecticides were plotted using SigmaPlot program version 12.0. The LC_{10}, LC_{30} and LC_{50} were analyzed using the PROC PROBIT procedure in SAS program version 9.2 (SAS Institute 2008).

The corrected mortality (CM) of data (in the experiments determining effects of insecticides, EPNs and EPN-insecticides combinations on the thrips) was calculated

using Abbot's formula (Abbott 1925). The data were arcsine transformed before subjected to statistical analysis. The analysis was carried out in completely randomized design using the PROC ANOVA procedure in SAS program version 9.2. Significant differences of means were evaluated using the LSD comparison test at 95% level (SAS Institute 2008).

Synergistic, additive, or antagonistic interactions between insecticides and EPNs were determined using a chi-square test followed by calculating the expected additive mortality, $M_E=M_N + M_I (1-M_N)$ (M_N and M_I are the observed mortality caused by EPNs and insecticides, respectively). The results from χ^2 tests, $\chi^2= (M_{N1}-M_E)2/M_E$ (M_{NI} is the observed mortality for nematode-insecticide combinations), were compared to the χ^2 table value for 1 d.f. (Koppenhofer *et al*., 2000b; Koppenhofer *et al*., 2002).

Results and Discussion

Insecticides efficacy on the thrips: In this study, we tested imidacloprid, deltamethrin, and abamectin that disrupt normal nervous system function of the insect by acting as a nicotinic acetylcholine receptor agonist, a sodium channel modulator, and a chloride channel activator, respectively, resulting in convulsion and paralysis of the insect (Yu, 2008).

Log dosage-probit lines and lethal dosages for imidacloprid, deltamethrin, and abamectin declared in Table 1. The LC10 of imidacloprid (≈50 mg/l) and deltamethrin (≈10 mg/l), and LC30 of abamectin (≈5 mg/l) were chosen for further experiments because for determining interactions (especially synergism), the mortality rate induced by presumed synergistic factor should be low enough to let for statistically significant improvements (Koppenhofer and Grewal, 2005). Also, these insecticides were used at low concentrations because in the integrated pest management (IPM) programs, pesticides should be able to control effectively at low application dosages (Jensen, 2000).

Table 1. Effects of different insecticides on the mortality of onion thrips second instar larvae

Insecticide name	Slope ± SE[a]	LC_{10} mg/l (95% FL)	LC_{30} mg/l (95% FL)	LC_{50} mg/l (95% FL)	χ^2 (d.f.)[b]	*P* [c]	Log dosage-probit line
Imidacloprid	0.674 ± 0.104	50.16 (18.07-87.02)	154.35 (89.72-216.73)	336.22 (243.6-455.59)	143.14 (18)	0.0001	y=1.397x + 1.572 R^2=0.887
Deltamethrin	0.958 ± 0.141	10.28 (4.89-15.6)	22.66 (14.72-30.09)	39.17 (29.4-50.92)	103.75 (13)	0.0001	y=2.12x + 1.7 R^2=0.9415
Abamectin	0.879 ± 0.133	2.18 (0.86-3.54)	5.16 (3.03-6.97)	9.36 (6.91-11.62)	66.14 (13)	0.0001	y=1.87x + 3.3 R^2=0.918

[a]SE, standard error; [b]Pearson χ^2 value; [c]*P* , the probability of the slope

Effects of insecticides, EPNs, and EPNs-insecticides combinations on the thrips: Several studies demonstrated that the impact of EPNs on WFT can be improved when the EPNs are combined with predatory mites (Premachandra *et al*., 2003; Ebssa *et al*., 2006). However, efficacy of this integration is highly dependent to greenhouse humidity and temperature conditions (Ebssa *et al*., 2006). A more applicable and effective strategy should be the combination of nematodes and insecticide (Koppenhofer *et al*., 2000a).

The different treatments of insecticides, EPNs, and combinations of EPNs and insecticides, resulted in significant differences in efficacy against the onion thrips (*S. carpocapsae* and insecticides: *F*=22.01; *d.f.*=10; *P*=0.0001, *H. bacteriophora* and insecticides: *F*=7.35; *d.f.*=10; *P*=0.0001) (Table 2 and 3). For all experiments, mean mortality values in the control treatments were less than 8% except for combinations of EPNs and insecticides at 48 and 72h with 23±0.82 and 31±6.78 control mortality, respectively. The control mortality after 72h (31±6.78) was so high, at the other hand, it was reported that the EPNs can kill their host within 24-48h as WFT prepupae and pupae were infected and killed by *S. feltiae* within only 2-4h after infection (Tomalak *et al*., 2005), so results for 72h (from all treatments) were supposed not reliable and they were omitted.

The sole application of *S. carpocapsae* at 400 IJs/cm^2

provided 9.83% and 11.02% thrips corrected mortality (CM) for 24 and 48h, respectively, whereas the CM for 24 and 48h at concentration of 1000 IJs/cm^2 were 25.26% and 22.92%, respectively. *S. carpocapsae* application at 1000 IJs/cm^2 caused significant higher thrips mortality than 400 IJs/cm^2 (Table 2).

Application of *H. bacteriophora* alone at 400 IJs/cm^2 induced 5.07% and 4.86% thrips CM, respectively, whereas the CM for 24 and 48h at concentration of 1000 IJs/cm^2 were 27.44% and 27.65%, respectively. Significant higher thrips mortality resulted at *H. bacteriophora* sole application at 1000 IJs/cm^2 (Table 3).

Sole applications of *S. carpocapsae* and *H. bacteriophora* caused low thrips larval mortality. High larval mobility may have reduced IJ attachment to the insects (Buitenhuis & Ship 2005). The immobile stages of the most serious greenhouse pests such as larvae of *Tuta absoluta* Meyrick (Garcia-del-Pino, Alabern and Morton, 2013), leafminer larvae (Williams and Walters, 2000), immature whitefly (Cuthbertson *et al*., 2003) and prepupal and pupal stages of the western flower thrips (Ebssa *et al*., 2001) have showed high susceptibility to EPNs (Fig. 1).

Each tested concentration of *S. carpocapsae* in combination with insecticides gave significantly higher thrips mortality than with the nematode alone, except for *S. carpocapsae* at 1000 IJs/cm^2 combined with imidacloprid at 48h and deltamethrin at 24 and 48h (Table 2). Additive/synergistic interactions were observed when *S. carpocapsae* was integrated with insecticide application. *S. carpocapsae* at 400 IJs/cm^2 combined with imidacloprid (24h: χ^2=8.366; *d.f.*=1; *P*=0.0038; 48h: χ^2=8.244; *d.f.*=1; *P*=0.0053) and deltamethrin (24h: χ^2=4.003; *d.f.*=1; *P*=0.045; 48h: χ^2=6.001; *d.f.*=1; *P*=0.009), and at 1000 IJs/cm^2 combined with abamectin (24h: χ^2=3.976; *d.f.*=1; *P*=0.0462; 48h: χ^2=3.976; *d.f.*=1; *P*=0.0462) interacted synergistically on the thrips mortality (Table 2). In the combined *S. carpocapsae*-insecticides treatments, the lower IJ concentration (400 IJs/cm^2) gave similar mortality compared to the higher IJ concentration (1000 IJs/cm^2), except for nematode-abamectin combination (Table 2). The efficacy of *S. carpocapsae* combinations showed no significant differences after 24 and 48h.

Table 2. Effect of the insecticides imidacloprid (LC_{10}≈50mg/l), deltamethrin (LC_{10}≈10mg/l), and abamectin (LC_{30}≈5mg/l), *Steinernema carpocapsae* at 400 and 1000 infective juveniles (IJs)/cm^2, or the combination of each insecticide as pre-treatment (8 hours) with *S. carpocapsae* as treatment (24 hours) on onion thrips mortality (mean corrected mortality ± SE). Different letters indicate significantly differences (*P*= 0.05, LSD test). An **/ * indicates significant synergistic interactions between each EPN and insecticide at *P*= 0.01 and 0.05, respectively (χ^2 test).

Experiment type	Insecticides (pre-treatment)	*S. carpocapsae* (IJs/cm^2) (treatment)	Corrected mortality±SE[a]		
			8h	24h	48h
Sole application of nematodes	-	400 IJs/cm^2	-	9.83±2.31^{e}	11.02±1.49^{e}
		1000 IJs/cm^2		25.26±4.74^{d}	22.92±5.01^{d}
Sole application of insecticides	Imidacloprid	-	7.36±1.56^{f}	-	-
	Deltamethrin		8.08±2.08^{f}		
	Abamectin		25.97±6.36^{d}		
Combinations of insecticides and nematodes	Imidacloprid	400 IJs/cm^2	-	41.47±7.39^{bcd**}	44.36±3.28^{bcd**}
		1000 IJs/cm^2		49.8±8.28^{bc}	44.98±6.34^{bcd}
	Deltamethrin	400 IJs/cm^2		33.36±1.11^{cd*}	32.68±2.12^{cd*}
		1000 IJs/cm^2		36.85±7.36^{cd}	40.41±3.35^{bcd}
	Abamectin	400 IJs/cm^2		60.09±9.6^{b}	63.36±1.9^{b}
		1000 IJs/cm^2		86.82±1.15^{a*}	87.11±2.39^{a*}

[a] Corrected mortality percentage after treatment

Table 3. Effect of the insecticides imidacloprid (LC_{10}≈50mg/l), deltamethrin (LC_{10}≈10mg/l), and abamectin (LC_{30}≈5mg/l), *Heterorhabditis bacteriophora* at 400 and 1000 infective juveniles (IJs)/cm^2, or the combination of insecticide with *H. bacteriophora* on onion thrips mortality (mean corrected mortality ± SE). Different letters indicate significantly differences (P= 0.05, LSD test).

Experiment type	Insecticides (pre-treatment)	*H. bacteriophora* (IJs/cm^2) (treatment)	Corrected mortality±SE[a]		
			8h	24h	48h
Sole application of nematodes	-	400 IJs/cm^2	-	5.07±1.96 [b]	4.86±2.36 [b]
		1000 IJs/cm^2		27.44±1.21 [a]	27.65±2.27 [a]
Sole application of insecticides	Imidacloprid	-	7.36±1.56 [b]	-	-
	Deltamethrin		8.08±2.08 [b]		
	Abamectin		25.97±6.36 [a]		
Combinations of insecticides and nematodes	Imidacloprid	400 IJs/cm^2	-	4.01±1.78 [b]	5.61±0.12 [b]
		1000 IJs/cm^2		3.84±1.24 [b]	2.97±2.01 [b]
	Deltamethrin	400 IJs/cm^2		3.65±1.96 [b]	5.49±0.09 [b]
		1000 IJs/cm^2		22.69±8.86 [a]	18.34±3.55 [a]
	Abamectin	400 IJs/cm^2		27.80±1.31 [a]	30.63±2.89 [a]
		1000 IJs/cm^2		36.80±8.83 [a]	28.25±3.92 [a]

[a] Corrected mortality percentage after treatment

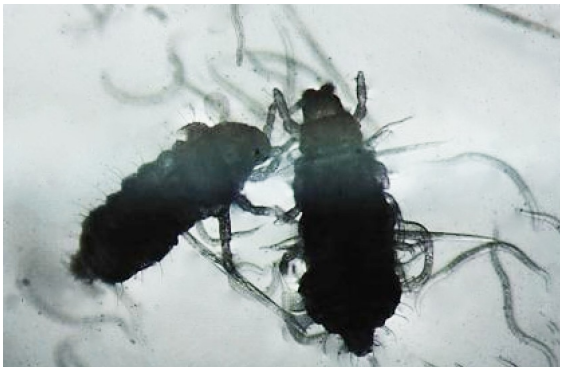

Fig. 1. *Steinernema carpocapsae* penetration to the onion thrips pupae

H. bacteriophora at 1000 IJs/cm^2 combined with imidacloprid and at 400 IJs/cm^2 combined with abamectin induced significantly different mortality compared to the nematode alone (Table 3). Imidacloprid pretreatment showed significantly adverse effect on the efficacy of *H. bacteriophora* at 1000 IJs/cm^2 (24h: 3.84%, 48h: 2.97% mortality), compared with the nematode alone (24h: 27.44%, 48h: 27.65% mortality). Application of abamectin significantly increase the efficacy of *H. bacteriophora* at 400 IJs/cm^2 (24h: 27.8%, 48h: 30.63% mortality), compared with the nematode alone (24h: 5.07%, 48h: 4.86% mortality). No significant different mortality was observed between *H. bacteriophora*-insecticide combinations and insecticides alone, expect for *H. bacteriophora* at 1000 IJs/cm^2 with deltamethrin at both time intervals. In the combined *H. bacteriophora*-insecticide treatments, lower concentrations of IJs gave similar effects as compared to the higher concentrations, except for EPN-deltamethrin combinations (Table 3). Additive interactions occurred in all combinations of *H. bacteriophora* and different insecticides (χ^2≤2.37; *d.f.*=1; P≥0.124).

S. carpocapsae in combination with insecticides demonstrated more control potential for management the larval stage of the onion thrips than *H. bacteriophora*. Elad *et al*. (2004) investigated the pathogenic effect of EPNs (*S. feltiae* and *H. bacteriophora*) alone or in combination with entomopathogenic fungi (*Beauveria bassiana, Lecanicillium muscarium* formerly *Verticillium lecanii*, and *Paecilomyces fumosoroseus*) against the onion thrips and showed that *S. feltiae* combinations with *P. fumosoroseus* and *L. muscarium* significantly reduced in the number of thrips per plant. Moreover, the integrated use of *S. carpocapsae* with thiaclopride and spiromesifen has resulted in higher *B. tabaci* control than using this nematode alone (Cuthbertson *et al*., 2008).

Many studies have assessed the compatibility and the tank-mixing potential of agrochemicals with EPN species (Head *et al*., 2000; Krishnayyaand and Grewal, 2002; De Nardo and Grewal, 2003; Alumai and Grewal, 2004). The compatibility of EPNs, *H. bacteriophora* and *S. carpocapsae* with selected pesticide formulations used in turf grass revealed that the majority of chemicals induced no significant

reduction in levels of IJs viability and pathogenicity of either nematode. *H. bacteriophora* pathogenicity actively increased after exposure to imidacloprid (Alumai and Grewal, 2004).

Different concentrations of EPNs were also examined to determine the best integrations for the thrips control. Our results showed that in the combined EPN-insecticide treatments, lower concentration of EPNs gave similar effects compared to the higher concentrations. Ebssa *et al*. (2006) compared different densities of *Amblyseius cucumeris* Oudemans and concentrations of *H. bacteriophora* and *H. indica* Poinar, Karunakar, and David to determine the best combination of these agents against WFT. The combined application of 10 adult mites with 200 IJs/cm^2 of EPNs resulted in about 83% thrips control.

This is the first report comparing individual and combined release of the EPNs and insecticides for the control of *Thrips tabaci*. We observed high thrips mortality after combination of EPNs and insecticides compared to individual control agents. In the present study, we selected second instar larvae for the bioassay experiments because previous study comparing different EPNs against the trips stages revealed that 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. Prepupae and pupae are quiescent, non-feeding, and inactive instars that move only when disturbed. Other pests controlled by EPNs, such as sugar beet beetle pupa, leafminer larvae, larvae of *Tata absolute* and immature whitefly are also immobile. There are also some reports that *steinernema* and *Heterorhabditis* spp. were more affective against prepupal and pupal stages of WFT than the late L2 (Kashkouli *et al*. 2014). Synergistic and additive interactions between different insecticides and EPNs for controlling the onion thrips larvae were observed. Synergistic/additive interactions may occur because of paralysis in the insecticides-treated thrips. Untreated thrips larvae are very mobile (Mortiz, 1997) and show evasive behavior toward nematodes (Buitenhuis and ship, 2005), but after treating the larvae with insecticides, activity is reduced and that may facilitate nematode attachment (Koppenhofer *et al*., 2002). This mechanism has also been reported as a main factor for synergistic interactions observed in white grubs treated with imidaclopride-EPN combinations (Koppenhofer *et al*., 2000b). Similar effects of synergistic interactions have been showed when entomopathogenic fungi combined with imidacloprid. The sensitivity of termite species, *Reticulitermes flavipes* (Kollar), was increased when it was treated with the combination of entomopathogenic fungi and imidacloprid (Koppenhofer *et al*., 2000b).

One another possible mechanism for additive/synergistic insecticide-pathogen interactions appears to be the effect of insecticides on cellular and humoral responses of the insect immunity system. Several studies have indicated the impact of insecticides on the hemocyte number, differentiation, phagocytosis, phenoloxidase and malanization activities, or antimicrobial peptide production (James and Xu, 2012). This weaken immunity system may raise pathogen infection risk as observed in *Nosema*-insecticide treated honeybees (Alaux *et al*., 2010).

In summary, the additive/synergistic interaction of some insecticides with entomopathogenic nematodes is an integrated control method against the larval stage of onion thrips. However, more information about using such combinations with simultaneous EPN-insecticide application against onion thrips is needed.

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