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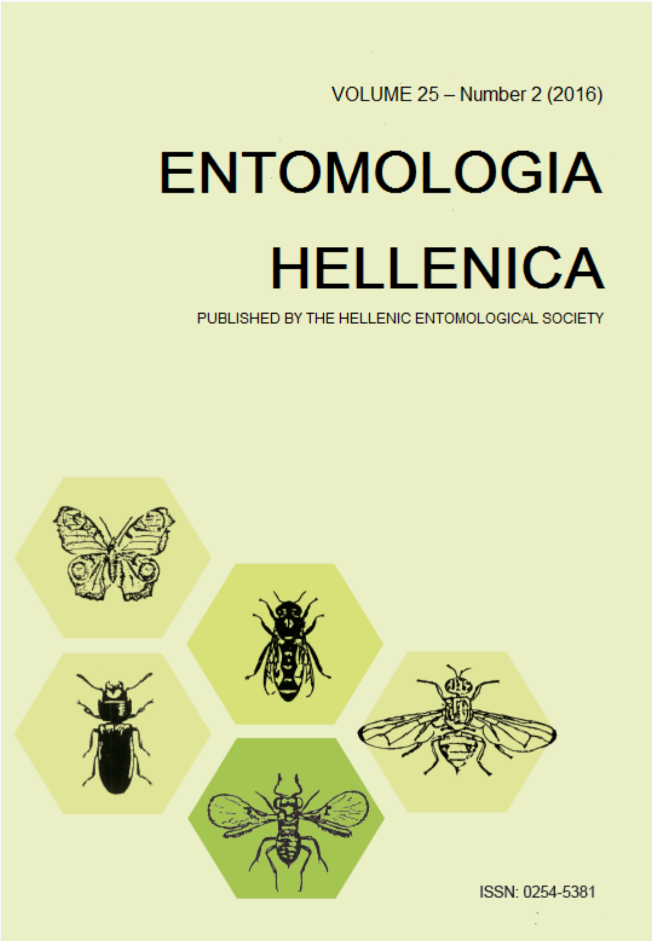

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Efficacy of a *Beauveria bassiana* strain, *Bacillus thuringiensis* and their combination against the tomato leafminer *Tuta absoluta*

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ABSTRACT

A single leaf bioassay was developed to investigate the entomopathogenic efficacy of a *Beauveria bassiana* strain, *Bacillus thuringiensis* (Costar®) and their interactions on larval mortality of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Tomato leaves were removed from the plants and sprayed with manufacturers' highest field recommended concentrations for tomato crops before or after infesting the leaves with the larvae. Third instar larvae proved the most susceptible, while susceptibility was lower in the second instar larvae. The combined use showed a higher potential indicating a positive synergistic effect. In addition, treated leaves were sprayed directly with concentrations of 0.0, 0.05, 0.10, 0.15 and 0.20 mL/L of *B. bassiana*. The efficacy was higher for the tested concentration of 0.20 mL/L that is higher than the recommended dose. The present study suggests that those bioinsecticides have a good potential in the control of *T. absoluta*.

KEYWORDS: bioinsecticides, entomopatogenic fungi, entomopathogenic bacteria, larval mortality, leaf bioassay, South American tomato leafminer.

Introduction

The South American tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is considered to be one of the most devastating pests of tomato crops worldwide. After its initial detection in Spain in 2006 (Urbaneja et al. 2007), it rapidly spread from the Mediterranean basin to African and Asian countries (Desneux et al. 2010).

Application of synthetic insecticides is the most common control strategy for this pest. However, chemical control may become ineffective due to the rapid development of resistance to insecticides (Lietti et al. 2005), let alone the negative impacts on natural enemies, the environment and human health (Desneux et al. 2010). Considering all those factors, the interest in alternative control methods and Integrated Pest Management (IPM) approaches has much increased (Consoli et al. 1998, Collavino and Gimenez 2008, Desneux et al. 2010).

Among the management methods used in IPM programs of *T. absoluta* (Amer et al. 2012, Cagnotti et al. 2012, Caparros Megido et al. 2012, Cocco et al. 2013), biological control is an environmentally and economically sound management method for this pest in Europe (Desneux et al. 2010, Zappalà et al. 2013).

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Beauveria bassiana and *Bacillus thuringiensis* var. *kurstaki* have been used to control many pests such as whiteflies, thrips, Lepidoptera, termites and other insects (Jacobson et al. 2001, Mburu et al. 2009). *B. thuringiensis* has already been included in the widely used control strategies for *T. absoluta* (Guistolin et al. 2001, Gonzalez- Cabrera et al. 2011, Youssef and Hassan 2013).

The present study aimed at examining the efficiency of an isolation of *B. bassiana* and a commercial product of *B. thuringiensis* and their interactions on larval mortality of *T. absoluta* on tomato plants under laboratory conditions.

Materials and Methods

Biological materials

In the experiments 6-week-old tomato plants, *Solanum lycopersicum* L. (Solanales: Solanaceae) were used. They were grown in climatic chambers (24 ± 1^{o}C, R.H. 65%, photoperiod 16L: 8D) and a nutrient solution was applied daily. *T. absoluta* was reared and fed in tomato plants in growth chambers (24 ± 1^{o}C, R.H. 70%, photoperiod 16L: 8D) inside cages (55 × 75 × 80 cm). During the experiments *T. absoluta* larvae were reared on tomato leaves at 22.8-24.0^{o}C, 12h photophase and R.H. 82-100%. The strain of *B. bassiana* was provided by BASF (BASF Agricultural Specialities Limited, Littlehampton, Great Britain) and *B. thuringiensis* var. *kurstaki* (Costar®) by CERTIS (Certis, Europe, B.V. Utrecht).

Leaf bioassay/ Experimental design

A single leaf bioassay was developed to investigate entomopathogenic efficacy of *B. bassiana*, *B. thuringiensis* and their interactions on larval mortality of *T. absoluta*. Small Petri dishes (60 × 15 mm) were filled up to 15mm with 1.5% water agar solidified and placed into experimental plastic cups, 6 × 5 × 8 cm. Tomato leaves were removed from the plants and sprayed with recommended doses on their both sides, from 55 cm distance with simple sprayer. Untreated controls were sprayed with water. Treatments were conducted by two experimental designs: the first bioassay included spraying the leaves after their infestation with *T. absoluta* and the second bioassay included spraying the leaves before infestation with the pest. In the first bioassay *T. absoluta* larvae were carefully extracted from their mines and deposited on tomato leaves. Then the leaves were left for 3 hours in the incubator, providing the time needed to dig galleries into the tomato leaves. Afterwards, the leaves were sprayed. The recommended concentration for *B. bassiana* was 0.1% and for *B. thuringiensis* 0.15%. The treatment applied prior to leaf infestation with the pest is identified as “before treatment” and application after the leaf infestation with *T. absoluta* as “treatment after”. For each larval instar untreated leaves were sprayed with water as control treatments. The same procedure was used for the combined use of both bioinsecticides. Firstly the leaves were sprayed with Costar® and allowed to dry at room temperature. Afterwards, the leaves were sprayed with *B. bassiana*.

Treated leaves were immediately transferred into the cups with their stalks passed into the solidified agar to ensure nutrients and water supply. Then, each cup was sealed with a lid. The experimental design for the mortality tests consisted of five replicates for 1st and 2nd larval instars using ten larvae per leaf (replicate) and 8 replicates for 3rd instar with five larvae per leaf. The experiments performed at 24 ± 1 ^{o}C, R.H. 70%, photoperiod 16L: 8D. Larval mortality was evaluated daily for 7 days. The application of treatment occurred on day 0.

Testing different concentrations of Beauveria bassiana

Tuta absoluta larvae were sprayed at a concentration of 0 (control), 0.05, 0.1, 0.15 and 0.2% (or 0, 0.5, 1, 1.5 and 2 mL of compound / 1000mL of water). The tests consisted of 4 replicates for 1st, 2nd and 3rd larval instars with 8, 8 and 6 larvae per leaf, respectively. The experiments were conducted in incubators at 24 ±1^{o}C, R.H. 85-100%, photoperiod 16L: 8D. *T. absoluta* larvae were added immediately after *B. bassiana* application and mortality was recorded daily for seven days.

Data Analysis

Percentages of mortality rates were corrected by using Abbott's formula (Abbott 1925). The data were tested for normality using MINITAB Version 17 (Minitab Inc., State College, Pennsylvania, USA). The effects of bioinsecticides on mortality of the pest were tested through Analysis of Variance [ANOVA], (CSS StatSoft, Tulsa, OK, USA). The significance of differences was tested by Tukey's HSD (Honest Significant Difference) test.

Results

Efficacy of Beauveria bassiana against Tuta absoluta

The efficacy of *B. bassiana* for each larval instar was similar between the two treatment methods (Figs 1, 2 & 3). In the case of treatment applied prior to infestation, there were no significant difference between 1st and 3rd larval instar, however the 2nd larval instar showed a significantly lower mortality compared to the other instars (F=18.61, df=17, P<0.01).

Regarding application of *B. bassiana* after infestation, statistical analysis confirmed that 3rd larval instars were more susceptible whereas the 2nd larval instars appeared the least susceptible (F=23.27, df=17, P<0.01) (Figs 1, 2 & 3).

Efficacy of Bacillus thuringiensis against Tuta absoluta

Bacillus thuringiensis caused a high mortality against all three larval instars. No significant differences in mortality between the two methods and among the instars were recorded (Figs 1, 2 & 3).

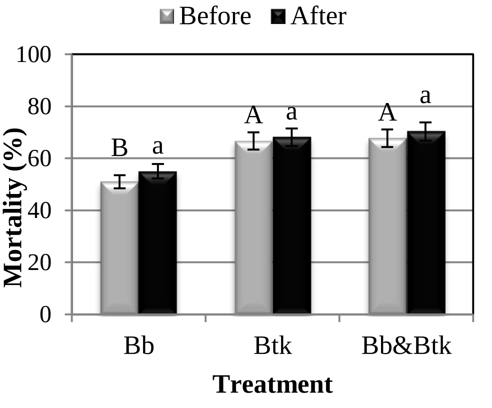

FIG. 1. Corrected mean percentage (%) mortality rates of 1st larval instars of *T. absoluta* for the three different treatments [*B. bassiana* (Bb), *B. thuringiensis* (Btk) and their combination (Bb & Btk)]; and for spraying before and after placing the larvae on the leaf. Different superscript letters above bars indicate significant differences in mortality. Capital and small letters above bars indicate separate statistical analysis.

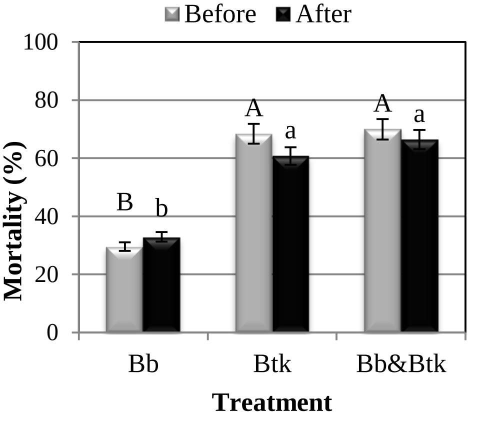

FIG. 2. Corrected mean percentage (%) mortality rates of 2nd larval instars of *T. absoluta* for the three different treatments [*B. bassiana* (Bb), *B. thuringiensis* (Btk) and their combination (Bb & Btk)]; and for different application methods. Different superscript letters above bars indicate significant

differences in mortality. Capital and small letters above bars indicate separate statistical analysis.

Efficacy of the combined use of Beauveria bassiana and Bacillus thuringiensis against Tuta absoluta

Between the two treatment methods no significant difference was recorded. Among the instars the 3rd larval instar was the most susceptible larval instar (F=10.76, df=17, P<0.01) (Figs 1, 2 & 3). The comparison between the combined and the separate use of each entomopathogen showed a significantly lower mortality for the *B. bassiana* in the 1st instar in the before treatment, in both treatments for the 2nd instar whereas in the 3rd instar, the mortality of the combined use in the "before" treatment was significantly higher than the separate use of *B. bassiana* or *B. thuringiensis.*

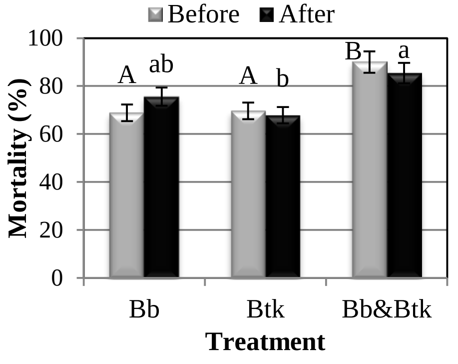

FIG. 3. Corrected mean percentage (%) mortality rates of 3rd larval instars of *T. absoluta* for the three different treatments [*B. bassiana* (Bb), *B. thuringiensis* (Btk) and their combination (Bb & Btk)]; and for different application methods. Different superscript letters above bars indicate significant differences in mortality. Capital and small letters above bars indicate separate statistical analysis.

Effects of different concentrations of Beauveria bassiana against Tuta absoluta

From the statistical analysis, the results indicated that there were significant differences in mortality among the larval instars for the concentrations of 0.15% (F=7.11, df=11, P=0.014) and 0.10% (F=1.23, df=11, P=0.337) but not for 0.20% (F=3.80, df=11, P=0.064). At the lowest concentration 0.05% no significant difference was recorded between the 1st and 3rd larval instars but the 2nd larval instar had significant lower mortality (F=59.38, df=11, P<0.01), as shown in figure 4.

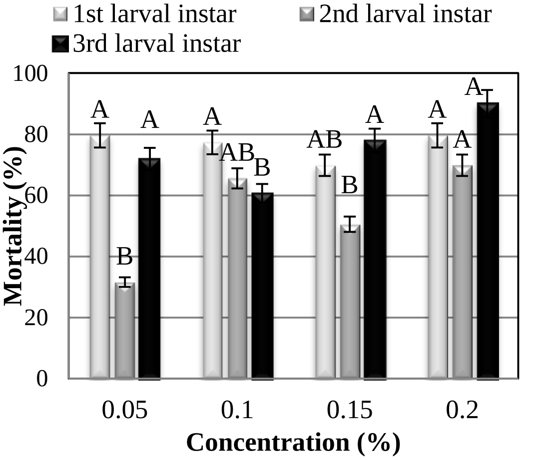

FIG. 4. Corrected mean percentage (%) mortality rates caused by different concentrations of *B. bassiana* on 1st, 2nd and 3rd larval instars of *T. absoluta*. Different superscript letters above bars indicate significant differences within each concentration tested.

The results indicated no significant difference for the 1st larval instar among the four treatment concentrations. However, for the 2nd larval instar, the results indicated high efficacy of 0.1% and 0.2% concentrations of a 65.6% and 69.8% respectively. For the 3rd larval instar, the results suggested that the recommended concentration (0.1%) was inefficient compared to 0.15% and 0.2% which caused 78.0% and 90.0% mortality rate, respectively. In overall, taking into account all larval instars tested, the recommended concentration 0.1% proved to be less efficient for the third instar than the concentration of 0.2%.

Discussion

The invasion and the rapid outbreaks of *T. absoluta* in Europe, Africa and Asia in the last decade reflect the serious economic impacts of this pest in both conventional and organic systems of tomato production. The purpose of this study was the evaluation of *B. bassiana* and *B. thuringiensis* in the control of *T. absoluta* since both biocontrol agents are considered as a key part in IPM programs of pests with paramount economic importance (Gonzalez-Cabrera et al. 2011, Terzidis et al. 2014).

The minor differences among the different application methods of treatment (before and after infestation) can be attributed to the habit of *T. absoluta* larvae of creating mines in the leaves which however, does not exclude them from emerging in the surface of the leaves and creating new mines (Picanço et al. 1998). The mortality rate in the 3rd instar may be higher due to the higher consumption of leaf tissue compared to 1st and 2nd larval instars. The significantly higher efficacy to the 1st than the 2nd larval instars has been also recorded by previous researchers (Torres Gregorio et al. 2009).

Due to the limitations of the laboratory experiments the current results should be further evaluated under more realistic, semi-field or field conditions. In addition, potential side-effects on natural enemies should be evaluated. Although previous research has found *B. bassiana* to be non-pathogenic to beneficial organisms (Thungrabeab and Tongma 2007), other studies indicate small-scale effects on mortality of natural predators due to *B. thuringiensis* applications (Hilbeck et al. 1998a, b, Mollà et al. 2011).

The specific strain of *B. bassiana* used in the present study has never been evaluated before against *T. absoluta*. The fairly high mortality rates on *T. absoluta* of those treatments pointed out the potential of those biological control agents in the control of this pest.

Gonzalez-Cabrera et al. (2011) has reported for the first time the high efficacy of different *B. thuringiensis* formulates that have reduced the damages up to 98% in treated plants in laboratory assays although there were significant differences among the formulates used in the experiment. They have identified an optimum concentration of Costar®, higher than the recommended for other pests, which led to a significant reduction in fruit damage. The results indicated high susceptibility of *T. absoluta* especially on third larval stage in both laboratory and open- field experiments.

However, when *B. bassiana* was combined with Btk (Costar®), the results obtained, indicated the positive effects of the combined use, which showed high percentage mortality of *T. absoluta* against all larval instars with one single application of the combined treatments. Compared to single application of *B. bassiana*, the combination of treatments suggested positive synergistic effects. The synergism of the bioinsecticides could potentially contribute to safer and more effective control of the invasive pest.

Testing different concentrations of *B. bassiana* have proved that the recommended concentration was inefficient compared to higher concentrations. All larval instars of *T. absoluta* were susceptible to the highest concentration used in laboratory experiments indicating the need for further experimentation in laboratory and open-fields.

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Αξιολόγηση των εντομοπαθογόνων *Beauveria bassiana* και *Bacillus thuringiensis* και του συνδυασμού τους στην αντιμετώπιση του υπονομευτή της τομάτας *Tuta absoluta*

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ΠΕΡΙΛΗΨΗ

Μελετήθηκε στο εργαστήριο η αποτελεσματικότητα των εντομο-παθογόνων μικροοργανισμών *Beauveria bassiana* και *Bacillus thuringiensis* καθώς και ο συνδυασμός αυτών στη θνησιμότητα του υπονομευτή της τομάτας *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Φυλλάρια τομάτας αποκόπηκαν από φυτά ηλικίας 42 ημερών και ψεκάστηκαν με τις υψηλότερες συνιστώμενες συγκεντρώσεις. Ο ψεκασμός των φύλλων έγινε είτε πριν την τοποθέτηση των προνυμφών 1ης, 2ης και 3ης ηλικίας είτε μετά. Οι προνύμφες 3ης ηλικίας ήταν οι πιο ευαίσθητες σε όλες τις μεταχειρίσεις, σε αντίθεση με τις προνύμφες 2ης ηλικίας που εμφάνισαν χαμηλότερη θνησιμότητα. Επιπλέον μελετήθηκε η αποτελεσματικότητα του στελέχους του *B. bassiana* εναντίον των προνυμφών 1ης, 2ης και 3ης ηλικίας του *T. absoluta* σε διάφορες συγκεντρώσεις (0.05, 0.1, 0.15 και 0.2%). Για τις προνύμφες 3ης ηλικίας η θνησιμότητα ήταν υψηλότερη όταν αυτές εκτέθηκαν στην συγκέντρωση των 0.2% του *B. bassiana*. Η παρούσα μελέτη δείχνει ότι τα βιοεντομοκτόνα που δοκιμάστηκαν έχουν καλές προοπτικές για τον έλεγχο του υπονομευτή της τομάτας *T. absoluta* ειδικά όταν χρησιμοποιήθηκαν σε συνδυασμό, ωστόσο περαιτέρω έρευνα θεωρείται αναγκαία.

