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ENTOMOPATHOGENIC EFFECT OF SOME *Beauveria* ISOLATES ON THE TOMATO LEAF MINER *Tuta absoluta* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) LARVAE UNDER LABORATORY CONDITIONS

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ABSTRACT

The present study aimed at examining the efficacy of two local Beauveria strains, used separately or simultaneously with a native predatory bug species and their interactions against the larvae of Tuta absoluta on tomato plants under laboratory conditions. The fungal treatments were done with concentrations of 1 × 10^5, 1 x 10^7 and 1 x 10^9 conidia ml^{-1}. Seven days after treatment application, both Beauveria isolates induced a Tuta absoluta mortality above 80%, the best results being registered in case of combination between the use of B. pseudobassiana isolate at a concentration of 1 x 10^7 conidia ml^{-1} and the predator Arma custos. No side-effects on natural enemies used in combination indicate these isolates as promising alternative for Tuta absoluta control.

INTRODUCTION

The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest recently spread from South America in Europe, Africa and Asia, that pose severe threat on tomatoes and other cultivated or wild species of Solanaceae including aubergines, tobacco and potato (Desneux et al., 2010). On tomatoes, it causes direct damage by attacking all green plant parts and crop developmental stages, although the larvae seem to prefer apical buds, new leaflets and green fruits (Haji et al. 1988, Lopez 1991, Apablaza 1992). The pest was detected in Romania in 2009, being found first in the market and in a grange, then in a greenhouse (Leaota 2009, Cean & Dobrin 2009, Baetan et al. 2013). Since 2018, the tomato leaf miner *T. absoluta* registered massive damage in Romanian horticultural farms (Ciceoi and Radulovici 2018) and has been considered as the key pest of tomato crops in southern Romania (Iamandei et al. 2021). Recent researches in newly invaded areas are focused to the development of alternative pest management strategies, based on the use of specific predators, parasitoids (Biondi et al. 2013, Chailleux *et al*. 2013), plant extracts (Moreno et al. 2011), nematodes (Garcia et al. 2013) and pathogenic microorganisms (Contreras et al. 2014; Klieber and Reineke 2015). The application of myco-

insecticide, that are primarily based on *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) represents a promising alternative for *T. absoluta* pest control and some studies reported high virulence of *B. bassiana* (Klieber & Reineke 2015; Silva et al. 2020), *Metarhizium anisopliae* (Contreras et al. 2014) and *B. pseudobassiana* (Haidar 2018) isolates against *T. absoluta.*

The study aim was to evaluate the insecticidal activity of two autochthonous *Beauveria* strains, used separately or simultaneously with a predatory bug species and their interactions against larval stages of tomato leaf miner *T. absoluta* (TLM) on tomato plants under laboratory conditions.

MATERIAL AND METHODS

Fungal isolates

One isolate of *B. pseudobassiana* (Bb1) and one isolate of *B. bassiana* (Bb2) were used. Both isolates were obtained from the Collection of Entomopathogenic Microorganisms maintained at the RDIPP Bucharest, Romania. The treatment was performed with an aqueous conidial suspensions obtained by washing fungal culture grown on agar slants (30 days old) with a solution of sterile distilled water and Tween 80 (0.01%). The concentration of conidia in each aqueous suspension was adjusted to concentrations of 1×10^5, 1×10^7 and 1×10^9 conidia ml^{-1} using Burker hematocytometer under Hund Medicus light microscope. For each treatment variant 50 ml of suspension were prepared. The control consisted of a solution of distilled water and Tween 80 (0.01%).The viability of conidia was checked by germination tests on PDA medium according to Francisco et al. (2006). The results of the viability tests indicated a viability percentage higher than 90% for both isolates.

Insect source

A rearing colony was established from a *T. absoluta* population collected from an infested tomato greenhouse at Dragomiresti Vale (Ilfov County), transferred to a mass-rearing facility at R.D.I.P.P. Bucharest in insect-proof cages on tomato variety PREKOS. After several generations obtained under controlled conditions second instar larvae of TLM were used in this experiment.

Predatory bugs were obtained from the R.D.I.P.P. Bucharest mass-rearing laboratory. Second instar larvae of *Arma custos,* were used in this experiment.

Laboratory assessment method

Second instar larvae of the TLM were tenderly released from the leaf tissue and transferred with fine brushes in the sterile Petri dishes, tapered with filter paper on the bottom, onto fresh tomato leaflets. The aqueous suspensions were sprayed using sterile hand-held spraying device immediately after TLM transfer. Applications made with sterile distilled water, instead of aqueous conidial suspensions, were used as control. One larva of predatory bug species *Arma custos*, was added into every unit of treatment no. 8 and 9. as described in table 1.

Table 1

Experimental treatments details

Treatment number	Treatment description
1	Untreated control (distiled water)
2	*Beauveria pseudobassiana* (Bb1) 1×10^5 conidia ml^{-1}
3	*B. pseudobassiana* (Bb1) 1×10^7 conidia ml^{-1}
4	*B. pseudobassiana* (Bb1) 1×10^9 conidia ml^{-1}
5	*B. bassiana* (Bb2) 1×10^5 conidia ml^{-1}
6	*B. bassiana* (Bb2) 1×10^7 conidia ml^{-1}

7	*B. bassiana* (Bb2) 1 x 10^9 conidia ml^{-1}
8	*B. pseudobassiana* (Bb1) 1 x 10^7 conidia ml^{-1} + 1 *Arma custos* second instar larvae
9	*B. bassiana* (Bb1) 1 x 10^7 conidia ml^{-1} + 1 *Arma custos* second instar larvae

Each treatment was replicated ten times and each Petri dish with 5 TLM second instar larvae served as a replicate. All experimental unit were maintained in laboratory at 25 ± 1 °C, 65% RH with a 14 L: 10 D photoperiod. The number of TLM dead larvae was noticed and recorded at 24 hours, and 3, 7 and 10 days after the applications (DAA). Surviving TLM larvae were also monitored until pupation.

Statistical analysis

The larval mortality data were corrected with the control using Schneider-Orelli's formula. The data were transformed to arcsin sqrt to normalise the variances and were subjected to One-Way ANOVA. Te signifcant differences among the treatment means were separated using Tukey HSD. The analyses were carried out with GraphPadPrism V.7 for Windows.

RESULTS AND DISCUSSIONS

Both autochthonous isolates of Beauveria tested during the experimental period were pathogenic to tomato leaf miner larvae, the first mortalities began to occur from the first day. Regarding the effect of the different concentration on the percentages of cumulative mortality, no significant differences were found between the variants of fungal treatment with Bb1 (F=0.8117; df=2.27; p=0.4546) and Bb2 (F=3.178; df=2.27; p=0.0576) (Fig. 1 and Fig. 2). At all the tested concentrations it was observed that there is no significant difference between the mortality percentages corrected at 7 days and those at 10 days (p>0.8) for both Beauveria species treatments.

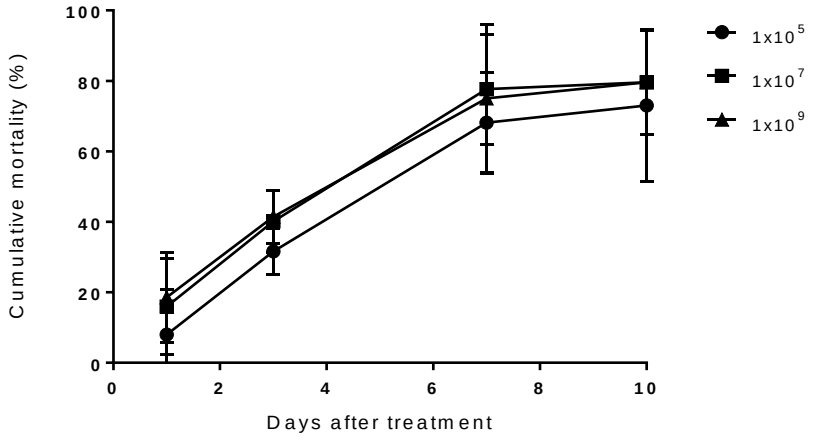

Figure 1. Cumulative mortality of *Tuta absoluta* larvae caused by different conidial concentrations of *Beauveria pseudobassiana* (Bb1)

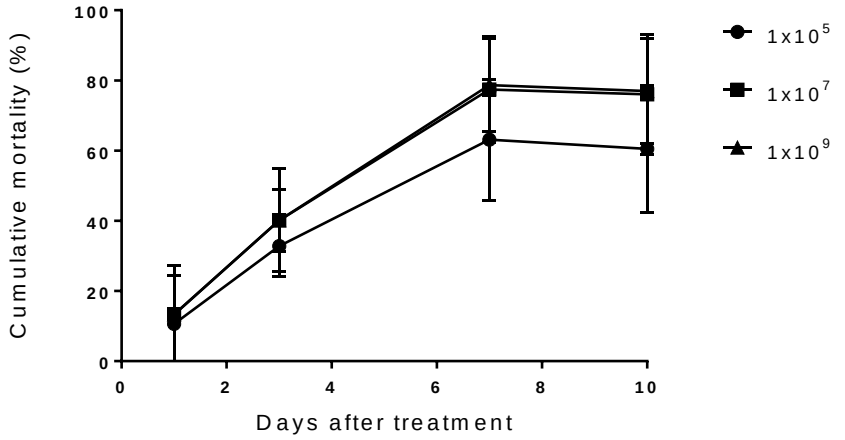

Figure 2. Cumulative corrected mortality of *Tuta absoluta* larvae caused by different conidial concentrations of *Beauveria bassiana* (Bb2)

For the fungal treatments applied at a concentration of 10^7 ml-1 simultaneously with the predator *A. custos*, there were no significant differences between the percentages of cumulative mortality recorded at 3, 7 and 10 DAA($p>0.09$). The treatments that included the predator caused a more rapid mortality of the *T. absoluta* larvae, this being significantly different from the fungi-only treatments (Table 2), the mortality being over 67% at 3 DAA.

Table 2

Percent corrected mortality of *Tuta absoluta* larvae treated with two different species of *Beauveria* and a mixture between *Beauveria* species and the predator *Arma custos*

Treatment	**Corrected mortality (%) mean±SD**			
	days after treatment			
	1	**3**	**7**	**10**
B. pseudobassiana (Bb1) 1 x 10^7 conidia ml^{-1}	15.94±13.7 a	40.16± 8.89 a	77.69±15.63 a	79.6±14.7 a
B. bassiana (Bb2) 1 x 10^7 conidia ml^{-1}	13.29±14 a	40.16±14.67 a	77.38±15.24 a	76±17.15 a
B. pseudobassiana (Bb1) 1 x 10^7 conidia ml^{-1} + Arma custos second instar larvae	36.59±7.7 b	72.79±15.3 b	86.62±8.28 a	84.9±12.3 a
B. bassiana (Bb1) 1 x 10^7 conidia ml^{-1} + Arma custos second instar larvae	34.17±6.5 b	67.6±16.41 b	78.65±13.17 a	83.2±10.58 a

The means within the columns followed by the same letters are not significantly different at $P < 0.05$, ANOVA followed by Tukey test for pair-wise comparisons

It was noticed that predator larvae were not affected by the entomopathogenic fungi during the observation period.

Comparing the effect of the fungi treatments applied in the concentration of 10^7 and the mixture between fungi and predator *A.cusos*, there were no significant differences between the treatments, at 7 DAI and 10 DAI (Table 2). However, the highest percentage of corrected mortality was recorded for the variant of B.b1 + A. custos (86.62%), at 7 days.

CONCLUSIONS

The present study revealed that local *B. pseudobassiana* and *B. bassiana* strains were successfully effective even when used separately or in combinations with *Arma custos* predatory bug under laboratory conditions. Both local *Beauveria* isolates in aqueous concentration of 1×10^7 conidia mL^{-1} induced high levels of control of *Tuta absoluta* larval stage. The best results have been registered in case of combination between the use of *B. pseudobassiana* isolate at a concentration of 1×10^7 conidia ml^{-1} and the predator *Arma custos*. No side-effects on natural enemies *Arma custos* used in combination indicate these isolates as promising alternative for *Tuta absoluta* control. Further studies need to be performed, focusing on the evaluation of the direct effects of both *Beauveria* isolates on *Tuta absoluta* populations in greenhouse condition.

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