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Efficacy of three local isolates of entomopathogenic nematodes against the tomato leafminer, *Tuta absoluta* (Meyrick)

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Eficacia de tres aislados locales de nematodos entomopatógenos contra el minador del tomate, *Tuta absoluta* (Meyrick)

RESUMEN. El minador del tomate, *Tuta absoluta* (Meyrick), plaga clave del cultivo de tomate tanto en invernadero como en campo abierto, se ha extendido rápidamente por todo Irán. Este trabajo evaluó la eficacia de aislados nativos de tres especies de nematodos entomopatógenos (*Steinernema feltiae*, *S. carpocapsae*, *Heterorhabditis bacteriophora*) contra larvas (fuera de la hoja y dentro de las galerías) y pupas de *T. absoluta* a varias concentraciones y tiempos en condiciones de laboratorio. Los experimentos se realizaron a 25 ± 2ºC, 65 ± 5% HR, y fotoperiodo 16L:8D h. El nematodo *S. feltiae* causó la mayor mortalidad para las larvas fuera de la galería (53,61%), seguido de una mortalidad del 45% para las larvas dentro de las galerías, y la menor mortalidad para las pupas (3,88%). El efecto de los juveniles infecciosos (JI) y del tiempo de exposición (TE) sobre la mortalidad larvaria en los diferentes tratamientos mostró una relación significativa (P< 0,01) entre JI y TE y sus interacciones. La mortalidad de las larvas y pupas de *T. absoluta* aumentó con más JI y más TE. La CL50 para *S. feltiae*, *S. carpocapsae* y *H. bacteriophora* fue de 156,01, 225,13 y 317,66 JIs/ml para las larvas de segundo estadio de *T. absoluta* (fuera de la galería) y de 296,31, 305,23 y 320,66 JIs/ml para larvas dentro de la galería, respectivamente. Por lo tanto, *S. feltiae* fue una especie más eficaz y puede sugerirse para estudios complementarios con el fin de encontrar un agente de biocontrol adecuado de la plaga.

PALABRAS CLAVE. *Steinernema feltiae*. *Steinernema carpocapsae. Heterorhabditis bacteriophora*. Control biológico.

ABSTRACT. The tomato leafminer, *Tuta absoluta* (Meyrick) a key pest of tomato both in greenhouses and open-fields, has spread rapidly throughout Iran. The efficiency of native isolates of three species of entomopathogenic nematodes (*Steinernema feltiae, S. carpocapsae, Heterorhabditis bacteriophora*) was evaluated against the tomato leafminer, *T. absoluta* larvae (outside leaf and inside galleries), and pupae at various concentrations and times in laboratory conditions. Experiments were conducted at 25 ± 2ºC, 65 ± 5% RH, and 16L:8D h photoperiod. *S. feltiae* nematode caused the highest mortality for larvae outside the gallery (53.61%), followed by 45% mortality for larvae inside galleries, and lowest mortality for pupae (3.88%). The effect of infective juveniles (IJ) and exposure time (ET) on larval mortality in different treatments showed a significant (P< 0.01) relationship between IJ and ET and their interactions. Mortality of the *T. absoluta* larvae and pupae rose with more IJ and longer exposure time. The LC_{50} for, *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* were 156.01, 225.13, and 317.66 IJs/ml for the second instar larvae of *T. absoluta* (outside the gallery), 296.31, 305.23 and 320.66 IJs/ml for inside gallery, respectively. Therefore, *S. feltiae* was a more effective species and can be suggested for complementary studies for finding a suitable biocontrol agent of the pest.

KEYWORDS. *Steinernema feltiae. Steinernema carpocapsae. Heterorhabditis bacteriophora. Bological control*.

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INTRODUCTION

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a key pest of tomato and native to the western part of South America, invaded Brazil around 1980 (Souza and Reis, 1992). It is now a devastating pest of tomato crops in South America, Europe, Africa, and Asia (Tropea Garzia et al., 2012; Zappalà et al., 2013). This pest is crossing borders and devastating tomato production in both protected and open fields (EPPO, 2008; 2009; Urbaneja et al., 2012). In Iran, *T. absoluta* was first discovered in November 2010 infesting open-field tomato crops of the Uromiyeh Plant Protection Organization from Uromiyeh in Azerbaijan province in Northwest region (Baniameri & Cheraghian, 2012). The insect has spread rapidly, and it is currently considered a key insect pest on tomato crops, both in greenhouses and open fields. Damage is caused by larval tunneling and they can penetrate young stems and fruits which finally result in the loss of over 80% of fruits (Desneux et al., 2011).

During the last decades, *T. absoluta* controls were directed to the utilization of chemical insecticides. Environmental safety of insecticides is the first and foremost criterion for *T. absoluta* control programs (Tropea Garzia et al., 2012). In most countries where *T. absoluta* occurs, the main control strategy includes frequent sprays with chemical insecticides, because, without control, *T. absoluta* causes about 100% yield losses and dramatically decreases the fruit quality in both field and greenhouse tomato crops (Gilardón et al., 2001; Tropea Garzia et al., 2012). Nevertheless, because of the development of resistance, chemical control has demonstrated limited efficacy even after the use of different types of pesticides and increased the application frequencies (Siqueira et al., 2000; Lietti et al., 2005; Tomé et al., 2012).

The use of environmentally friendly pest control strategies is important to minimize the use of insecticides on tomato fields. Environmentally friendly strategies include cultural control (e.g. crop rotation, selective removal and destruction of infected plant materials), and the use of natural enemies (parasitoids, predators, and entomopathogens) (Dlamini et al., 2020). Entomopathogenic nematodes (EPNs) are good alternatives to synthetic insecticides. They are soil-dwelling organisms that attack insect pests that live in, on, or near the soil surface and can be used effectively to control harmful insect pests (Adams & Nguyen, 2002). Entomopathogenic nematodes in the Steinernematidae and Heterorhabditidae families do not affect non-target species, do not leave residues (Georgis et al., 2006), and are essential biocontrol agents used for controlling insect pests (Grewal & Georgis, 1999).

Gözel & Kasap (2015) studied the efficacy of the infective juveniles of four native EPNs species, *Steinernema affine*, *S. carpocapsae*, *S. feltiae* and *Heterorhabditis bacteriophora* against the larvae of *T. absoluta* in the field and it was found that the most effective nematode species on *T. absoluta* larvae was *S. feltiae* with 90.7% and 94.3% mortality in 2012 and 2013, respectively. Van Damme et al. (2016) evaluated the potential of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* against larvae of *T. absoluta* inside leaf mines and observed that the species were effective against all four larval instars of *T. absoluta* but caused higher mortality in the later instars (77.1–97.4% mortality) than in the first instars (36.8–60.0% mortality). Overall, *S. feltiae* and *S. carpocapsae* yielded better results than *H. bacteriophora*. Mutegi et al. (2017) demonstrated that native EPNs including *Heterorhabditis* sp. and *S. karii* have a potential for management of *T. absoluta* Kamali et al. (2018) examined the effect of temperature, soil type, and exposure time on the efficacy of the EPN species including *S. carpocapsae* and *H. bacteriophora* against last-instar *T. absoluta* larvae in the laboratory. Also, Ndereyimana et al. (2019) assessed the potential of six local isolates of EPNs in the management of *T. absoluta* in Rwanda. Finally, Dlamini et al. (2020) investigated the virulence of two sub-tropical EPN species, *S. yirgalemense* and *S. jeffreyense* on *T. absoluta* larvae.

Despite the successes that have been recorded for the use of EPNs to control the tomato leafminer, environment determines the success or failure of EPNs because of the potential differences in persistence, virulence, host range, and familiarity to habitats between local and non-local EPN isolates (Lacey & Georgis, 2012). The target host and the environment where EPNs will be applied should be considered when designing a control program using EPNs. Thus, screening several nematode isolates against a particular target host in a specific environment is a prerequisite in the development of any control program using EPNs (Biondi et al., 2018).

Our overall goal in this study was to determine the potential for the use of new EPN isolates for *T. absoluta* suppression. The comparison between susceptibility of *T. absoluta* larvae inside and outside galleries and pupae to EPNs has not been reported yet. A critical component for success in any biocontrol program with entomopathogenic nematodes is matching the most suitable nematode with the target host, and relative virulence among nematodes to the target pest is a major factor in suitability (Shapiro-Ilan et al., 2009).

MATERIAL AND METHODS

Insect rearing

Colonies of *T. absoluta* were originally collected from leaves or leaf parts with larvae from infested greenhouses of Qom province of Iran. All experiments were carried out in the biological control laboratory, Faculty of Agriculture, Shahed University, Tehran, Iran. After the emergence of adults, *T. absoluta* moths were collected by suction and

released into a transparent polyester jar containing fresh, detached, composite tomato leaf (Goldie cultivar) with the cut end fixed in a vial (4 × 1 cm) filled with sterile water. The insects were reared in a gross chamber at 27 ± 2°C, 65 ± 5% RH, and 16L:8D h photoperiod (Rostami et al., 2017). They were provided with water and 10% sucrose solution and allowed to oviposit for 24–48 h. Upon observation of an adequate number of eggs (at least 260 eggs), the infested leaves of potted plants were placed in an insect-proof rearing cage to allow larval development to the second instar (Mohamed et al., 2022). In particular, the infested tomato leaves were placed gently on a potted tomato plant to ensure food availability. Newly emerged 2nd instar larvae inside and outside leaves and pupae were used for bioassay experiments.

Preparation of EPNs inoculum

Native preparations of *S*. *feltiae*, *S*. *carpocapsae,* and *H*. *bacteriophora* were initially obtained from Biological Control Laboratory at Ferdowsi University of Mashhad, Iran. These were cultured in last-instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lep.: Pyralidae), as per methods of Kaya and Stock (1997). Each nematode species was passed through *G. mellonella* less of seven times before use in bioassays. After harvesting, infective juveniles (IJs), they were stored in 250 ml flasks at 13°C and a maximum concentration of 5000 IJs per ml for <1 week before use in the experiments. Infective juveniles stock cultures were serially diluted to achieve concentrations of 25, 50, 100, 200, and 400 IJs per ml of distilled water. Microscopic examinations were done to determine the viability of each nematode in all bioassays.

Pathogenicity test

For the larvae inside the gallery experiment, two 2nd instar larvae were placed on a tomato leaf disc (Goldie, 3 cm diameter) in a petri dish (4 × 1 cm) and allowed to mine into the leaf disc (van Damme et al., 2016). For each nematode species and concentration, five petri dishes were used in five replications and were sprayed with 1 ml for each concentration above mentioned and for each combination of EPN species. Totally, 375 petri dishes were used in experiment. A control treatment was included, consisting of distilled water only.

For larvae outside the gallery experiment, ten 2nd instar larvae were placed in 9 cm diameter plastic Petri dishescovered with filter paper (Whitman No. 1) and sprayed with EPN species. Individual concentrations, including 25, 50, 100, 200, and 400 IJs per ml of distilled water for each nematode species were treated on the filter paper. Also, a control treatment was included, consisting of distilled water only. Five replications were considered for each concentration.

Newly emerged pupae of *T*. *absoluta* were obtained from breeding larvae of last instar. The susceptibility of the pupal stage of *T*. *absoluta* was also assessed in soil, ten newly emerged pupae in five replications were placed in 9 cm diameter plastic Petri dishes covered with filter paper and the dish was filled with 23 g of moistened (10% w/w) sterile sandy loam soil (Kamali et al., 2018). The same concentrations used in the larval treatment were applied on the soil surface of the pupae for each nematode species. Control plates were treated with distilled water only. Five replications were considered for each concentration.

Arenas were maintained in an environmental chamber at 25 ± 2ºC, 65 ± 5% RH, and 16L: 8D h photoperiod. Arenas were examined and larval mortality was recorded at 24, 48, 72, and 96 h after treatment. Dead larvae were recognized according to change in their body color. Due to symbiotic bacteria associated with EPNs, the infected cadavers of Steinernematidae turn tan, ochre, gray or dark gray, whereas those of Heterorhabditidae turn red, purple, orange, yellow, brown or sometimes green (Kaya and Gaugler, 1993). For the pupae experiment, results were studied after 8 days, and pupae were dissected to verify the presence of nematodes and that were not transformed into an adult after this period were considered dead.

Statistical analysis

Mortality data were normalized by the square root transformation. The effects of three factors of species, concentration, and exposure time were subjected to trifactorial ANOVA using SPSS version 15.0 (SPSS 2006), with a level of significance at $P < 0.05$. Probit analysis of significantly different treatments were performed using Tukey's honestly significant difference (HSD) test.

RESULTS

Microscopic examinations found that ≥95% of IJs were viable in each nematode preparation in all bioassays. Analysis of variance on different species of nematodes showed that the effect of nematodes, different concentrations, exposure time, and the interaction of concentration and time on the mortality rate of 2nd instar larvae inside and outside of the gallery were significantly different ($P < 0.05$). Also, analysis of variance on nematode species showed that only the effect of concentration on the mortality of *T. absoluta* pupae was significant ($P < 0.05$) (Table I).

Results of the mean comparison of the mortality rate of *T. absoluta* larvae under different species of nematodes showed that *S. feltiae* caused the highest mortality, followed by *S. carpocapsae* and *H. bacteriophora* (Fig. 1).

The results showed that among different concentrations of the three nematode species, the highest larval mortality was observed when EPNs were applied at a dose of 400 IJs/ml. (Fig. 2a, b). The average mortality rate decreased with a lower concentration of all three nematode species. The mortality of 2nd instar larvae of *T. absoluta* was significant at different concentrations, and the mortality of 200 and 400 IJs/ml concentrations were higher than those at low concentrations.

Table I. Analysis of variance (ANOVA) of the mortality of 2nd instar larvae inside and outside gallery and pupae the tomato leafminer, *Tuta absoluta* affected by treatment with three species of nematodes at different concentrations and exposure times.

Variable	df	Larvae outside gallery			Larvae inside gallery			Pupae		
		Mean squares	F	P-value	Mean squares	F	P-value	Mean squares	F	P-value
Nematode (N)	2	976.39	31.48^{**}	0.001	946.30	15.49^{**}	0.001	22.22	2.00^{ns}	0.150
Concentration (C)	4	22516.39	725.90^{**}	0.001	19455.19	318.36^{**}	0.001	154.11	13.90^{**}	0.001
Exposure time (ET)	3	49254.78	1.59^{**}	0.001	60347.53	987.51^{**}	0.001	-	-	-
N × C	10	45.28	1.46^{ns}	0.160	28.52	0.47^{ns}	0.909	6.67	0.60^{ns}	0.803
N × ET	6	39.97	1.29^{ns}	0.266	77.16	1.26^{ns}	0.278	-	-	-
C × ET	15	1842.56	59.40^{**}	0.001	24488.27	40.72^{**}	0.001	-	-	-
N × C × ET	30	13.30	0.43^{ns}	0.996	33.46	0.55^{ns}	0.972	-	-	-
Error	144	31.02			61.11			11.11		

^{ns} not significant, ** P ≤ 0.01.

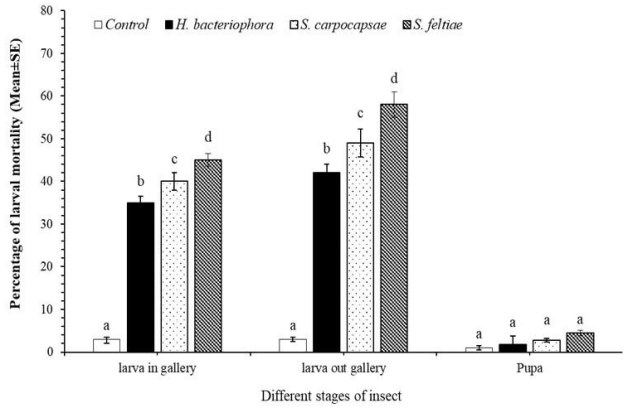

Fig. 1. Mortality (%) of *Tuta absoluta* 2nd instar larvae inside and outside gallery and pupae following application of entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *S. feltiae*. Data are expressed as mean ± SEM, bars of each nematode species followed by the same letter are not significantly different (*P* = 0.05) according to Tukey HSD Test for mortality.

The pupae exhibited less pathogenicity in all nematode species tested. Results showed that there was no significant difference between 0 to 100 IJs/ml concentrations on the mortality of *T. absoluta* pupae, but between 200 and 400 IJs/ml concentrations as well as between these two concentrations and others there were significant differences. In general, the concentrations used resulted in low mortality in the pupae of *T. absoluta*, with the highest mortality (about 13%) caused by *S. feltiae* at the highest concentration (Fig. 2c).

The mortality percentage of 2nd instar larvae increased with exposure duration. When larvae inside and outside gallery were exposed to EPNs for a similar period time, larvae outside gallery were more vulnerable than those inside. After 72 h, *S. feltiae* caused 100% and 93% mortality in 2nd instar larvae outside and inside the gallery, respectively, when applied at 400 IJs/ml (Table II).

Lethal concentrations (LC_{50}s) for each nematode species against 2nd larvae inside and outside gallery were 296.30 and 156.01 IJs/ml for *S. feltiae*, 305.23 and 225.13 IJs/ml for *S. carpocapsae*, and 320.66 and 317.66 IJs/ml for *H. bacteriophora*, respectively. These estimates differed significantly based on the 95% confidence intervals except for *H. bacteriophora* (Tables III, IV).

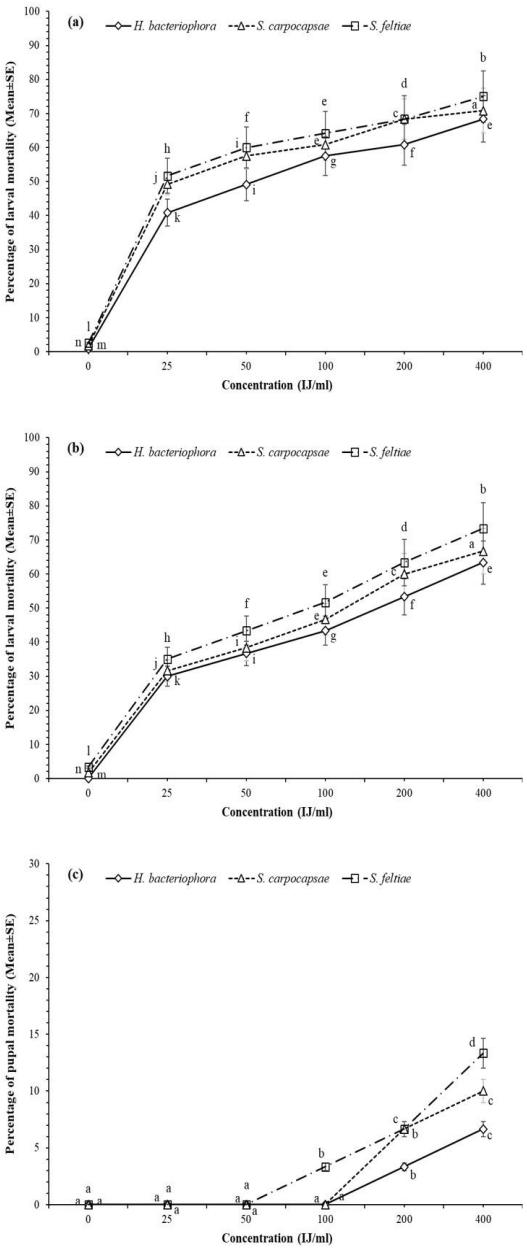

Fig. 2. Nematode induced mortality of 2nd instar larvae of the tomato leafminer, *Tuta absoluta* a) outside gallery, b) inside gallery, and c) pupae within each trial (control is concentration=0 (IJ/ml), bars (mean±SE) of each nematode species followed by the same letter are not significantly different (*P* = 0.05) according to Tukey HSD Test for mortality.

Table II. The effect of different concentrations of *Steinernema feltiae* on mortality of 2nd instar larvae of the tomato leafminer, *Tuta absoluta* inside and outside gallery at different concentrations and exposure times.

No. of IJs/ml[a]	% dead larvae outside gallery (mean ± SE)[b]				% dead larvae inside gallery (mean ± SE)[b]			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	6.66±2.68 b	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a	6.66±2.68 b
25	8.89±6.56 b	31.11±2.23 ef	61.11±5.60 h	88.78±8.23 k	0.00±0.00 a	2.22±0.23 a	46.66±4.60 f	80.00±8.00 h
50	12.22±1.02 bc	36.67±3.60 f	72.22±6.65 i	96.67±8.01 I	0.00±0.00 a	13.33±1.03 bc	53.33±4.05 f	91.11±8.01 i
100	15.56±1.23 cd	44.44±4.20 g	81.11±8.04 j	100.00±9.05 I	2.22±0.23 a	20.00±1.20 cd	66.66±5.50 g	100.00±9.05 j
200	21.11±2.10 d	47.78±4.23 g	87.78±7.60 k	100.00±6.57 I	15.56±1.60 c	33.33±3.03 e	86.67±7.60 h	100.00±8.57 j
400	27.78±2.60 e	96.67±5.60 I	100.00±6.62 I	100.00±7.60 I	24.44±2.40 d	53.33±4.60 f	93.33±6.62 i	100.00±7.60 I

[a] the number of infective juveniles per ml. [b] Mean values followed by different letters in the same column are significantly different according to Tukey HSD test (P < 0.05).

Table III. LC_{50} and LC_{90} values of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* applied against 2nd larval instars of the tomato leafminer, *Tuta absoluta* inside gallery at different concentrations of nematodes.

Nematode Species	LC_{50}*		LC_{90}		χ^2	df	Slope ± SE
	IJs/ml	CI[a]	IJs/ml	CI			
S. feltiae	296.30	158.35-302.85	2817.86	1559.82-6656.6	1.39	3	1.67 ± 0.50
S. carpocapsae	305.23	267.65-345.34	3254.66	1776.82-4564.67	12.8	3	1.35 ± 0.41
H. bacteriophora	320.66	250.72-398.42	3569.7	1815.57-5078.89	32.1	3	1.40 ± 0.21

* LC index, number of third instar larvae of nematode (IJ) required for 50 and 90% mortality of *X. luteola* [a] Upper and lower limits of 95% confidence level.

Table IV. LC_{50} and LC_{90} values of *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis bacteriophora* applied against 2nd larval instars of the tomato leafminer, *Tuta absoluta* outside gallery at different concentrations of nematodes.

Nematode Species	LC_{50}*		LC_{90}		χ^2	df	Slope ± SE
	IJs/ml	CI[a]	IJs/ml	CI			
S. feltiae	156.01	128.35-188.85	1712.86	1559.82-6859.6	0.55	3	1.34 ± 0.36
S. carpocapsae	225.13	149.95-286.54	1158.8	528.89-3256.68	9.52	3	1.07 ± 0.31
H. bacteriophora	317.66	239.72-391.52	2505.7	1715.57-5578.89	1.27	3	1.40 ± 0.21

* LC index, number of third instar larvae of nematode (IJ) required for 50 and 90% mortality of *X. luteola*. [a] Upper and lower limits of 95% confidence level.

DISCUSSION

This study showed that entomopathogenic nematodes can infect larvae and kill them inside larval tunnels. In all three species of nematodes, high success was achieved to control the larvae, but in the case of pupae, no specific results were obtained. The difference in the nematode's ability to infect larvae and pupae is consistent with studies by Henneberry et al. (1995). They reported 91.9% mortality for pink bollworm, *Pectinophora gossypiella* (Saunders) (Lep.: Gelechiidae) larvae, and 13% for pupae by *S. carpocapsae* in vitro and moist soil. A study by Lindegren et al. (1993), revealed that *P. gossypiella* pupae are not a sensitive stage for nematode attack, which can be justified due to the lack of input pathways (mouth and anus) for the nematode. As previously reported by Gözel & Kasap (2015), EPNs most likely entered feeding canals in the leaves of tomatoes and many larvae of *T. absoluta* died inside these galleries, which indicates that IJs were able to find and infect them.

Summary of research data showed that the susceptibility of the *T. absoluta* larvae to EPNs depends on concentration of nematode larvae, time of exposure, nematode species used, and nematode species seeking strategies. In the case of Petri dishes, the nematodes move only on the surface of the filter paper (one dimension), and the 2nd instar larvae of *T. absoluta* can stick to parts of the container (moving in several dimensions), thus becoming inaccessible and dying as recorded randomly. In an experiment by Batalla-Carrera et al. (2010), the effect of three species of nematodes; *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* were studied on larvae, pupae, and adult insects of *T. absoluta*, which were tested in Petri dish, as the mortality rate of out-of-leaf larvae at a dose of 25 IJs/ml were equal to 78.6, 85.7 and 100% for applied nematodes, respectively. At 50 IJs/ml dose for the above nematodes, it reached 100, 86.6, and 100%, respectively. Pupae were more resistant to nematodes and no significant mortality was observed in the treatments. In our experiment within the Petri dish, the mortality of larvae outside the gallery at low doses was relatively high for *S. feltiae*, *S. carpocapsae* and *H. bacteriophora*, respectively.

In another study, the efficacy of soil treatments of three native EPNs (*S. carpocapsae*, *S. feltiae* and *H. bacteriophora*) against *T. absoluta* larvae, pupae and adults was determined under laboratory conditions (Garcia-del-Pino et al., 2013). When the larvae dropped into the soil to pupate, soil application of nematodes resulted in high mortality of larvae: 100%, 52.3%, and 96.7% efficacy for *S. carpocapsae*, *S. feltiae* and *H. bacteriophora*, respectively. No mortality of pupae was

observed and mortality of adults emerging from soil was 79.1% for *S. carpocapsae* and 0.5% for *S. feltiae*. Azarnia et al. (2018) reported the lethal concentration (LC_{50}) for the same nematode species at 35.97 IJs/ml for *S. feltiae*, 44.0 IJs/ml for *S. carpocapsae*, and 104.5 IJs/ml for *H. bacteriophora* against the last instar larvae of clearwing moth, *Paranthrene diaphana* (Dalla Torre & Strand) (Lepidoptera: Sesiidae). These values were much lower than the present study, which may be due to the greater resistance of *T. absoluta* to entomopathogenic nematodes.

CONCLUSION

Obtained results suggest that *S. feltiae* can be used as biological control agent against the larvae of *T. absoluta*. Typical feeding galleries made by *T. absoluta* larvae provide EPNs an excellent environment to penetrate the pest easily and avoid negative factors (desiccation, ultraviolet light, etc.). However, future field studies are recommended to focus on field efficacy and field application against *T. absoluta* for using this nematode as a biopesticide.

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