Biological Control Using Microorganisms

*Metarhizium anisopliae, Beauveria bassiana and Lecanicillium lecanii Against Tuta absoluta (Meyrick)*

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SHORT COMMUNICATION

Abstract

*Tuta absoluta* (Meyrick) is a serious pest of tomato crop with larvae causing even 100% loss, if not effectively controlled. Native from South America, currently, it can be found throughout Europe, Africa and parts of Asia. In Romania, it was reported for the first time in 2009 (Leaotă, 2009). In this study, 12 concentrations of *Metarhizium anisopliae* and *Lecanicillium lecanii* were prepared and tested on *T. absoluta* larvae to study the impact of entomopathogenic fungi on larvae mortality, under laboratory conditions. The results showed that only four experiments had a mortality rate of over 50% on *T. absoluta* larvae. The higher concentration had the highest mortality rate. Research shows that microorganisms are effective, but at very high concentrations, leading to a higher cost of production. In these conditions, we could recommend either to increase the colony forming units in the commercial products, or to enrich the crop area with beneficial microfauna before establishing the crop. The present study might be useful in developing future integrated management strategies for tomato leaf miner.

Keywords: biopesticides; entomopathogenic fungi; tomato leaf miner.

INTRODUCTION

It is well-known that tomato is one of the most widely cultivated vegetable, with over 5 million hectares planted worldwide and up to 180 million tons produced yearly (FAOSTAT, 2019). *Tuta absoluta* (Meyrick) also known as tomato leaf miner or tomato pinworm is a serious pest of tomato crop with larvae causing even 100% loss if not effectively controlled. Invasive species represent a major threat to natural and managed systems (Asplén et al., 2015; Clavero and Garcia-Berthou, 2005; Haye et al., 2015; Piçanco et al., 1998). Native from South America, *T. absoluta* (Meyrick) has been a key pest of tomato since the 1950s, causing drastic tomato yield losses, but also other solanaceous vegetables (Desneaux et al., 2013). Before invading Spain, *T. absoluta* was included on the list of quarantine pests of the European Plant Protection Organization, but was not listed in the Plant Health Directive (2000/29/EC) of the European Commission. Thus, imported tomato materials were not subjected to plant health inspection before entry into Europe, and the omission likely helped initial introduction (Biondi et al., 2017). After invading Spain in 2006, the status of tomato pinworm completely changed and steady spread across the Europe, Afro-Eurasian continents, becoming a major threat not only to South America, but to global tomato...
production (Campos et al., 2017; Desneaux et al., 2013). In 2009, after entering Europe, the USDA-APHIS issued a federal import quarantine order for materials potentially carrying T. absoluta, including on fresh tomatoes and alternative host plants (USDA, 2014).

In Romania, first report of T. absoluta was recorded in 2009 in Botosani county (Leaoță, 2009) and then spread in almost all the vegetables area in the country (Cean and Dobrin, 2009). In order to diminish the attack of tomato pinworm, control strategies have been developed, but the widespread use of insecticides have resulted in insecticide resistance in many T. absoluta populations as well as in side effects on most of the non-target arthropods present in the tomato agro-ecosystems. The increase of insecticide has disturbed pre-existing integrated pest management (IPM) programs in invaded areas, as seen in Europe. As a result, biocontrol of T. absoluta has been developed, and it relies largely on the augmentation and conservation of omnivorous mirid predators, conservation of parasitoid complexes, and use of microbial organisms such as Bacillus thuringiensis (Biondi et al., 2017), Metarhizium anisopliae, Beauveria bassiana and Lecanicillium lecanii (Abdel-Raheem et al., 2020).

The aim of this study was to evaluate the impact of commercial Metarhizium anisopliae, Beauveria bassiana and Lecanicillium lecanii on tomato leaf miner larvae, on different concentrations.

MATERIALS AND METHODS

Biological material
Tomato seeds were grown in a nursery in 70 cell pallets for 45 days and transplanted in the greenhouse. A colony of T. absoluta was establish in the greenhouse and after the developments of insects, larvae and pupae were dislodge from the leaves and transfer to laboratory.

Fungi experiment
The experiments were conducted under laboratory conditions of Vegetable Research Development Station Buzau, Romania. Promising commercial entomopathogens Lecanicillium lecanii (Verticillium lecanii-10^6 UFC/g), Beauveria bassiana and Metarhizium anisopliae (10^6 UFC/g) where taken in the experiment in 12 different concentrations (Table 1).

Table 1. The concentrations of the entomopathogenic fungi used in the research

<table>
<thead>
<tr>
<th>Entomopathogenic fungi</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecanicillium lecanii - L2</td>
<td>2 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii - L3</td>
<td>3 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii - L4</td>
<td>4 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii - L6</td>
<td>6 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii - L8</td>
<td>8 ml/1l water</td>
</tr>
<tr>
<td>Beauveria bassiana and Metarhizium anisopliae - BM5</td>
<td>5 ml/1l water</td>
</tr>
<tr>
<td>Beauveria bassiana and Metarhizium anisopliae - BM6</td>
<td>6 ml/1l water</td>
</tr>
<tr>
<td>Beauveria bassiana and Metarhizium anisopliae - BM8</td>
<td>8 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii, Beauveria bassiana and Metarhizium anisopliae - LBM2</td>
<td>2 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii, Beauveria bassiana and Metarhizium anisopliae - LBM4</td>
<td>4 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii, Beauveria bassiana and Metarhizium anisopliae - LBM6</td>
<td>6 ml/1l water</td>
</tr>
<tr>
<td>Lecanicillium lecanii, Beauveria bassiana and Metarhizium anisopliae - LBM8</td>
<td>8 ml/1l water</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSIONS

Tomato leaf miner is a serious pest of solanaceous family and is notoriously difficult to control because of the early colonization of fields. The attack of pinworm (Figure 1, a, c) can be hard to distinguished in the first stages of invasion, because the injuries are quite narrow. Also, in the early stages, the attack of T. absoluta is similar to another pest, Liriomyza trifoli (serpentine leaf miner, figure 1, b). The difference between them will become noticeable when the galleries caused by tomato pinworm will acquire greater proportions (Figure 1, c).

The mortality rate of larvae was studied in laboratory conditions and was recorded after 48 and 72 hours, as exposed in Figure 2. The experimental variants L2, L6 and LBM2 had no reportable incident of death registered.
after 48 hours. It can be seen in Figure 2 that the concentration with Lecanicillium lecanii – L2 and Lecanicillium lecanii, Beauveria bassiana and Metarhizium anisopliae - LBM2, had a mortality rate after 72 hours with less than 12%. In other cases, the variants L3 and L4, had a mortality rate around 22% after 48 hours, but afterwards no other death larva occurs.

![Image](https://via.placeholder.com/150)

**Figure 1.** (a) Incipient attack of *T. absoluta* and (b) *Liriomyza trifolii* (c) Stronger attack of *T. absoluta*

The experimental variants, L8, BM8, LBM6 and LBM 8 had registered a mortality rate range between 30.72% to 60.42% after 48 hours. The same concentrations had a higher mortality rate varying from 61.26% to 83.88% after 72 hours.

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** The mean mortality rate of *T. absoluta* larvae after 48 and 72 hours

Compared with other studies (Halder et al., 2017), the treatment with *B. bassiana* had a larval mortality varying from 33.35 to 53.36% after 48 and 72 hours, but the concentrations where lower than the one studied in this research. Bioactivity of *B. bassiana* against larvae of pinworm tomato was confirmed also by other studies (Molla et al. 2011; Kaoud, 2014). Some studies (Rodriguez et al., 2006a) have shown that the mortality percentage of the third instars of pinworm was higher than 90% when exposed to both *M. anisopliae* and *B. bassiana*. Formerly, the same authors (Rodriguez et al., 2006b) have evaluated the efficacy of *M. anisopliae* against *T. absoluta* eggs and the results were not as promising as they obtained 60% mortality rate. In terms of efficacy using *L. lecanii*, it was affirmed that the mortality rate can range between 70-100% (Abdel-Raheem et al., 2015; Abdel-Raheem et al., 2020).

The mean results of the experiments were statistical analysed using analysis of variance followed by Duncan test and the results are presented in Figure 3. It can be noted that we recorded significant differences between all concentrations. As a general rule, it was observed that the percentage of mortalities had an increase with the increase of concentrations, as can be seen, L8, BM8, LBM6 and LBM8 had a higher impact on pinworm larva than lower concentrations taken in the study. The mixed combination of *L. lecanii*, *B. bassiana* and *Metarhizium anisopliae* has shown that it can be efficient against larvae of *T. absoluta* under laboratory conditions, but at higher doses than the commercial recommendations. In a study made on *B. bassiana* and *Metarhizium anisopliae*, Neves and Alves (2000) stated that as more conidia penetrate the cuticle, more toxins or enzymes are released, increasing the insect mortality.
Figure 3. The impact of biopesticides under laboratory conditions a). Means chart b). Results of Duncan test

CONCLUSIONS

In the current study, it was shown that higher doses of entomopathogenic fungi can have a significant impact on the *T. absoluta* larvae in laboratory conditions. As the results had demonstrated some efficacy, this might lead to a higher cost of production. In these conditions, we could recommend either to increase the colony forming units in the commercial products, or to enrich the crop area, especially the soil with beneficial microfauna as part of an integrated pest management program.

**Author Contributions:** E.B. Wrote the paper, performed the statistical analysis, conceived and designed the analysis; G.I. and B.E. Collected the data; A.O. Contributed data and performed the analysis; C.V. supervised the experiments.

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**Conflicts of Interest**

The authors declare that they do not have any conflict of interest.

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