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Virulence of some Entomopathogenic Fungi as Abio Control Agent on Tomato leaf miner, *Tuta absoluta* (Meyrick) and *Bemisia tabaci* in Tomato Crop.

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Abstract: In this study, three concentrations (10^2; 10^3 and 10^4 Conidia/ ml.) of *Verticillium lecanii,M. anisopliae* and *Beauveria bassiana* were prepared and tested on *T. absoluta* eggs and larvae (1st instar,2nd instar &3rd instar) to study the virulence of these Entomopathogenic fungi on larval mortality In addition, eggs hatchability under laboratory conditions. Results showed that; the estimated LC_{50} of values of *V. lecanii,M. anisopliae* and*B. bassiana* were (0.20×10^2, 0.25×10^2&0.35×10^2), (0.23×10^2, $0.26 \times 10^2$$0.27 \times 10^2$) and ($3.3 \times 10^2$, 5.0×10^2&3.4×10^2conidia/ml) for 1st instar,2nd instar&3rdinstar*T. absoluta* larvae, respectively. The higher concentration (10^4) was the higher mortality. Also, the three Concentration used against adult stage of *Bemisia tabaci.* Results showed that; 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 70.2to 100 and 65.5to 100% with *V. lecanii* and *B. bassiana*, respectively, in the 7thday after treatment.
Keywords: Entomopathogenic Fungi, *Tuta absoluta*, *Bemisia tabaci*.

Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), first described in Peru in 1917, is now found throughout South America, where it is considered to be one of the most devastating pests for tomato crops [1-3].In Spain, this pest was first detected at the end of 2006 in the north of Castellon (Eastern Spain) [4]. During 2007, *T. absoluta* was detected in several locations throughout the Spanish Mediterranean Basin, the most important tomato growing region in the country. Since then, its presence has also been confirmed in Algeria, Canary Islands, France, Italy, Morocco, and Tunisia in 2008, and in Albania, Bulgaria, Cyprus, Germany, Malta, Portugal, Switzerland, The Netherlands, and the United Kingdom in 2009 [5, 6]. The tomato leaf miner *T. absoluta* (Meyrick) is one of the most devastating pests of tomato in South America [1]. This pest was initially reported in eastern Spain in late 2006 [4]and has subsequently spread throughout the Mediterranean Basin and Europe [7] Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas [8] and it is currently considered a key agricultural threat to European and North African tomato production. If no control measures are taken, then the pest can cause up to 80-100% yield losses by attacking leaves, flowers, stems and especially fruits [9].

The current management of *T. absoluta* in the Mediterranean Basin is mainly based on treatments with chemical insecticides.

Nevertheless, few active ingredients are effective against *T. absoluta* and selectively beneficial to pollinators at the same time. (*T. absoluta* is considered a key pest in many areas where it is present, including Latin America [10, 11]. A key pest is one that occurs regularly and will cause economic losses if left uncontrolled). Some consider *T. absoluta* to be the major limiting factor in tomato production in South America [12]. It is known as the most devastating tomato pest in Brazil, at times causing 100% loss of production [13].

Tomatoes may lose their commercial value when severely attacked. *T. absoluta* can potentially become a pest of tomatoes in both field and greenhouses [14]. Its major host is *Solanum lycopersicum* (tomato) [15]other hosts also exist Such as *Capsicum* spp. (pepper), [16-18].

The other control methods (cultural, biological and biotechnological methods) becomes imperative, as the continued use of chemical insecticides could harm non-target organisms[19-32.]

Whitefly *Bemisia tabaci* (Genn) causes significant damage to potato as direct feeding pest and vector of viruses [33]. *B. tabaci* is one of the most severe pests of crops in subtropical and tropical climates. The widespread distribution of *B. tabaci* is attributed to their exceptionally wide host rang and short generation time. The cause of this increase is unknown but it may be due to the extended use of synthetic organic insecticides and subsequent augmented resistance to pesticides, changing climatic conditions and international movement of plant materials in the nursery and horticultural trade [34].

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin has high activity against whitefly [35]. 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 blastospores[36, 37.]

The aim of this study was evaluate the virulence of*V. lecanii, M. anisopliae andB. bassiana T. absoluta* larvae to *V. lecanii* and*B. Bassiana* and the effect of *V. lecanii and* B. *bassiana* on egg hatchability.

Also, to estimate the susceptibility of *Bemisia tabaci* nymphs & adults to *V. lecanii* and*B. bassiana* and the effect of *V. lecanii and B. bassiana* on egg hatchability.

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^2wide 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 [38, 39].

Fungi cultures

Three concentrations of *V. lecanii,M. anisopliae* and *B. bassiana* were (10^2; 10^3 and 10^4conidia/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 Conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added [31].

Preparation of the concentrations

Conidia 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. Conidia were counted in the suspension by using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm2). To restore the virulence of the isolates it was passed through their natural host, wax moth larvae *Galleria mellonella*. Three concentrations were prepared, (C1) 10^2, (C2) 10^3 and (C3) 10^4 conidia/ml in all isolates.

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. Nine 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 1stlarvae 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 three entomopathogenic materials on larvae from the second instar and third instar. In addition, eggs of *T. absoluta* were exposure to *V. lecanii, M. anisopliae*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&3rd 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 [40.]

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

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 10^2, 10^3 and 10^4 conidia / 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- 4) revealed virulence of three concentrations of *V. lecanii*,*M. anisopliae* and*B. bassiana* were prepared with concentrations of (10^2; 10^3 and 10^4) and tested on *T. absoluta* larvae (1st, 2nd &3rd instar) to study the virulence of these materials on larval mortality. In addition, eggs of *T. absoluta* were exposed to *V. lecanii, M. anisopliae*and *B. bassiana* to evaluate their virulence on hatchability under laboratory conditions. In table (1) The estimated LC_{50} of *V. lecanii, M. anisopliae* and *B. bassiana* were (0.20 x 10^2, 0.25x 10^2&0.35x 10^2), (0.23 x 10^2, 0.26 x $10^2$0.27 x 10^2) and (3.3 x 10^2, 5.0 x 10^2&3.4 x 10^2 conidia/ml) for 1st instar , 2nd instar & 3rd 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 10^4 conidia /ml. followed by 10^3conidia /ml while the other concentrations (10^2conidia /ml.).

Table (1): Efficacy of three entomopathogenic Fungi against *Tuta absoluta*

Entomopathogenic fungi	**LC_{50}**		
	1st instar larvae	**2nd instar larvae**	**3rd instar larvae**
V. lecanii	0.20 x 10^2	0.25x 10^2	0.35x 10^2
M. anisopliae	0.23 x 10^2	0.26 x 10^2	0.27 x 10^2
B. bassiana	3.3 x 10^2	5.0 x 10^2	3.4 x 10^2

The results in table (2)revealed that when eggs exposure to*V. lecanii* the pathogen virulence was evident by the 4th day of evaluation after exposure in the three concentrations (10^2; 10^3; 10^4 Conidia /ml.) with recorded hatchability (30, 20, 15%) 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						
10^2	0.0	0.0	0.0	30	35	35	35
10^3	0.0	0.0	0.0	20	30	30	30
10^4	0.0	0.0	0.0	15	22	22	22
Control	0.0	0.0	0.0	45	80	83	84

The results in table (3) revealed that when eggs exposure to *M. anisopliae*the pathogen virulence was evident by the 4th day of evaluation after exposure in the three concentrations (10^2; 10^3; 10^4 Conidia /ml.) with recorded hatchability (40, 30, 27%) respectively.

Table (3): % Hatchability of *Tuta absoluta* eggs treated with *M. anisopliae*

Concentration	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
	% Hatchability						
10^2	0.0	0.0	0.0	40	42	44	44
10^3	0.0	0.0	0.0	30	36	38	38
10^4	0.0	0.0	0.0	27	33	36	36
Control	0.0	0.0	0.0	70	83	83	83

The results in table (4) revealed that when eggs exposure to *B. bassiana* the pathogen virulence was evident by the 4th day of evaluation after exposure in the three concentrations (10^2; 10^3; 10^4 Conidia /ml.) with recorded hatchability (43, 35, 33%) respectively.

Table (4): % Hatchability of *Tuta absoluta* eggs treated with *B. bassiana*.

Concentration	**1st day**	**2nd day**	**3rd day**	**4th day**	**5thday**	**6th day**	**7th day**
	% Hatchability						
10^2	0.0	0.0	0.0	43	46	48	48
10^3	0.0	0.0	0.0	35	43	43	43
10^4	0.0	0.0	0.0	33	3	39	39
Control	0.0	0.0	0.0	83	83	83	83

Bemisia tabaci

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

The result revealed in Table (5) 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 70.2to 100 and 65.5to 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 9.2 in the seventh day.

Table (5): Virulence 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]	25.0± 1.1[b]	23.0± 1.5[b]	27.5 ± 3.5[b]	25.5 ± 1.5[b]	45.5± 5.5[c]	42.22 ± 3.15[c]	6.8
5th	0.0[a]	50.6± 2.2[c]	35.5± 2.2[b]	64.6± 4.5[d]	55.3± 2.5[d]	70.5± 3.5[e]	65.55± 2.4[e]	7.3
6th	0.0[a]	60.0±2.5[b]	55.2 ± 2.5[b]	75.61 ±2.2[c]	70.0± 2.2[b]	80.5± 5.5[e]	80.2 ±2.5[e]	8.5
7th	0.0[a]	70.2± 5.2[b]	65.5± 2.3[b]	85.± 2.3[c]	80.6± 2.2[c]	100± 2.3[d]	100 ± 1.5[d]	9.2

Discussion

Finally, these data clear that the entomopathogenic fungi *V. lecanii*, *M. anisopliae* 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 *M. anisopliae* and *B. bassiana*against eggs hatchability and larval stages on *Tuta absoluta* this results according with [41], when the author use three concentration from the entomopathogenic fungi.Also,*V. lecanii* was more virulence than *B. bassiana* against the adult stage of *Bemisia tabaci* this data according with [22].

The percent of mortalities are increased with increase of concentrations. The percent of mortalities ranged between 70.2to 100 and 65.5to 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 9.2 in the seventh day.

This result compatible with [42] who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively. [27, 43] 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*. [43-50]mentioned that entomopathoginic fungi caused good mortality to whitefly.

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