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EFFICACY OF THE ENTOMOPATHOGENIC NEMATODES AND FUNGI FOR CONTROLLING THE TOMATO LEAF MINER, *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae)

[43]

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ABSTRACT

Susceptibility of the tomato leaf miner, *Tuta absoluta* **(Meyrick) (Lepidoptera : Gelechiidae)** larvae, pupae and adults to entomopathogenic nematode, *Steirnernema carpocapsae* and two fungal species; *Beauveria bassiana* and *Metarhizium anisopliae* was investigated under laboratory conditions. Applied concentrations against the last instar larvae and different ages of the pupae, using leaf and soil treatments, were 250, 500,1000 IJs/ml for the nematode and 10^8, 10^9,10^{10} spores/ml for the fungi. Soil applications of the nematode and fungi resulted to high mortality (100, 100 and 93.3%) of4thinstar larvae while low pupal mortality (46.7,30and 23.3%),respectively. In leaf treatment a high level of larval mortality (93.3, 90 and 80%) was recorded revealing *S. carpocapsae*, *B. bassiana* and *M. anisopliae*, respectively. The present study also showed also susceptibility of *Tuta absoluta* adults to the three pathoens. The results demonstrated suitability of entomopathogenic nematode and fungi for controlling *T. absoluta*.

INTRODUCTION

Egypt is one of the most important tomato producers in the world **(WP TC, 2011)**. The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is among the major insect pests of tomato crop. Recently, it is considered as a key pest, causing damage to leaves, stems and fruits, and may cause complete loss or reduce crop yield by up to 90**% (Andrew et al 2013).** Females usually lay the eggs on the underside of leaves or on stems and sometime on fruits. Neonate larvae penetrate leaves, stems or fruits, on which they feed and develop. Last instar larvae usually drop to the soil to pupate although pupation may also occur on leaves. After a few days adults emerge from soil **(Frenando et al 2013).**

Current control strategies for *T. absoluta* arebased mainly on the use of insecticides. The problems associate with this pest beside its population rapid growth may increase its ability to develop resistance to insecticides within few years **(Bielza, 2010).**Therefore, some integrated pest management programs in tomato crops against *T. absoluta* are directed toward biological control (**Fernando et al 2013)**.

Entomopathogenic nematodes (EPNs) are considered as biological control agents for a variety of economically important pests **(Grewal et al 2005)**. Most of these EPNs belong to the families; Steirnernematidae and Heterorhabditidae, whichare obligate parasites that kill insects with the help of mutualistic bacteria in their intestine **(Poinar, 1990 and Boemare, 2002).** They have been used with variable success against insects .Most success has been achieved against soil dwelling pests as well as in cryptic habitats **(Williams and Walters, 1999 and Tomalak et al 2005).** Susceptibility of *T. absoluta* to entomopathogenic fungi (EPFs) has been investigated **(Rodriguez et al 2006).** Effectiveness of the fungus *M. anisopliae* on different developmental stages of *T. absoluta* revealed a complete efficacy against the pupae of this insect (**Contreras et al 2014).** *B. bassiana* was recorded affecting also on all differ-

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ent developmental of growth stages of *T. absoluta* **(Aziz et al 2012)**

The aim of this work was to study the efficacy of nematodes and fungi against larvae, pupae and adults of *T. absoluta* under laboratory conditions.

MATERIALS AND METHODS

Insect culture

A laboratory colony of *T. absoluta* was established by larvae and pupae which, collected from infested tomato fields at Fayoum Governorate, Egypt. Larvae were reared on tomato seedlings under a climatic chamber at 27±2°C and 55%±5 RH. Adults were collected using an aspirator, fed on 10% honey solution and provided with tomato plants which, placed in pots in rearing wooden cages (60 cm^2 high, 50 cm^2 wide, 50 cm^2 long) for oviposition. Last instar larvae and pupae, 1, 3, 7 day sold, were carefully collected to be used in the experiments **(Faragalla and Shalaby, 2013).**

Entomopathogenic nematode culture

The EPN species, *Steirnernema carpocapsae* was supplied by Nematology, Pest Plant Protection Department, National Research Center, Giza, Egypt. The nematode was propagated as mentioned by **(El-Kifl, 1980).** Water suspension of infective juveniles (IJs) was washed and prepared at a concentration of 1000IJs/ml in sterile distilled water and maintained at 4°C till been used (**(El-Kifl and Sammour, 1989).** Three different concentrations of nematode (250, 500,1000 IJs/ml) were used in this study.

Fungi culture

Two EPF species were used in this study, the first one was *B. bassiana*, which isolated from infected whitefly *Bemisia tabaci* in Fayoum Governorate and the second was *M. anisopliae* which obtained from Plant Protection Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Both species were cultured at 25±1°C on potatoes Dextrose Agar (PDA). Conidia were harvested at 15 days old plates by scraping into sterile tween – 80. Conidial concentration of the stock suspension was estimated using a hemocytometer. Three concentrations (10^8, 10^9 and 10^{10} spores/ml) in sterile saline solution were prepared.

Treatments

A- **Soil treatment**

The 4thinstar larvae and different ages of pupae (1st, 3rd, and 7th day) of *T. absoluta* were used in this experiment, which provided from the laboratory culture. The experiments were carried out in Petri dishes(9 cm diameter), filled with 20g of sterile sand adjusted to 10% water content by adding tap water (**Batalla-Carrera et al 2010).** Serial dilutions were prepared in 100 ml distilled water for *S. carpocapsae* (250, 500, 1000 IJs/ml), and the two fungi *B. bassiana* and *M. anisopliae* (10^8, 10^9, 10^{10} spores/ml). Inoculation with 10 ml of the three concentrations of each pathogen suspensions was done using micropipette onto the soil surface in each Petri- dish. Three replicates were used for each concentration (ten 4th in star larvae or pupae / replicate). Control treatments were identical to those of the treatments, except that nematode or fungi were not added. Petri- dishes were kept in a climate chamber at 25±2°C. Insect mortality was estimated at 24, 48 and 72 hours after exposure to the nematode, and at 2, 4 and 6 days to fungi treatment. Dead larvae and pupae were dissected to confirm nematode and fungi parasitism by using stereoscopic microscope. Mortality rates were corrected using **(Abbott's, formula 1925).**

B- Leaf treatment

Tomato leaves and filter papers were sprayed with the same concentrations of nematode and fungi and left for 5 seconds to avoid water excess and transferred to Petri- dishes (10 larvae/ dish). Three replicates were used for each concentration and supplied with moisture as needed to avoid desiccation of leaves and ensure a continuous and adequate moisture for spore germination **(Hicks et al 2001; Shalaby et al 2013).** Pupae; 1,3 and 7-days old were sprayed with the same concentrations and kept in Petri- dishes (10pupae/dish). The median lethal concentration (LC_{50}) values of the entomopathogenic nematode and fungi were estimated by software computer probane.

Statistical analysis

Analysis of variances of obtained data was computed using the General Linear Model (GLM) procedure according to **SPSS, v. 17 (2008)**. Significant differences between means were calculated using Duncan's multiple range test **(Duncans, 1955)**.

RESULTS AND DISCUSSION

A. Soil treatment

Susceptibility of larvae

The results of the soil experiment revealed that last instar larvae of *T. absoluta* were highly susceptible to the tested EPN and the two fungal species. Mortality percentages of the 4thinstar larvae treated with *S. carpocapsae, B. bassiana and M. anisopliae* are shown in **(Tables 1 and 2)**. The nematode was highly virulent at 72 h, after exposure to the three concentrations of (250, 500 and 1000 IJs/ml) and the mortality rates recorded were 80,100 and 100%, respectively **(Table, 1)**. Percentages of corrected mortalities of the 4th instarlarvae increased gradually until the end of experiment (6th day), while in case of both entomopathogenic fungi, were recorded (86.7, 100 and 100%) for *B. bassiana* and (76.7, 83.3 and 93.3%) for *M. anisopliae,* at concentrations of (10^8,10^9 and 10^{10} spores/ml), respectively **(Table 2)**.

From the present work, it is obvious that larvae of *T. absoluta* seemed highly susceptible to the tested nematode and fungi**. Shalaby et al (2013)** found that the pathogenicity of *B. bassiana* and *M. anisopliae* gave the highest effect on larvae of *T. absoluta* by time. Similar results were reported about the efficacy of the entomopathogenic fungi; *M. anisopliae* or *B. bassiana* (caused 96% mortality) on larvae of *T. absoluta* **(Rodriguez et al 2006). Sabbour and Singer (2014)** reported that the infestations with *T. absoluta* was significantly decreased in plots treated with *M. anisopliae* as compared to control. **Sabbour (2014)** reported that control of *T. absoluta* by *Bacillus thuringensis* var *kurstaki*, *B. bassiana* and *M. anisopliae* occurred under laboratory and field conditions.

Also the, mortality percentage was positively correlated with the nematode and fungal concentrations by time. These results are in agreement with **Shairra (2000) who mentioned that** the mortality percentage of some Lepidopterous larvae increased with the increase of IJs dose of either of the nematodes, *Heterorhabditis indicus* or *H. bacteriophora.*. **Paul, (2013)** stated that the two EPNs, *S. feltiae* and *S. carpocapsae* were capable to kill *T. absoluta* larvae within 2-6 days of application. **Fernando et al (2013),** recorded high mortality (100%) on *T. absoluta* larvae buried in soil caused by *S. carpocapsae*. **Batalla-Carrera et al (2010**) showed that the 4th instar larvae of *T. absoluta* were susceptible towards *S. carpocapsae* causing mortality of 86.6%.The application of EPNs on soil would control the last instar larvae, when they slide down from the leaves to soil for pupation, as well as emerging adults from the buried pupae.

Susceptibility of pupae

In contrast to the larvae, pupae were hardly infected by both nematode and fungi, especially for the old pupae (7 day old). Percentage mortality of one day pupae, treated with nematode recorded (20,33.3 and 46.7%), the 3 day old pupae was (13.3,20 and 26.7%), while for the 7 day old pupae, it was (6.7,13.3 and 16.7%) at concentrations of (250, 500 and 1000 IJs), respectively after 72h. of exposure **(Table, 1).** Mortality percentages of one, 3 and 7 day old pupae of *T. absoluta* treated with *B. bassiana* attained 20, 26.7 and 30%, 10, 13.3 and 20% and 6.7,10 and 13.3%, respectively. Correspondence values for *M. anisopliae* were 13.3,20 and 23.3%, 6.7,10 and 13.3% and 3.3, 6.7 and 10% at the concentrations of 10^8, 10^9 and 10^{10} spores/ml **(Table 2)**. Results indicated that one day old pupae were more susceptible to the three tested pathogens compared with the3and 7day old pupae. **Arthurs et al (2004),** reported that insect habitat determined the efficacy of the EPNs**.** However, tomato leaf miner larvae produce tunnels with large entry holes that can be used by nematodes to penetrate and avoid desiccation and ultraviolet light and finally infect the larvae **(Batalla-Carrera et al 2010).**

Differences in susceptibility between larvae and pupae in this study are in agreement with the observations recorded by **Batalla-Carrera et al (2010)** as high larval mortality (78.6-100%) and low pupal mortality (<10%) were determined under laboratory experiments. **Henneberry et al (1995)** reported 91.9% and only 13% larval and pupal mortality of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), treated with *S. carpocapsae* under laboratory conditions in moist soil.

Estimated LC_{50} of *S. carpocapsae* was 119.4, 1039.5, 5169 and 12740.6 IJs/ml for 4th instar larvae and 1, 3, 7 day old pupae of *T. absoluta* at 72h, respectively. Respective LC_{50} values of *B. bassiana* and *M. anisopliae*a on day 6 were ($7.5x10^6$, $1.6x10^{13}$, $45x10^{12}$ and $1.4x10^{13}$), (2.133x 10^7, 5.7 x 10^{13}, 2.7 x 10^{13} and $2.5x10^{14}$ spores/ml) **(Table, 3).** According to the LC_{50} values, *B. bassiana* seemed more effective on *T. absoluta* larvae and pupae than *M. anisopliae.*

Susceptibility of adults

Percentage of mortality among the adults emerged from the soil treated with *S. carpocapsae* were presented in **Table (1)**. They varied from 43.31% at 250IJs, 35% at 500IJs and 12.5% at 1000 IJs/ml. Percentages of mortality among the adults emerged from the soil treated with both fungi were presented in **Table (2)**. They were 75, 63.64 and 59.1% for *B. bassiana* while they were 84.62, 70.8 and 69.96%) for *M. anisopliae* at concentrations of 10^8, 10^9 and 10^{10} spores/ml, respectively.

It could be concluded, that adults of *T. absoluta* were also susceptible to the three tested pathogens which suggest that the pathogens could attack the adults while they emerging from the pupae in the soil. These results are in agreement with **those obtained by Batalla-Carrera et al (2010) and Fernando et al (2013)** who reported a high susceptibility to *T. absoluta* adults to *S. carpocapsae*.

B. Leaf treatment

Susceptibility of larvae

The efficacy of EPN *S. carpocapsae* and the two fungal species *B. bassiana* and *M. anisopliae* on last instar larvae of *T. absoluta* on (leaf treatment) under laboratory investigation is presented in **(Tables 1 and 2).** Obtained results indicated that the tested 4th instar larvae was susceptible to infection by the three pathogens. *S. carpocapsae* caused the highest mortality percentage at the highest concentration (93.3%) after 72 h. of exposure. A significant difference between *S. carpocapsae* and the fungi, *B. bassiana* (90%) and *M. anisopliae* (80%) at the end of the experiment (6th day) was found. **Batalla-Carrera et al (2010),** stated that the applied nematodes at a dose of 60IJs cm^{-2} were able to penetrate inside the leaf galleries and caused between 76.3% and 92% mortality of *T. absoluta* larvae.

Susceptibility of pupae

Laboratory investigation was carried out to study the effect of EPN pathoges and fungi on 1, 3, and 7 day old pupae of *T. absoluta*. In **Tables (1 and 2)** nematode showed mortality on the 1, 3, and 7 day old pupae at the highest concentration at 72 h. after treatment recorded (36.7, 20 and 13.3%) while with fungi *B. bassiana* and *M. anisopliae* it was (26.7, 16.7 and 10%), (16.7, 10 and 6.7%), respectively.

According to LC_{50} values, in **Table (3)** *B. bassiana* was the most effective on *T. absoluta* larvae and pupae than *M. anisopliae.*

Table 1. Accumulated corrected mortality of *T. absoluta* treated with different concentrations of *S. carpocapsae* as soil and leaf treatment

Treated larvae and pupae Ages/h.	*S. carpocapsae* Concentrations used (IJs/ml)		
	250	**500**	**1000**
Soil treatment			
4th instar larvae			
24	66.7	73.3	76.7
48	77	83.3	90
72	80	100	100
Mean	74.57a	85.53a	88.9a
one day old pupae			
24	3.3	10	13.3
48	6.7	16.7	30
72	20	33.3	46.7
Mean	10def	20cde	30c
3 day old pupae			
24	0	6.7	10
48	6.7	10	20
72	13.3	20	26.7
Mean	6.67ef	12.23def	18.9cdef
7 day old pupae			
24	0	0	6.7
48	3.3	10	13.3
72	6.7	13.3	16.7
Mean	3.33ef	7.77ef	12.23def
Emerged adult	43.31	35.00	12.5
Leaf treatment			
4th instar larvae			
24	43.3	70	73.3
48	63.3	73.3	80
72	70	77	93.3
Mean	58.87b	73.43a	82.2a
one day old pupae			
24	0	10	16.7
48	10	13.3	23.3
72	13.3	23.3	36.7
Mean	7.77ef	15.53cdef	25.57cd
3 day old pupae			
24	0	3.3	6.7
48	3.3	10	13.3
72	10	13.3	20
Mean	4.43ef	8.87ef	13.33def
7 day old pupae			
24	0	0	3.3
48	0	3.3	6.7
72	6.7	10	13.3
Mean	2.23f	4.43ef	7.77ef
Emerged adult	59.26	47.83	26.32
F. between concentrations			15.55***
F. between instars			265.2***
F. between types of application			7.25*

*Significant*** Highly significant

Table 2. Accumulated corrected mortality of *T. absoluta* treated with different concentrations of entomopathogenic fungi as soil and leaf treatment

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	Concentrations used					
	10^8	**10^9**	**10^{10}**	**10^8**	**10^9**	**10^{10}**
Soil treatment						
4th instar larvae/ day						
2	13.3	53.3	60	10	40	43.3
4	56.7	83.3	90	53.3	80	83.3
6	86.7	100	100	76.7	83.3	93.3
Mean	52.23bcd	78.87ab	83.33a	46.67cdef	67.77abcd	73.3abc
1 day old pupae						
2	0	6.7	6.7	0	3.3	6.7
4	13.3	23.3	26.7	10	16.7	16.7
6	20	26.7	30	13.3	20	23.3
Mean	11.100ghi	18.900fghi	21.13efghi	7.77hi	13.33ghi	15.57fghi
3 day old pupae						
2	0	6.7	10	0	3.3	6.7
4	6.7	13.3	16.7	3.3	6.7	13.3
6	10	13.3	20	6.7	10	13.3
Mean	5.57hi	11.10ghi	15.57fghi	3.33i	6.67hi	11.10ghi
7 day old pupae						
2	0	0	6.7	0	0	3.3
4	3.3	6.7	10	0	3.3	6.7
6	6.7	10	13.3	3.3	6.7	10
Mean	3.33i	5.57hi	10.00ghi	1.10i	3.33i	6.67hi
Emerged adult	75	63.64	59.1	84.62	70.8	69.96
Leaf treatment						
4th instar larvae/ day						
2	6.7	6.7	36.7	3.3	6.7	6.7
4	46.7	70	73.3	40	56.7	63.3
6	70	80	90	67.7	73.3	80
Mean	41.13defg	52.23bcd	66.67abcd	37.00defgh	45.57cdef	50.00bcde
1 day old pupae						
2	0	3.3	10	0	0	0
4	10	13.3	20	6.7	6.7	10
6	10	20	26.7	6.7	10	16.7
Mean	6.67hi	12.2ghi	18.9fghi	4.47hi	5.5hi	8.9ghi
3 day old pupae						
2	0	0	6.7	0	0	3.3
4	3.3	6.7	10	0	3.3	6.7
6	6.7	13.3	16.7	3.3	3.3	10
Mean	3.33i	6.67hi	11.13ghi	1.10i	2.20i	6.67hi
7 day old pupae						
2	0	0	3.3	0	0	0
4	3.3	3.3	6.7	0	0	3.3
6	3.3	6.7	10	3.3	3.3	6.7
Mean	2.2i	3.33i	6.67hi	1.10i	1.10i	3.33i
Emerged adult	81.48	79.17	69.57	89.29	81.48	79.0
F. between concentrations		5.70**				
F. between instars		85.04***				
F. between types of applications		7.06**				

n.s*Significant *** Highly significant

Table 3. Lethal concentrations of entomopathogenic nematode and fungi for larval and pupal stages of *T. absoluta,* on 3rd and 6th day

Pathogen	Soil Treatment				Leaf Treatment			
	4th instar larvae	**1 day old/pupae**	**3 day old/pupae**	**7day old/pupae**	**4th instar larvae**	**1 day old/pupae**	**3 day old/pupae**	**7 day old/pupae**

	LC_{50}	LC_{50}	LC_{50}	LC_{50}	LC_{50}	LC_{50}	LC_{50}	LC_{50}
B. bassiana	7.5×10^{5}	1.6×10^{10}	45×10^{12}	1.4×10^{13}	7×10^{6}	6.2×10^{11}	4.7×10^{13}	2.5×10^{14}
Slope	0.992	0.16	0.31	0.34	0.29	0.33	0.25	0.29
M. anisopliae	2.133×10^{6}	5.7×10^{11}	2.7×10^{13}	2.5×10^{14}	1.2×10^{7}	4.5×10^{12}	9.7×10^{13}	2.2×10^{15}
Slope	0.253	0.19	0.297	0.29	0.29	0.33	0.33	0.61
S. carpocapsa	119.4	1039.54	5169.018	12740.65	122.4	1790.69	14307	88011
Slope	2.41	1.7	0.81	0.84	1.47	1.32	0.74	0.58

Susceptibility of adult

Percentage of mortality among the adults emerged from the one day old pupae treated with *S. carpocapsae* were presented in **Table (1)**. These percentages were 59.26, 47.83, 26.32 % at 250, 500, and 1000 IJs/ ml. Percentage of mortality among the adults emerged from the one day old pupae treated with both fungi were presented in **Table (2)**. They were 81.48, 79.1, 69.57% and 89.29, 81.4, 79.0 % for *B. bassiana* and *M. anisopliae* at the concentrations of 10^{8},10^{9} and 10^{10} spores/ml, respectively.

Results of this study indicate that EPN pathogens and fungi can be an efficient bio control agent against *T. absoluta*. *S. carpocapsae* was more virulence than the other two fungi. The fungui take days or weeks to kill whilel, nematodes, working with their symbiotic bacteria, can kill insects in 24-48 h. **Jacobson and Martin (2011)** described how high volume sprays of the entomopathogenic nematode, *S. feltiae*, could also make an important contribution to the overall IPM program me by slowing down the growth population of *T. absoluta*.

According to LC_{50} values, in **Table (3)** *B. bassiana* was the most effective on *T. absoluta* larvae and pupae than *M. anisopliae* in both soil and leaf treatments.

From the above mentioned data the infection by nematode and fungi soil treatment caused complete mortality for the 4th instar larvae after 72h. or 6th day after treatment compared to leaf treatment which caused(93.3 %,90% or80%) mortality. Highly significant differences in mortality percentages were observed between soil and leaf treatments. The application of this nematode in the soil surface at tomato plant ions could create a nematode barrier that the tomato leaf miner adult would have to pass through before reaching the tomato plant. Soil is the natural environment of entomopathogenic nematodes which shares this habital with many other micofuna and flora, including antagonists and other pathogen **(Kaya, 2002).**

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