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Entomopathogenic Effect of *Beauveria bassiana* (Bals.) and *Metarrhizium anisopliae* (Metschn.) on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) Larvae Under Laboratory and Glasshouse Conditions in Ethiopia

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Abstract

Tomato leaf miner, *Tuta absolute (*Meyrick) is one of the major pest that infest tomato plant in all agro-ecological regions of the world where it present. Currently, the management strategies highly rely on chemical insecticides, which do not provide effective control and at the same time have environmental concern in addition to the residue left on the fruits. Hence, looking for alternative control measure is vital. Studies were conducted to determine the pathogenicity and virulence of three different concentrations of *Beauveria bassiana* and *Metarhizium anisopliae* against larvae of *T. absoluta* using the concentrations of 2.5×10^7, 2.5×10^8, and 2.5×10^9 conidia ml^{-1} under laboratory and glasshouse conditions. The experiments were carried out in the laboratory and glasshouse. Mortalities caused by *B. bassiana* isolate at the different concentrations ranged from 79.17% to 95.83% under laboratory and 73.0% to 84.04% under glasshouse, the highest mortality percentage being found at 2.5×10^9 conidia ml^{-1}. The isolate of *M. anisopliae* caused the highest mortality also at the highest concentration. The lowest lethal time for *B. bassiana* and *M. anisopliae*, were achieved by the concentration 2.5×10^9 (5.01 days) and 2.5×10^8 (5.21 days), respectively. The isolates of *B. bassiana* and *M. anisopliae*, at 2.5×10^9 conidia ml^{-1} are promising for use the integrated control of *T. absoluta* larvae.

Keywords: *Beauveria bassiana*; *Metarhizium anisopliae*; Efficacy; Conidia concentrations; Larval mortality; Virulence; Chemical insecticides

Introduction

Tomato leafminer, *Tuta absoluta* (Meyrick) is an oligophagous notorious pest of a number of economic crops including tomato. To overcome the problem of this pest, insecticides play a significant role globally. Tomato is a perishable commodity with a relatively short shelves life after harvest. This pest was initially reported in the central Rift Valley region of Ethiopia in 2012 [1]. Since the time of its initial detection, the pest has caused serious damages to tomato in invaded areas [2] and it is currently considered as a key threat to Ethiopian tomato production. If no control measures are taken, the pest can cause up to 80% to 100% yield losses by attacking leaves, flowers, stems and fruits [3]. Currently, chemical insecticides are heavily used by tomato growers against *T. absoluta*. However, the chemicals which are under use have negative impacts as the other chemical have. Hence, combination with other control methods like use of entomopathogen becomes imperative, as the continued use of chemical insecticides could harm non-target organisms [4] and the environment among others. The recommended waiting period which is required between application of conventional organophosphate pesticides group and consumption can hardly be afforded. Therefore, the current experiment was initiated to evaluate the efficacy of *M. anisopliae* and *B. bassiana* isolates against T. absoluta in the laboratory and glasshouse conditions

Materials and Methods

Description of the study area

The research was conducted under laboratory and glasshouse conditions at Ambo University glasshouse and plant Science laboratory. Ambo is far away from Addis Ababa 110 km and at geographical coordinate of 8°59`N latitude and 37.85°E longitude with an altitude of 2100 meter above sea level (m.a.s.l.) [5]. The mean daily temperatures were 22°C ± 2°C and 32°C ± 2°C for laboratory and glasshouse experiments, respectively.

Experimental design and materials used

The laboratory and glasshouse experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. Eight treatments were considered such treatments were *Beauveria bassiana* isolate at three different concentrations (2.5×10^7, 2.5×10^8 and 2.5×10^9 conidia ml^{-1}), similar concentrations were performed in *Metarhizium anisopliae* isolate. Chlorantraniliprole (Coragen 200 SC) as a standard check and untreated control was also considered for comparision.

Tomato cultivar known as "Coshoro" was brought from Melkasa Agricultural Research Center. The seeds were sown on the field for natural infestation of *T. absoluta*. Harboring *T. absoluta* larvae were collected from the fields of tomato and brought to the laboratory and glasshouse. The tomato leaf miner present on these collected tomato leaves were wrapped with wet cotton kept in plastic box (20×15 cm^2) in laboratory and glasshouse. After the emergency of adults rearing cages were prepared under glasshouse.

The insect was reared and maintained on tomato plants in the glasshouse until use. Leaves were examined under binocular microscope and *T. absoluta* larvae were counted. Spore suspensions were sprayed using a hand sprayer (1 liter of capacity). After treatment applications, the percent mortalities of the agents were observed at: 3, 5 and 7days in the laboratory and 3, 5, 7 and 10 days under glasshouse conditions.

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Fungus culture and viability test

Isolates of *Beauveria bassiana* (PPRC-56) and *Metarhizium anisopliae* (PPRC-2) were obtained from Ambo Plant protection research center. These entomopathogenic fungi were cultured on potato dextrose Agar (PDA) medium containing 20 g glucose, 20 g starch, 20 g agar, and 1000 ml of distilled water in test tubes. The test tubes containing PDA medium was autoclaved at 121°C for 15-20 min and incubated at 27°C ± 1°C, 80% ± 5% relative humidity and photophase of 12 h for 15 days. The relative humidity was measured using Huger Hygrometer. The conidia were harvested by scraping the surface of 14-15 days old culture gently with inoculation needle. The mixture was stirred with a magnetic shaker for 10 min. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using Haemocytometer. Serial dilutions were prepared in distilled water containing 0.1% Tween-80 and preservedat 5°C until used.

Conidial viability was assessed according to Goettel and Inglis [6]. Three different concentrations were evaluated. The droplet of a diluted suspension was placed on a thin film of potato dextrose agar medium incubated at 27°C ± 1°C and 80% ± 5% relative humidity in the dark for 24 h. The conidia were stained with lacto-phenol cotton blue and germination was observed under microscope.

Mortality of *T. absoluta* under laboratory

The concentration of the stock suspension was adjusted to 2.5×10^7. 2.5×10^8 and 2.5×10^9 conidia ml^{-1} using an improved neubaour heamocytometer. To evaluate the efficiency of each of the fungal isolates on *T. absoluta*, 20 larvae were placed on a filter paper in 9 cm diameter petri-dish and 100 µl of the suspension was then spread. On similar trend the suspension was spread in glasshouse using hand sprayed was performed and after 3rd days of observation all counted larvae were collected from plants to brought in the laboratory to determined how many *T. absoluta* larvae were dead without being infested with fungal isolates. A control treatment was sprayed with only sterile distilled water as negative control.

Statistical analysis

The mean number of live larvae per plant or per leaf was tested for percent mortality**.** The data was subjected to analysis of variance (ANOVA) and the means were compared by least significant different (LSD) test at 0.05 levels, using SAS program version 9.1 [7]. Efficacy analysis was done based on data transformation to Arcsine $\sqrt{x+0.5}$ when necessary according to **Gomez and Gomez [8]**.

$$\text{CM (\%)} = \frac{[T(\%) - C(\%)]}{[100 - C(\%)]} X100$$

Where: CM (%) - Corrected mortality

T- Mortality in treated insects

C- Mortality in untreated insects

Isolate code	Host	Place of collection	Scientific Name	% Germination	Source
PPRC-56	*P. interrupta*	Berbere	*B. bassiana*	79	PPRC Ambo
PPRC-2	*P. interrupta*	Ashan	*M. anisopliae*	93	PPRC Ambo

Table 1: Pieces of information about indigenous entomopathogenic fungi in Ethiopia.

Treatments	Conc.	Mean percent mortality after treatment application		
		3 days	**5 days**	**7 days**
Beauveriabassiana **(PPRC-56)**	2.5×10^7	37.50^c	58.33^c	79.17^b
	2.5×10^8	70.83^{bc}	70.83^{bc}	83.33^{ab}
	2.5×10^9	79.17^{ab}	91.67^{ab}	95.83^a
Metarhiziumanisopliae **(PPRC-2)**	2.5×10^7	58.33^{bc}	58.33^{bc}	66.67^b
	2.5×10^8	58.33^{bc}	79.17^{bc}	79.17^{ab}
	2.5×10^9	66.67^{bc}	83.33^{abc}	87.50^{ab}
Chlorantraniliprole (Coragen 200 SC)		95.83^a	95.83^a	95.83^a
Control (water)		0.0^d	0.0^d	0.0^c

Note: Means with the same letter(s) in rows are not significantly different for each other. All treatment effects were highly significant at p<0.01 (DMRT)

Table 2: Mean percent mortality of *T. absoluta* treated with fungal isolates at different concentration over time under laboratory condition.

Results and Discussions

Under laboratory condition

The laboratory result also showed that percent mortality of *T. absoluta* larvae due to entomopatogenic fungi significant (P<0.01) differences among the concentrations of *B. bassiana* and *M. anisopliae* (Table 1). All concentrations of *B. bassiana* caused mortality of *T. absoluta* above 75% after treatment application of 7 days, indicating that 2.5×10^9 conidia ml^{-1} caused the highest mortality. For *M. anisopliae*, at the concentration of 2.5×10^9 conidia ml^{-1}, mortalities obtained with all concentrations were higher than 50%; however, the concentrations did differ statistically from each other after treatment application, and the highest mortality of *T. absoluta* larvae were observed with concentration 2.5×10^9 (87.5%) under laboratory condition (Table 2).

After 7th day of treatment application *B. bassiana* raveled that 79.17%, 83.33% and 95.83% mortality at 2.5×10^7, 2.5×10^8 and 2.5×10^9 concentrations, respectively. Similarly, *M. anisopliae* concentrations showed that 66.67%, 79.17% and 87.50% mortality at 2.5×10^7, 2.5×10^8 and 2.5×10^9 concentrations, respectively. There was a highly significant variation among the concentrations in causing mortality of *T. absoluta* larvae. The lowest mean percent mortality was caused by the *B. bassiana* at 3rd days of observation 37.50% which was not significantly different from *M. anisopliae* at 3rd days of 58.33 %. The highest mortality of *T. absoluta* was caused by *B. bassiana* 95.83% which did not significantly differ from the *M. anisopliae* which was 87.50% mortality. Based on the results of the virulence assays of *B. bassiana* and *M. anisopliae* had time taken by the three concentrations to caused percent mortality of *T. absoluta*. The effects of the concentrations varied significantly (P<0.01) with the lowest (3 days) recorded from concentration $2.5 \text{x} 10^7$ in *B. bassiana followed* by *M. anisopliae* (5 days) which recorded 58.33%. In the 7th day of the three concentrations the highest was recorded due to *B. bassiana* which was significantly (P<0.01) different from *M. anisopliae* concentrations.

The comparison among the different concentrations and treatments against *T. absoluta* the results indicated good performance and gradually increased from 3 to 7 days treatment application. The percent mortality according to Abbott formula [9], both agents at 2.5×10^9 conidial/ml gave statistically no significant (P<0.01) differences from the standard check (Coragen 200 SC) while highly significant different from untreated check after 3 days of treatment application (Table 2).

Concentration-response test

Percent mortality of *T. absoluta* larvae at different concentrations of *B. bassiana* and *M. anisopliae* shown in Table 2. There were no significant differences in mortality rates within each concentration except for the concentration of 2.5×10^9 conidia/ml in which the *B. bassiana* showed significantly higher mortality than *M. anisopliae*. The results of all concentrations except the first concentration 2.5×10^7 in

B. bassiana revealed the lowest at 3rd days of application but also highly significantly ($P<0.01$) among the concentrations requiring higher concentration (2.5 $10^9 \times 10^9$conidia ml^{-1}).

B. bassiana, strain presented the highest pathogenicity on *T. absoluta* larvae with 95.83% an average mortality, $LC_{50}=2.5 \times 10^9$ conidia ml^{-1} and $LT_{50}=5.01$ days (Table 3). *M. anisopliae* strain was the most virulent on *T. absoluta* larvae presenting 87.50% mortality, $LC_{50}=2.5 \times 10^9$ conidia ml^{-1} and $LT_{50}=4.82$ days. The LT_{90} values to *B. bassiana* strains on *T. absoluta* larvae ranged from 8.06 to 9.32 days, and for *M. anisopliae* strains on *T. absoluta larvae* ranged from 8.14 to 9.04 days (Table 3). The *M. anisopliae* strain presenting the lowest LC_{90} on *T. absoluta* larvae was 2.5×10^9 conidia ml^{-1}) and the highest LC_{90} was presented by *B. bassiana* 2.5×10^7 conidia ml^{-1}). Finally, for *T. absoluta* larvae the LC_{90} of both *B. bassiana and M. anisopliae* varied from 2.5×10^7 to 2.5×10^9 conidia ml^{-1} concentration (Table 4).

Under glasshouse conditions

The entomopathogenic fungal isolates were tested at three different concentrations for their percent mortality against *T. absoluta* in glasshouse to explore their potential to manage the pest population. Percent mortality of *T. absoluta* larvae were calculated for the different concentrations of the two isolates and showed increasing mortality with increasing spore concentration. Cumulative mortality of *T. absoluta* larvae over exposure period (3, 5, 7 and 10 days) was significantly ($P<0.01$) different for fungi isolates (Table 5). On the 3rd days of exposure maximum mortality 91.84 recorded from standard check, while the untreated control had 2.78% mortality. These were significantly different from all concentrations of the fungal isolates. Among the concentrations of entomopathogenic fungi maximum percent mortality was recorded at 2.5×10^9 conidial ml^{-1} of *B. bassiana* (84.04%) followed by *M. anisopliae* (76.31%) on 10th day after treatment application. At the highest concentration of conidial ml^{-1}, all *B. bcassiana* concentration gave the highest percent mortality (Table 5). The results indicated for pathogenicity of all the concentrations revealed that all of them are virulent, even three days after application causing significant mortality up to 64.05% when compared with untreated control.

Treatments		95% Confidence Limit		95% Confidence Limit	
	(Conidia ml^{-1})	LT_{50} (days)	Slope ± SE	LT_{90} (days)	Slope ± SE
B. bassiana **(PPRC-56)**	2.5×10^7	5.45	3.17 ± 0.52	9.32	2.28 ± 0.48
	2.5×10^8	5.21	3.80 ± 0.61	8.87	2.66 ± 0.37
	2.5×10^9	5.01	4.29 ± 0.82	8.06	3.06 ± 0.68
M. anisopliae **(PPRC-2)**	2.5×10^7	5.14	3.64 ± 0.56	9.04	2.86 ± 0.46
	2.5×10^8	5.02	3.63 ± 0.48	8.56	3.04 ± 0.58
	2.5×10^9	4.82	3.31 ± 0.64	8.14	3.31 ± 0.72

Table 3: Median lethal time (LT_{50}) and (LT_{90}) of *B. bassiana* and *M. anisopliae* against *T. absoluta*.

Treatments				95% Confidence Limit	
	(Conidia ml^{-1})	LC_{50}	Slope ± SE	LC_{90}	Slope ± SE
B. bassiana (PPRC-56)	2.5×10^7	4.23 ± 0.52	4.29 ± 0.82	9.68 ± 0.82	3.36 ± 0.41
	2.5×10^8	3.93 ± 0.61	3.80 ± 0.61	9.22 ± 0.61	2.64 ± 0.38
	2.5×10^9	3.50 ± 0.82	3.17 ± 0.52	8.46 ± 0.52	2.45 ± 0.28
M anisopliae (PPRC-2)	. 2.5×10^7	3.59 ± 0.56	3.31 ± 0.64	8.71 ± 0.64	2.47 ± 0.77
	2.5×10^8	3.26 ± 0.48	3.63 ± 0.48	8.13 ± 0.48	2.63 ± 0.58
	2.5×10^9	2.91 ± 0.64	3.64 ± 0.56	7.52 ± 0.56	3.54 ± 0.72

Table 4: Median lethal (LC_{50}) and (LC_{90}) of *B. bassiana* and *M. anisopliae* (100µ / larva) of *T. absoluta*.

Treatments	Conc.	Mean percent mortality after treatment application			
		3 days	5 days	7 days	10 days
Beauveriabassiana **(PPRC-56)**	2.5×10^7	43.85^c	57.57^{bc}	75.17^{ab}	81.64^{ab}
	2.5×10^8	56.27^{bc}	56.27^{bc}	76.62^{ab}	73.0^{abc}
	2.5×10^9	63.84^b	67.05^b	67.05^{bc}	84.04^{ab}
Metarhiziumanisopliae **(PPRC-2)**	2.5×10^7	38.76^c	42.93^c	53.37^c	53.37^d
	2.5×10^8	44.07^c	51.98^{bc}	61.49^{bc}	64.65^{cd}
	2.5×10^9	64.05^b	68.21^{bc}	71.98^{abc}	76.31^{bc}
Chlorantraniliprole (Coragen 200 SC)		91.84^a	91.84^a	91.84^a	91.84^a
Control		2.78^d	4.76^d	4.76^d	7.14^e
Note: Means with the same letter(s) in rows are not significantly different for each other. All treatment effects were highly significant at p<0.01 (DMRT)					

Table 5: Mean percent mortality of Entomopathogenic fungi at different concentration on larvae *T. absoluta* under glasshouse condition.

A positive relationship was recorded between mortality percentages and concentrations among the *B. bassiana* and *M. anisopliae* concentrations. Concurrently, with the increase in conidia concentration, a reduction in LT_{50} was observed. Concentrations of 2.5×10^9 from *B. bassiana*, at the concentrations 2.5×10^8 and 2.5×10^9 conidia ml^{-1}, presented the shortest lethal time (Table 5). These low values are probably associated to the presence of enzymes that aid in the process of penetration of the fungi [10].

The effect of entomopathogenic fungi were evaluated to determine the concentrations with high efficacy against larvae *T. absoluta* under laboratory and glasshouse conditions. Both fungal isolates were found to be pathogenic to *T. absoluta*. Though, there was a variation in their virulence against *T. absoluta*. The percent mortality for all the concentration gradually increased. The spore formation appeared on the larvae of *T. absoluta* took place after treatment exposure of the concentrations of the two isolates starting from the day three after treatment exposure, and thereby no hatched larvae were appeared in the concentrations of both isolates comparing the control treatment. The *M. anisopliae* in all concentrations were significantly less effective when compared with that of *B. bassiana* in terms of virulence. Virulence due to *B. bassiana* on 10th day was not significantly different from each other. This indicated that all *B. bassiana* concentrations were the best management option of *T. absoluta*. This finding confirms with earlier reports [11] who obtained high percent mortality during the evaluation time for *B. bassiana* and *M. anisopliae*.

The amount of conidia used should to attain a certain concentration and thus, achieving an efficacious penetration of the fungus on the insect cuticle and causing host death. Similar findings by Garcia *et al.* [12] were obtained, evaluating the insecticidal activity of *B. bassiana* strains and *M. anisopliae* on *Spodoptera frugiperda* and *Epilachna varivestis* larvae at six concentrations (10^4 to 10^9); *B. bassiana* strain was more virulent for *E. varivestis* larvae with a 93.3% mortality, $LC_{50}=1.20 \times 10^6$ conidia ml^{-1} and $LT_{50}=5.1$ days. *B. bassiana* strain presented the highest mortality on *S. frugiperda* larvae (96.6%, $LC_{50}=5.92 \times 10^3$ conidia ml^{-1} and $LT_{50}=3.6$ days). It was also reported by another authors differences among lethal times is a tool widely used in selecting strains, because it is interesting that the fungus quickly eliminate its host, as well [13]. These results are disagreed with Khalid et al. [14], evaluating the virulence of various strains of *B. bassiana* and *M. anisopliae* on *G. mellonella* larvae using 10^2, 10^3, 10^4, 10^5 and 10^6 conidia ml^{-1} concentration.

Thus, laboratory and glasshouse experiments suggested that *B. bassiana* and *M. anisopliae* have good effect on both egg and larvae of *T. absoluta.* Sabbour [15] also confirmed the effectiveness of both *B. bassiana* and *M. anisopliae* against larvae of *T. absoluta* under laboratory and greenhouse. The same results were obtained by Sabbour and Singer [16]; Sabbour and Abdel-Raheem [17]. These results agree with our findings and Cabello et al. [18] where stated that; the higher mortality of larvae under laboratory studies indicated *B. bassiana* could cause good larval mortality. At present, the knowhow of entomopathogenic fungi on *T. absoluta* was very limited because of very few studies that are available to indicate that the isolates causes the high mortality on other lepidopteran insects [19]. In this study it has been shown that all the fungal concentrations are effective against *T. absoluta*.

Our results confirmed that, the previous study of Shalaby et al. [11], they stated that when the second instar larvae fed on *M. anisopliae* the pathogen effect was evident by the 3rd day of evaluation after exposure in the concentration (10^7 and 10^8 conidia/ml). Dahliz et al. [20] have reported similar results with *Metarhizium*. Our result was confirmed the work of İnanl and Oldargc [21], they reported the studies conducted in Turkey, researchers compared the efficacy of *B. bassiana* and *M. anisopliae* on *T. absoluta* eggs and larvae; these two agents provided highly effective to control of *T. absoluta* larvae. Our results also indicated the potential of *B. bassiana* and *M. anisopliae* to control the larvae of *T. absoluta* in an integrated pest management programs. Neves and Alves [22] also noted, as more conidia penetrating, more toxins or enzymes are released, increasing the insect mortality. Though, the fungus action speed depends, besides the concentration, of the host species involved [23]. According Kleespies and Zimmermann [24], variation in virulence of entomopathogenic strains is a result of differences in the enzymes and toxins production in conidia germination speed, mechanical activity in the cuticle penetration, colonization capacity and cuticle chemical composition.

Conclusion and Recommendation

The most effective percent mortality of fungal isolates was found in *B. bassiana* followed by *M. anisopliae* at all concentrations. Both agents could be very well utilized as alternative to bio pesticides for the management of *T. absoluta*. It might be concluded that *B. bassiana* and *M. anisopliae* fungi present different capacity cause mortality of the insects, with the 2.5×10^9 conidian ml^{-1} *B. bassiana* strains as the most pathogenic for *T. absoluta*, as well as 2.5×10^9 conidian ml^{-1} *M. anisopliae* strains was also good virulence for *T. absoluta* and also presenting the lowest LC_{50} and LT_{50} values. Hence, insecticidal substances that have potential for use as alternative control measure. Therefore, further study on field conditions should be undertaken to evaluate effectiveness of experimental mycopesticide formulations in the management of *T. absoluta* under Tropical conditions in various economically important insect pests.

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