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Biological control of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), in open fields using Egyptian entomopathogenic nematode isolates

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Abstract

Entomopathogenic nematodes (EPNs) of families Heterorhabditidae and Steinernematidae are known to be effective against a variety of pests. In the present work, different EPNs that are isolated from the Egyptian environment were tested against the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), under field conditions. The Egyptian EPNs were tested at different concentrations and against different onion thrips stages (adult and nymph). When used as a foliage spray, the tested EPN isolates were efficient against both adult and nymph stages. Differences in pathogenicity were observed within the same EPN species as in *Heterorhabditis indica* (EGAZ3) that caused higher reduction in population of *T. tabaci* (adult and nymph) after 24 and 48 h at a concentration of 10,000 infective juveniles (IJs)/ml compared to *H. indica* (EGAZ2) after 24 h post treatment at a concentration of 15,000 IJs/ml. The same pattern was observed with *Heterorhabditis bacteriophora* (HP88), where a higher reduction percent was recorded after 24 h at a concentration of 20,000 IJs/ml. On the other hand, *Steinernema carpocapsae* (All), *S. carpocapsae* (EGAZ9), and *S. carpocapsae* (BA2) isolates were less effective in controlling onion thrips (adult and nymph) population. In general, controlling *T. tabaci* at the nymphal stage was more efficient than at the adult stage (12–73% reduction at the nymph stage compared to 6–65% at the adult stage). It was also found that increasing inoculation concentration above 15,000 IJs/ml had no significant difference in controlling onion thrips populations.

Keywords: Entomopathogenic nematodes (EPNs), *Thrips tabaci*, Field conditions, Biological control

Background

Onion, *Allium cepa* L. (Amaryllidaceae (Alliaceae), is an important cash crop in Egypt for local consumption and exportation. Onion plants are infested with different insect pests throughout their growing season (Mahmoud, 2008 and Diaz-Montano et al., 2011). One of the major destructive pests of onion is onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), which feeds on onion plants during their vegetative growth and fruit filling (Mahmoud, 2008).

Management of *T. tabaci* has proved to be problematic, due to its minute size and its thigmotactic behavior (Lewis, 1997). Traditionally, controlling thrips is conducted via the usage of chemical applications, which may explain the widespread chemical-resistance development in onion thrips (Jensen, 2000). Entomopathogenic nematodes (EPNs) as one of the biological control agents are obligate pathogens, which possess free-living third stage infective juvenile (IJ) characteristics. This characteristic, in particular, enables them to kill insects via releasing their symbiotic mutualistic bacteria (*Xenorhabdus* and *Photorhabdus* in Steinernema and Heterorhabditis, respectively), which, in turn, causes insect death within 24–48 h (Kaya and Gaugler, 1993 and Gaugler, 2002).

EPNs have been used successfully as an alternative to traditional pesticides in controlling several pests localized in soil, caterpillars, leaf miners, and thrips (Williams and Walters, 2000 and Laznik et al., 2010). EPNs have been used to control larval stages of western flower thrips

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(WFT), *Frankliniella occidentalis*, in ornamental plants and greenhouse vegetables (Wardlow et al., 2001 and Trdan et al., 2007b). Foliar applications of the IJ stage for both *Steinernema feltiae* (Filipjev) and *Lecanicillium muscarium* (Petch) were found to offer various levels of control over other quarantine invertebrate pest species including leaf miners and whiteflies (Williams and Walters, 2000 and Cuthbertson and Walters, 2005).

Onion thrips feed on the transition-zone parts of leaves (pale green color) that provides an ideal situation to control infestation at early stages (Theunissen and Legutowska, 1991). Therefore, foliage spray to this zone could increase the efficiency of EPN application against onion thrips (Shiberu and Mohammed, 2014). For this reason, the weak activity associated with foliage applications could be due to the negative efficiency of high temperature, low humidity, and direct exposure to sunlight (Shapiro-Ilan et al., 2006 and Jung, 2008).

In the present work, the efficiency of foliar application of several Egyptian EPN isolates was tested against the onion thrips under field conditions.

Methods

All EPN isolates used in this study were reared at 25 ± 2 ° C in greater wax moth larvae *Galleria mellonella* L. (Lepidoptera: Pyralidae), following the procedure of Ehlers and Shapiro-Ilan (2005). Six isolates of EPNs were used: four were isolated from Egyptian soil (*Heterorhabditis indica* (EGAZ2), *H. indica* (EGAZ3), *Steinernema carpocapsae* (EGAZ9), and *S. carpocapsae* (BA2), unpublished data) and two were imported from Biosys Palo Alto, CA (USA) (*Heterorhabditis bacteriophora* (HP88) and *S. carpocapsae* (All)) (Table 1).

In general, IJs were acclimatized through soaking in tap water for at least 5 h at room temperature before usage. Different concentrations (i.e. 10,000, 15,000, and 20,000 IJs/ml) of EPNs were prepared following the nematode quantification method (Woodring and Kaya, 1988 and Kaya and Stock, 1997). A formulation of 0.1% Tween 80 was added to the nematode suspension to maximize the efficiency of onion leave coating while spraying.

Table 1 Six entomopathogenic nematode isolates (EPNs) tested to control *Thrips tabaci* in field treatments

Strains of EPN	Source
Heterorhabditis bacteriophora (HP88)	Biosys, Palo Alto, CA (USA)
Steinernema carpocapsae (All)	
Heterorhabditis indica (EGAZ2)	Isolated from Ismailia Governorate, Egypt,by Azazy et al. (unpublished data)
Accession no. KY088204	
Heterorhabditis indica (EGAZ3)	
Accession no. KY088205	
Steinernema carpocapsae (EGAZ9)	Isolated from Sharkia Governorate, Egypt, by Azazy et al. (unpublished data)
Accession no. KY088211	
Steinernema carpocapsae (BA2)	Isolated by Hussein and Abou El-Soud (2006)

The experiment was conducted at the experimental research station, Faculty of Agriculture, Benha University. Onion seedlings, var. Giza 20, were planted in December 2015 using the recommended agriculture practices, while in 2016 the onion seedlings were planted on the last week of March at the experimental site. A complete randomized block design (~ quarter feddan) with four replicates was implemented; each replicate consisted of four 12-m-long rows; the distance between rows was 10 cm with 5 cm distance among the plants; and a 1-m-long distance was used to separate each block.

The trail started when thrips population (adults and nymphs) was at its peak. The thrips number on plants was counted immediately before treatment and 24 and 48 h post treatment by randomly selecting five plants and vigorously shaking them over a white sheet.

Foliage application was performed in the open field 3 h before sunset (at ~ 4:00 PM). The spray was conducted by directing the nozzle to the plant base, as well as the surrounding soil. The control plots were sprayed with water. The reduction in thrips population was calculated using Henderson and Tilton equation (Henderson and Tilton, 1955). Thrips were considered dead when they did not respond to mechanical stimulation and showed discoloration symptoms. Furthermore, random cadavers were selected for dissection and examination under a binocular microscope to confirm the presence of EPN inside.

For statistical analysis, the percentage reduction values in the present study were normalized using arcsine transformation. The significance of the main effects was performed using analysis of variance (ANOVA), Duncan's multiple range test ($P < 0.05$), and SAS program (SAS Institute, 2002).

Results and discussion

Effect of EPN applications against onion thrips population

In general, all EPN applications caused measurable reductions in *T. tabaci* nymph and adult population 24 and 48 h post treatment.

For the adult stage, at a concentration of 10,000 IJs/ml, a significant reduction in thrips population (65.6%) was achieved using "EGAZ3" isolate, followed by "All" isolate (55.8%), 24 h post treatment (Fig. 1a), while after 48 h "EGAZ3" isolate still caused the highest mortality rate, followed by "EGAZ9," "All," and "EGAZ2" (47.1, 46.8, 37.9, and 33.4%, respectively (Fig. 2a). At a concentration of 15,000 IJs/ml, the thrips adult population was reduced significantly to 62.1 and 55.2% 24 h post treatment with "EGAZ2" and "HP88" isolates, respectively

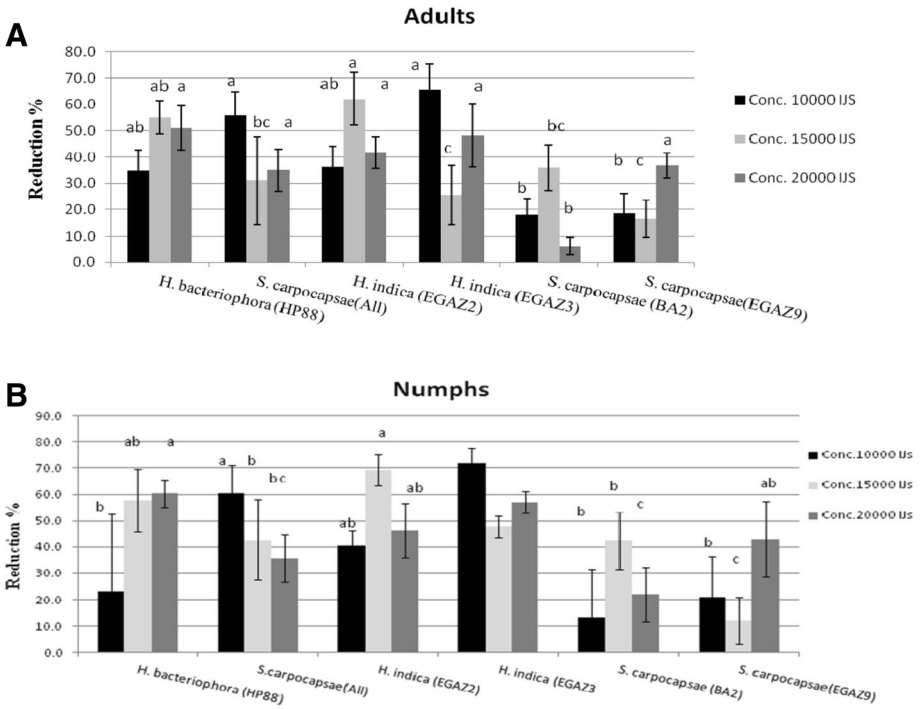

Fig. 1 Reduction percentage for different treated stages using the EPNs after 24 h at different concentrations: **a** for *T. tabaci* adults, **b** for *T. tabaci* nymphs. Columns annotated with the same letter are not significantly different ($P < 0.05$)

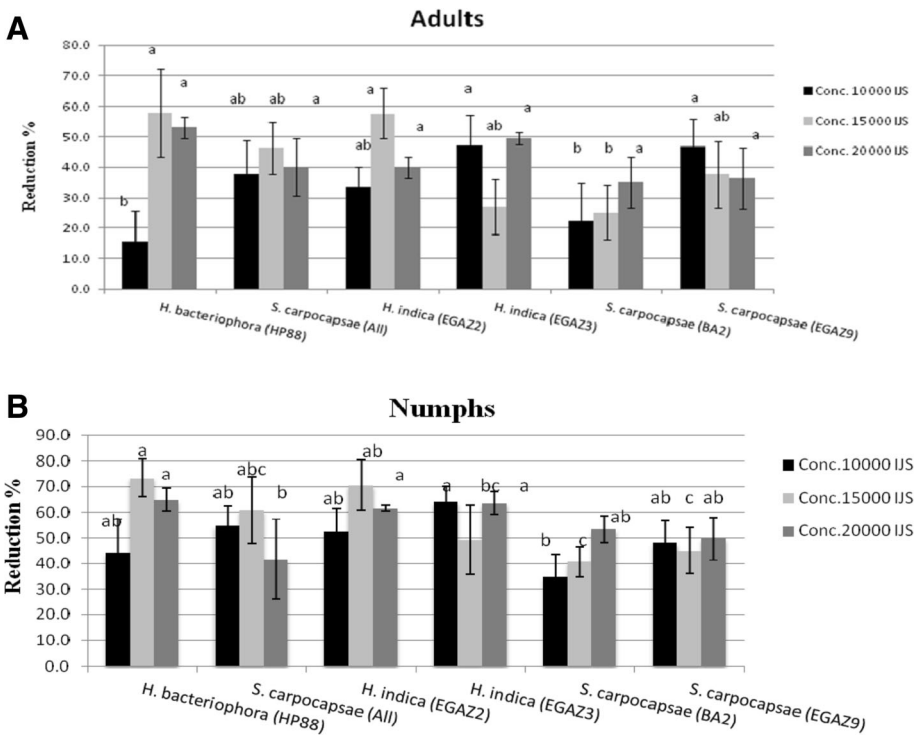

Fig. 2 Reduction percentage for different treated stages using the EPNs after 48 h at different concentrations: **a** for *T. tabaci* adults, **b** for *T. tabaci* nymphs. Columns annotated with the same letter are not significantly different ($P < 0.05$)

Table 2 Mean reduction (%) of life stages of onion thrips as induced by six different entomopathogenic nematode isolates (EPNs) at different concentrations

Concentrations (IJs/ml)	Mean reduction % ± SE*											
	H. bacteriophora (HP88)		*S. carpocapsae* (All)		*H. indica* (EGAZ2)		*H. indica* (EGAZ3)		*S. carpocapsae* (BA2)		*S. carpocapsae* (EGAZ9)	
	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
10,000	52.28 ± 17.1 b	33.77 ± 20.1 b	46.83 ± 9.7 a	57.6 ± 8.6 a	34.78 ± 7.3 b	46.48 ± 7.1 b	56.4 ± 4.8 a	66.0 ± 1.2 a	20.2 ± 16.6 a	24.05 ± 12.9 a	32.76 ± 10.9 a	34.58 ± 11.1 a
15,000	56.52 ± 10.1 a	65.62 ± 9.4 a	38.75 ± 8.4 a	51.82 ± 10.1 ab	59.9 ± 6.4 a	69.93 ± 7.5 a	26.23 ± 14.3 b	60.33 ± 7.5 a	30.5 ± 5.1 a	41.58 ± 5.1 a	27.1 ± 9.7 a	28.48 ± 4.5 a
20,000	52.03 ± 4.5 a	62.58 ± 3.8 a	37.6 ± 8.3 a	38.78 ± 11.8 b	40.85 ± 7.3 b	54.03 ± 5 b	48.8 ± 6.2 a	48.57 ± 4.5 b	20.7 ± 1 a	37.63 ± 4.5 a	36.58 ± 9.2 a	46.48 ± 4.5 a
F	6.47	5.54	0.71	2.42	13.57	7.65	6.72	7.37	0.69	1.75	0.26	1.46
P	0.0094	0.0157	0.51	0.123	0.0004	0.0051	0.0083	0.0059	0.52	0.208	0.783	0.263
LSD	20.015	26.89	18.1	18.63	10.7	13.05	18.22	10.87	21.15	20.98	22.9	22.7

*% reduction within a row followed by the same letters are not significantly different at ($P < 0.05$)

(Fig. 1a), while 48 h post treatment, the reduction caused by "HP88" and "EGAZ2" isolates were almost the same (57.9 and 57.7%, respectively). Increasing the concentration to 20,000 IJs/ml did not significantly improve the mortality rate in thrips either after 24 h (51.0 and 48.2% reduction in "HP88" and "EGAZ3" isolates, respectively) or after 48 h (53.0 and 49.3% reduction in "HP88" and "EGAZ3" isolates, respectively) (Figs. 1a and 2a).

For the nymphal stage, at a concentration of 10,000 IJs/ml, a significant reduction in thrips population was observed 24 h post treatment (71.8% using "EGAZ3" isolate, followed by 60.4% for "All" isolate) (Fig. 1b). At 15,000 IJs/ml of "EGAZ2" and "HP88," the highest nymph mortality with 69.6 and 57.8%, respectively, was observed. Similar to the obtained results on adults, increasing the concentration to 20,000 IJs/ml did not improve the mortality rate in nymphs, 24 h post treatment (Fig. 1b). In general, measuring mortality rates in nymphs 48 h post treatment was not significantly different than after 24 h (Fig. 2b).

Data in Table 2 revealed that isolate "EGAZ2" had the highest reduction (59.9 and 69.9%) of adults and nymphs, respectively, at a concentration of 15,000 IJs/ml. There were significant differences in the mortality levels achieved when comparing adults vs. nymphs; for example, at a concentration of 10,000 IJs/ml, "EGAZ3" isolate caused more reduction in nymphs than adults (56.4 and 66.0%, respectively); these differences were tested at all concentrations. Moreover, isolates "All," "EGAZ9," and "BA2" were found to be the least effective (Table 2). The results clearly revealed that significant reduction in onion thrips population (at nymph and adult stages) was achieved upon EPN treatments; significant differences were observed among the different isolates used, yet no differences were observed in onion thrips mortality percentage among the different concentrations (Table 3).

It is well-known that EPN species/strains could vary tremendously in their virulence (expressed in terms of pathogenicity) against different host insects (Laznik et al., 2010), and their efficiency is highly affected by different factors including concentration, host density, and temperature (Trdan et al., 2009). EPN foliage application to control thrips in onion could be an applicable method (Trdan et al., 2007a, b), where spraying application could provide an effective way to cover leaves and parts of plants that are not exposed to direct sunlight (preferable infective areas for thrips).

Results presented by Premachandra et al. (2003) and Ebssa et al. (2004) suggested that western flower thrips (WFT) was more susceptible to *Heterorhabditis* than to *Steinernema* species/strains. Therefore, Ebssa et al. (2004) used *S. carpocapsae* strain "S.S2" isolated from Egypt and compared it to "A1 B5" isolated from Italy, and found that the Egyptian strain caused significantly higher mortality in WFT than the Italian strain, which

Table 3 Summary of ANOVA results for reduction % of different *T. tabaci* life stages caused by different treated concentrations (10,000, 15,000 and 20,000 IJs/ml) of entomopathogenic nematodes (EPNs)

	df	*F*	*P*
Sources			
EPNs	5	11.35	0.0007***
EPN concentration	2	1.03	0.3935ns
Life stage	1	11.48	0.0069**
Interaction			
EPNs × EPN concentration	7	3.32	0.0358*
EPN concentration × life stage	3	1.37	0.2984ns
EPNs × life stage	6	0.93	0.6121ns
EPNs × EPN concentration × life stage	8		

*, **, and *** indicate significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

may be explained by differences in geographical origin and environmental adaptation.

On the other hand, Ebssa et al. (2001) reported that *H. indica* "LN2" isolate was more effective, in terms of its foraging behavior, than *S. bicornutum*. Similarly, *H. bacteriophora* was five times more effective than *S. carpocapsae*, in controlling prepupa of *T. tabaci* (Kamali et al., 2013).

The usage of nematodes to control thrips has been reported and suggested by some authors (Cuthbertson and Walters, 2005; Buitenhuis and Shipp, 2005). EPNs may offer a more suitable solution to deal with sedentary/settled insect pests (immature stage of *Bemisia tabaci* (Gennadius)) (Cuthbertson and Walters, 2005) or in the case of leaf-mining larvae (Williams and Walters, 2000). It was suggested that susceptibility of the nymph stage in onion thrips to EPNs is directly related to the nymph's limited mobility (Buitenhuis and Shipp, 2005); therefore, it was no surprise that *Thrips palmi* larvae were more susceptible to *S. feltiae* infection compared to adults (North et al., 2006).

In our results, a significant difference was found between the Egyptian isolates and the isolates from the USA. This finding is in line with Premachandra et al. (2003) who found no differences in mortality rate of WFT when commercial isolates (*S. fetiae* Nemaplus®) vs. local isolates (*H. bacteriophora* HD01) were compared, while contradicting the results reached by Helyer et al. (1995).

Foliage application of EPN was recommended by different groups to control WFT in Verbena leaves (Wardlow et al., 2001) by spraying *S. feltiae* to control the population of *F. occidentalis* (Arthurs and Heinz, 2006).

In the present work, use of different studied IJ/ml concentrations of the tested EPNs was found to have a little effect on increasing the reduction rate of onion thrips, which is in agreement with Premachandra et al. (2003), Ebssa et al. (2004), Kamali et al. (2013), and Kashkouli et al. (2014).

Conclusions

In conclusion, we demonstrate the efficiency of using EPNs in biological control of onion thrips under an open field condition in Egypt.

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Author Contributions

AMA designed and supervised the overall experiment. IAE and AEHA carried out the field experiment and recording data. MFMA performed sequence analysis to identify the isolated by molecular techniques and interpreted the results and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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