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USE OF *VERTICILLIUM LECANII* AND *BEAUVERIA BASSIANA* AGAINST TOMATO LEAF MINER, *TUTA ABSOLUTA* (MEYRICK) AND *BEMISIA TABACI* (GENN.) IN TOMATO CROP

[1*]Abdel-Raheem M.A., [2]M.A. I. Youssif and [2]Sherin M.M.Y. Helaly

[1]Pests & Plant Protection Department, Agricultural and Biological Research Division, National Research Centre, 33rd El Bohouth St, (Postal code: 12622) Dokki, Giza, Egypt.

[2]Plant Protection Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

Corresponding Author: E-mail: abdelraheem_nrc@yahoo.com, abdelraheem_nrc@hotmail.com

Abstract

The tomato leaf miner *Tuta absoluta* (Meyrick) has invaded tomato (*Solanum lycopersicum* L.) crop in Egypt and is representing today a major threat to the production of this crop. Also, *Bemisia tabaci* is the most widespread insect pest of broad range of greenhouse and field crops. In this study, three concentrations ($1X10^1$; $1X10^2$ and $1X10^3$ spore / ml.) of *Verticillium lecanii,* and *Beauveria bassiana* were prepared and tested on *T. absoluta* eggs and larvae (1st instar, & 2nd instar) to study the impact of these Entomopathogenic fungi on eggs hatchability and larval mortality, under laboratory conditions. The estimated LC_{50} of values of *V. lecanii, M. anisopliae* and *B. bassiana* were (0.18×10^1, & $0.22x\ 10^2$), (0.20×10^1, & 0.22×10^2) and (3.1×10^1, & 4.3×10^2 spore /ml) for 1st instar, & 2nd instar *T. absoluta* larvae, respectively. The higher concentration ($1X10^3$) was the higher mortality. Also, the three concentrations used against nymph stage of *Bemisia tabaci.* Percent mortalities are increased gradually and reached to the maximum in the 7th day from treatment. Percent of mortalities are increased with increase of concentrations. The percent of mortalities ranged between 70.2to 100 and 65.5 to 100% with *V. lecanii* and *B. bassiana*, respectively, in the 7th day after treatment.

Keywords **:** Entomopathogenic Fungi, *Tuta absoluta*, *Bemisia tabaci*, Tomato Crop.

Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) has invaded tomato (*Solanum lycopersicum* L.) crop in Egypt and is representing today a major threat to the production of this crop. Also, *Bemisia tabaci* is the most widespread insect pest of broad range of greenhouse and field crops (Abdel-Raheem and Lamya Ahmed Al-Keridis, 2017). *T. absoluta* was detected in several locations throughout the Spanish Mediterranean Basin, the most important tomato growing region in the country. It is considered a key agricultural threat to European and North African tomato production. The pest can cause up to 80-100% yield losses by attacking leaves, flowers, stems and fruits (Lopez, 1991). *T. absoluta* can potentially become a pest of tomatoes in both field and greenhouses (EPPO, 2008). Its major host is *Solanum lycopersicum* (tomato), (Ismail and Abdel-Raheem, 2010 and Abdel-Raheem *et al*., 2019), other hosts also exist Such as *Capsicum* spp. (pepper), (Vargas, 1970; NAPPO, 2008 and Korycinska and Moran, 2009). Control methods (cultural, biological and biotechnological methods) becomes imperative, as the continued use of chemical insecticides could harm non-target organisms (Marta Rodríguez *et al*., 2006; Landgren *et al*., 2009; Zaki and Abdel-Raheem, 2010 and Abdel-Raheem, 2016). Narmen, 2015, mentioned that 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.

Whitefly *Bemisia tabaci* (Genn) is the most severe pests of crops in subtropical and tropical climates. *B. tabaci* is attributed to their exceptionally wide host rang and short generation time (WANG *et al*., 2007).

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin has high activity against whitefly (Al-Dehairi and Bioassay, 2008). Blastospores and conidia can infect the host directly; mycelium needs to grow and from infectious propagates first. Conidia can be produced easily and are more stable in challenging environmental conditions than blastopores (Del Prado *et al*., 2008 and Scorsetti *et al*., 2008).

The aim of study was evaluate the impact of *V. lecanii,* and *B. bassiana* on *T. absoluta* egg hatchability and larvae. Estimate the susceptibility of *Bemisia tabaci* nymphs to *V. lecanii* and *B. bassiana.*

Materials and Methods

Tomato plants

Tomato seeds were sown in the nursery in 100 cell foam trays and kept for 45 days until transplanted to the laboratory under conditions (22 °C, 65±2% R.H.). Seedlings of 45 days old were transplanted in 35 cm diameter plastic pots containing a sterilized soil- peat moss mixture, one seedling per pot. Pots were held in rearing cages (70 cm^2 high, 60 cm^2 wide and 60 cm^2 long).

Tuta absoluta colony

A laboratory colony of *T. absoluta* was established with larvae and pupae from field strain. This colony was maintained in the laboratory. Larvae and Pupae were dislodged from leaves and were housed in a wooden and glasses cage. Adults were fed on 10% honey solution (Taphla leaves were used as a carrier for honey droplets as a food source for adults) and provided with tomato terminal buds and leaves for oviposition overnight so that *T. absoluta* pupation could take place either on leaves or on the soil. When pupation was completed, the cocoons were carefully collected to be used for starting the experiment. *T. absoluta* adults were reared on tomato plants (45 days old). Tomato plants were placed in Pots and held in rearing cages (70 cm^2 high, 60 cm^2 wide and 60 cm^2 long) provided weekly by

seedlings for feeding and egg laying. When required for our assays, newly emerged adults were collected using an aspirator (Fargalla and Shalaby, 2013 and Hussein *et al*., 2014).

Fungi cultures

Three concentrations of *V. lecanii,* and *B. bassiana* were ($1x10^1$, $1x10^2$ and $1x10^3$ spore/ml). The entomopathogenic fungi were grown on peptone media (10g Peptone, 40g Dextrose, 2g Yeast extract 15g Agar and 500 ml. Chloramphenicol and completed to one liter with distilled water). The media was autoclaved at 120 °C for 20 minutes, and poured in Petri-dishes (10 cm diameter x 1.5 cm) then inoculated with the entomopathogenic fungi and kept at 22 ±2◦C and 85 ±5 R.H. The fungal isolates were re-cultured every 14-30 days and kept at 4 °C.

The spores were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added (Lacey, 1997).

Preparation of the concentrations

Spores of fungal isolates were harvested by rinsing with sterilized water 0.5% Tween 80 from 14 days old culture rice media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. Spores were counted in the suspension by using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm^2). To restore the virulence of the isolates it was passed through their natural host, wax moth larvae *Galleria mellonella.* Three concentrations were prepared, (C1) $1x10^1$, (C2) $1x10^2$ and (C3) $1x10^3$ Spore/ml in all isolates (Mohamed Abdel-Raheem, 2020).

Treatment procedures

T. absoluta couples in rearing cages for 24 hrs. Then *T. absoluta* adults were removed and the plants were checked daily until egg hatching. Potted plants were removed after exposure period and transferred in other cages until eggs start to hatching. 9 randomly selected leaves for each concentration were cut and dipped into the suspensions (three leaves per replicate), transferred onto clean white paper for water evaporation then treated leaves were put in Petri dishes with filter papers and supplied with moisture as needed, then treated leaves infested with 1st larvae obtained from the laboratory colony (15 larvae/replicate). The treated disks were only used once at the beginning of the bioassay. Subsequently, the larvae were fed with untreated leaves when needed. Similar method of experiments was performed to estimate the effect of the two entomopathogenic materials on larvae from the second instar. In addition, eggs of *T. absoluta* were exposure to *V. lecanii,* and *B. bassiana* to evaluate their effect on hatchability.

In these cases, the experiments were conducted in the same way. In order to obtain larvae of the 2nd instar used in these experiments, larvae were reared to the desired instar on tomato plants. The leaves were collected from the tomato plants, arranged in Petri dishes and infested with larvae obtained from the laboratory colony. Larvae were allowed to feed on untreated leaves until they reached the second and third instar. Discs were transferred to Petri dishes and larvae in the appropriate instar were placed in the dishes. The bioassay lasted for 7 days and the median lethal concentration (LC_{50}) values were obtained by the software computer probane. The larval mortality was evaluated daily until the end of the experiment. The mortality was corrected using Abbott's formula (Abbott, 1925).

$$\text{Corrected Mortality\%} = 100 \times 1 - \frac{\text{Insect population in treated after treatment}}{\text{Insect population in control after treatment}}$$

Laboratory inoculation

Adults whitefly, *B. tabaci* were transferred to the laboratory from the field and put in Petri-dishes with tomato leaf disk and incubated in 22±2°C and 65 ±5 % RH. (Five adults / replicate) were used in all treatments. The Entomopathogenic fungi (*V. lecanii* and *B. bassiana) were* sprayed using a manual sprayer in a suspension containing $1x10^1$, $1x10^2$ and $1x10^3$ spore/ml; while sterilized water was sprayed to the leaves disks as blank control. The mortality of whitefly was observed daily.

Results

The results in tables (1-3) revealed the impact of three concentrations of *V. lecanii,* and *B. bassiana* were prepared with concentrations of ($1x10^1$; $1x10^2$ and $1x10^3$) and tested on *T. absoluta* larvae (1st instar & 2nd instar) to study the impact of these materials on larval mortality. In addition, eggs of *T. absoluta* were exposed to *V. lecanii,* and *B. bassiana* to evaluate their impacts on hatchability under laboratory conditions. In table (1) the estimated LC_{50} of *V. lecanii,* and *B. bassiana* were (0.25×10^1 & $0.28x\ 10^1$) and (3.7×10^1 & $5.4x\ 10^1$ spores /ml) for 1st instar & 2nd instar *T. absoluta* larvae, respectively.

Thus, it was evident that the higher effective concentration of *V. lecanii* on 1st instar larvae of *T. absoluta* was $1x10^3$ spores /ml. followed by $1X10^2$ spores /ml while the other concentrations ($1X10^1$ Spores /ml.).

Table 1 : The impact of two Entomopathogenic Fungi against *Tuta absoluta* larvae.

Entomopathogenic fungi	LC_{50}	
	1st instar larvae	2nd instar larvae
V. lecanii	0.25×10^1	$0.28x\ 10^1$
B. bassiana	3.7×10^1	5.4×10^1

The results in table (2) revealed that when eggs exposure to *V. lecanii* the pathogen impacts were evident by the 4th day of evaluation after exposure in the three concentrations ($1x10^1$; $1x10^2$; $1x10^3$ spores /ml.) with recorded hatchability (29, 19, 14%) respectively.

The results in table (3) revealed that when eggs exposure to *B. bassiana* the pathogen impacts was evident by the 4th day of evaluation after exposure in the three concentrations ($1x10^1$; $1x10^2$; $1x10^3$ Conidia /ml.) with recorded hatchability (40, 36, 35%) respectively.

Table 2 : % Hatchability of *Tuta absoluta* eggs treated with *V. lecanii*

Concentration	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
	% Hatchability						
$1x10^1$	0.0	0.0	0.0	29	34	37	37
$1x10^2$	0.0	0.0	0.0	19	31	32	32
$1x10^3$	0.0	0.0	0.0	14	21	23	23
Control	0.0	0.0	0.0	44	79	84	85

Table 3 : % Hatchability of *Tuta absoluta* eggs treated with *B. bassiana*.

Concentration	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
	% Hatchability						
10^1	0.0	0.0	0.0	40	47	49	49
10^2	0.0	0.0	0.0	36	44	45	46
10^3	0.0	0.0	0.0	35	38	39	40
Control	0.0	0.0	0.0	47	80	85	85

Bemisia tabaci

Three concentrations of two isolates *V. lecanii* and *B. bassiana* were evaluated against the nymph stage of *B. tabaci* under laboratory conditions.

The result revealed in Table (4) there are no effect for *V. lecanii* and *B. bassiana* to *B. tabaci* after three day from treatment.

Mortalities are occurred in the 4th day. The percent of mortalities are increased gradually and reached to the maximum in the 7th day from treatment. With the all concentrations, the percent of mortalities are increased with increase of concentrations. The percent of mortalities ranged between 70.0 to 100 and 65.0 to 100% with *V. lecanii* and *B. bassiana*, respectively, in the seventh day after treatment. The statistical analysis shows that there are significant differences between all concentrations in both isolations. The less significant difference (L.S.D) increased gradually and reached at 9.0 in the seventh day.

Table 4 : Impact of *V. lecanii* and *B. bassiana* on *B. tabaci* under laboratory conditions.

Days after application	%Mortalities							L.S.D
	Control	C_1		C_2		C_3		
		V. lecanii	*B. bassiana*	*V. lecanii*	*B. bassiana*	*V. lecanii*	*B. bassiana*	
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4th	0.0^a	24.0 ± 1.2^b	22.0 ± 1.3^b	25.6 ± 3.2^b	21.5 ± 1.2^b	44.2 ± 5.3^c	40.10 ± 3.1^c	6.6
5th	0.0^a	48.3 ± 2.3^c	33.8 ± 2.1^b	62.3 ± 4.2^d	51.6 ± 2.3^d	69.3 ± 3.2^e	64.2 ± 2.1^e	7.1
6th	0.0^a	58.1 ± 2.3^b	53.7 ± 2.4^b	73.21 ± 2.3^c	68.2 ± 2.2^b	80.9 ± 5.3^e	80.0 ± 2.2^e	8.3
7th	0.0^a	70.0 ± 5.0^b	65.0 ± 2.2^b	$83.\pm 2.2^c$	78.9 ± 2.0^c	100 ± 2.1^d	100 ± 1.7^d	9.0

Discussion

Finally, these data clear that the entomopathogenic fungi *V. lecanii*, and *B. bassiana* can be used as a promising agent in pest control and integrated pest management programs instead of conventional pesticides to reduce the environmental pollution especially when the pests were under the economic threshold.

The data obtained that *V. lecanii* was more virulence than *B. bassiana* against eggs hatchability and larval stages on *Tuta absoluta* this results according with (Abdel-Raheem *et al*., 2015), when the author use three concentration from the entomopathogenic fungi. Also, *V. lecanii* was more impacts than *B. bassiana* against the nymphs stage of *Bemisia tabaci* this data according with (Abdel-Raheem *et al*., 2009).

The percent of mortalities are increased with increase of concentrations. The percent of mortalities ranged between 70.0to 100 and 65.0 to 100% with *V. lecanii* and *B. bassiana*, respectively, in the seventh day after treatment. The statistical analysis shows that there are significant differences between all concentrations in both isolations. The less significant difference (L.S.D) increased gradually and reached at 9.0 in the seventh day.

This result compatible with (Maniania, 1991) who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively. (Zaki and Abdel-Raheem, 2010 and Zaki, 1998) reported that *B. bassiana* as an entomopathogenic fungi showed high effects on the aphid *Aphis craccivora*, the white fly *B. tabaci* infesting cucumber and *Spodoptera littoralis*, *Spodoptera exigua* and nymphs of *Aphis craccivora* (Zaki, 1998 and Saleh, 2016) mentioned that entomopathoginic fungi caused good mortality to whitefly.

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