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 absoluta (Meyrick) is one of the major pests that infest tomato plants in all agro-ecological regions of the world where it is 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 Metarrhizium anisopliae against larvae of T. absoluta using the concentrations of 2.5 × 10^6, 2.5 × 10^9, and 2.5 × 10^10 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 × 10^9 (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; Metarrhizium 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 × 10^9, 2.5 × 10^10 and 2.5 × 10^9 conidia ml^-1), similar concentrations were performed in Metarrhizium anisopliae isolate. Chlorantraniliprole (Coragen 200 SC) as a standard check and untreated control was also considered for comparision.

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 7 days in the laboratory and 3, 5, 7 and 10 days under glasshouse conditions.

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Received March 06, 2017; Accepted May 24, 2017; Published May 27, 2017

Citation: Tadele S, Emana G (2017) Entomopathogenic Effect of Beauveria bassiana (Bals.) and Metarrhizium anisopliae (Metschn.) on Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) Larvae Under Laboratory and Glasshouse Conditions in Ethiopia. J Plant Pathol Microbiol 8: 411. doi: 10.4172/2157-7471.1000411

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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 preserved at 5°C until used.

Conidial viability was assessed according to Göttel 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 using an improved neubaur 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 replicated three times. The mixture on each isolate was spread in glasshouse using hand sprayed was replicated three times. The mixture on each isolate was spread in glasshouse using hand sprayed was replicated three times. 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 preserved at 5°C until used.

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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 √x+0.5 when necessary according to Gomez and Gomez [8].

\[ CM(\%) = \frac{[T(\%) - C(\%)] X 100}{[100 - C(\%)]} \]

Where: CM (%) = Corrected mortality

T- Mortality in treated insects

C- Mortality in untreated insects

Results and Discussions

Under laboratory condition

The laboratory result also showed that percent mortality of *T. absoluta* larvae due to entomopathogenic fungal 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 × 10^8 conidia ml^-1 caused the highest mortality. For *M. anisopliae*, at the concentration of 2.5 × 10^6 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 × 10^7 (87.5%) under laboratory condition (Table 2).

After 7th day of treatment application *B. bassiana* ravelled 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 × 10^6, 2.5 × 10^7 and 2.5 × 10^8 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 2.5x10^6 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 × 10^6 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 × 10^6 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

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Host</th>
<th>Place of collection</th>
<th>Scientific Name</th>
<th>% Germination</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPRC-56</td>
<td>P. interrupta</td>
<td>Berbere</td>
<td><em>B. bassiana</em></td>
<td>79</td>
<td>PPRC Ambo</td>
</tr>
<tr>
<td>PPRC-2</td>
<td>P. interrupta</td>
<td>Ashan</td>
<td><em>M. anisopliae</em></td>
<td>93</td>
<td>PPRC Ambo</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conc.</th>
<th>Mean percent mortality after treatment application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td>2.5 × 10^7</td>
<td>37.50%</td>
</tr>
<tr>
<td>(PPRC-56)</td>
<td>2.5 × 10^8</td>
<td>70.83%</td>
</tr>
<tr>
<td><em>Metarhizium anisopliae</em></td>
<td>2.5 × 10^5</td>
<td>79.17%</td>
</tr>
<tr>
<td>(PPRC-2)</td>
<td>2.5 × 10^6</td>
<td>58.33%</td>
</tr>
<tr>
<td></td>
<td>2.5 × 10^7</td>
<td>79.17%</td>
</tr>
<tr>
<td></td>
<td>2.5 × 10^8</td>
<td>66.67%</td>
</tr>
<tr>
<td><em>Chlorantriuliprole</em> (Coragen 200 SC)</td>
<td>95.83%</td>
<td>95.83%</td>
</tr>
<tr>
<td>Control (water)</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

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.
B. bassiana revealed the lowest at 3rd days of application but also highly significantly (P<0.01) among the concentrations requiring higher concentration (2.5×10⁹ conidia ml⁻¹). The strain presented the highest pathogenicity on T. absoluta larvae with 95.83% an average mortality, LC₅₀=2.5×10⁹ conidia ml⁻¹ and LT₅₀=5.01 days (Table 3). M. anisopliae strain was the most virulent on T. absoluta larvae presenting 87.50% mortality, LC₅₀=2.5×10⁹ conidia ml⁻¹ and LT₅₀=4.82 days. The LT₅₀ 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 LC₅₀ on T. absoluta larvae was 2.5×10⁹ conidia ml⁻¹ and the highest LC₅₀ was presented by B. bassiana 2.5×10⁹ conidia ml⁻¹. Finally, for T. absoluta larvae the LC₅₀ of both B. bassiana and M. anisopliae varied from 2.5×10⁷ to 2.5×10⁹ 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 2.5×10⁹ 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. bassiana 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.

A positive relationship was recorded between mortality percentages and concentrations among the B. bassiana and M. anisopliae concentrations. Concurrently, with the increase in conidial concentration, a reduction in LT₅₀ was observed. Concentrations of 2.5×10⁷ from B. bassiana, at the concentrations 2.5×10⁸ and 2.5×10⁹ 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. These results were in the treatments of two isolates and then the least effective against both egg and larvae of T. absoluta. Sabbix [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 Sabbix and Singer [16]; Sabbix and Abdel-Raheem [17]. These results agree with our findings and Cabello et al. [18] where stated that; the higher mortality

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LC₅₀ (Conidia ml⁻¹)</th>
<th>LC₉₀ (Conidia ml⁻¹)</th>
<th>95% Confidence Limit</th>
<th>95% Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. bassiana (PPRC-56)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5×10⁷</td>
<td>4.82 ± 0.82</td>
<td>3.16 ± 0.64</td>
<td>8.14</td>
<td>3.31 ± 0.72</td>
</tr>
<tr>
<td>2.5×10⁸</td>
<td>3.26 ± 0.48</td>
<td>3.80 ± 0.61</td>
<td>9.32</td>
<td>3.63 ± 0.82</td>
</tr>
<tr>
<td>2.5×10⁹</td>
<td>5.14</td>
<td>3.64 ± 0.56</td>
<td>9.04</td>
<td>3.93 ± 0.61</td>
</tr>
<tr>
<td>M. anisopliae (PPRC-2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5×10⁷</td>
<td>6.22 ± 0.51</td>
<td>3.17 ± 0.52</td>
<td>8.46 ± 0.52</td>
<td>2.45 ± 0.28</td>
</tr>
<tr>
<td>2.5×10⁸</td>
<td>3.31 ± 0.64</td>
<td>9.04</td>
<td>2.86 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>2.5×10⁹</td>
<td>4.82</td>
<td>3.31 ± 0.64</td>
<td>9.04</td>
<td>3.93 ± 0.61</td>
</tr>
</tbody>
</table>

**Table 3**: Median lethal time (LT₅₀ and LT₉₀) of both B. bassiana and M. anisopliae against T. absoluta.

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 (10⁴ to 10⁸); B. bassiana strain was more virulent for E. varivestis larvae with a 93.3% mortality, LC₅₀=1.20×10⁶ conidia ml⁻¹ and LT₅₀=5.1 days. B. bassiana strain presented the highest mortality on S. frugiperda larvae (96.6%, LC₉₀=5.92×10⁵ conidia ml⁻¹ and LT₉₀=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 10⁴, 10⁵, 10⁶ and 10⁷ conidia ml⁻¹.

Thus, laboratory and glasshouse experiments suggested that B. bassiana and M. anisopliae have good effect on both egg and larvae of T. absoluta. Sabbix [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 Sabbix and Singer [16]; Sabbix 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 Inanlı and Oldarg [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 x 10^7 conidial ml^-1 *B. bassiana* strains as the most pathogenic for *T. absoluta*, as well as 2.5 x 10^8 conidial 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 toxins or enzymes are released, increasing the insect mortality. Though, the larvae of *T. absoluta* and also indicated the potential of *B. bassiana* and *M. anisopliae* for control of adult *Haematobia irritans* (Diptera: Muscidae). *J Econ Entomol* 99: 1943-1947.

References


Citation: Tadele S, Emana G (2017) Entomopathogenic Effect of *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) Larvae Under Laboratory and Glasshouse Conditions in Ethiopia. *J Plant Pathol Microbiol* 8: 411. doi: 10.4172/2157-7471.1000411