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BIO-INSECTICIDAL EFFICACY OF *BEAUVERIA BASSIANA* VUILL. STRAINS AGAINST *TETTIGONIA VIRIDISSIMA* L. IN TOBACCO

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Abstract

The green grasshopper (*Tettigonia viridissima* L.) is a species which attacks tobacco plants. The purpose of the present study was to establish the bio-insecticidal efficacy of entomopathogenic fungi *Beauveria bassiana* Vuill. strains 214, 644, and 733 on the growth and development of *T. viridissima* in tobacco. The research was conducted under laboratory conditions. The study found insecticidal effects of the *B. bassiana* strains 214, 644, and 733 against *T. viridissima*. Surviving of larvae was monitored at 5-day intervals until the 15th day after treatment with a spore suspension of the tested *Beauveria* strains. The highest virulence of the tested fungi was shown by strain 644, followed by strains 214 and 733. On the 5th day after treatment, no efficacy against *T. viridissima* larvae was detected. On the 10th day post-treatment strain 644 showed 66.7% efficacy, while efficacy against larvae of grasshoppers for the other two strains 214 and 733 was significantly lower: 33.3%. Efficacy gradually increased and on the 15th day post-treatment 100% mortality of *T. viridissima* was observed for all isolates. The results obtained from the virulence test of *B. bassiana* 214, 644, and 733 ascertain their application as biocontrol agents in tobacco production under field conditions.

Key words: entomopathogenic fungi, grasshopper, ecology, biocontrol, pests

Introduction. Pest control in agriculture is very important to reduce damages and raise yields. In recent years, an increasing tolerance of pesticides towards

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pests has been reported, while at the same time, there is a tendency to reduce the use of pesticides. Chemical pesticides are mostly used for pest control worldwide. Their use leads to resistance in many pests and can influence non-target organisms [1]. Insecticides pose risks to human health and the environment [2, 3].

The use of microorganisms can reduce the need for chemical pesticides and increase the sustainability of agriculture [4], but biological control has a more specific approach and takes longer time to achieve the desired results [5]. The use of entomopathogenic fungi for pest control provokes big interest [6]. The efficacy of entomopathogenic fungi infection is impacted by many factors [7]. The fungus penetrates the internal organs in insects and releases toxins, which leads to the pest's death [8].

Some bio-insecticidal preparations have a narrow range of hosts, but *B. bassiana* is characterized by a large range of insects [9] and can be applied against more pests. Entomopathogenic fungi secrete beauvericin, bassianolone, bassianolid and bassiacredin which have toxic effect on grasshoppers by disrupting the cell wall [10]. Secondary metabolites of *Beauveria brongniartii* (Saccardo) Petch are used in many bioinsecticide preparations [11].

Tobacco plants are attacked by several different pests and one of them is the green grasshopper *T. viridissima* which could cause serious damages. *T. viridissima* has colour polymorphism: green and brown [12].

B. bassiana was used against various insect species due to the proliferation into the insect's body, producing toxins and draining the insect of nutrients. The different strains in most expreiments published have different biochemical activity, and, correspondingly different effectiveness towards the target entomopathogen and plants.

The purpose of the present study was to establish the bio-insecticidal efficacy of *Beauveria bassiana* Vuill. strains 214, 644, and 733 on *Tettigonia viridissima* L.

Materials and methods. The present study was conducted under laboratory conditions at the Tobacco and Tobacco Products Institute, Markovo (Bulgaria). For the experiment, oriental tobacco plant ecotype Krumovgrad (variety Krumovgrad 90) has been used as plant material. *Tettigonia viridissima* L. larvae were collected from wild populations at Tobacco and Tobacco Products Institute, Markovo. The research was conducted with previously grown tobacco plants (with a length of up to 40 cm and 8–13 number of leaves) in pots. The plants were colonized with 12 larvae from the tested insect. After that, the plants were sprayed with 10 ml *of Beauveria bassiana* Vuill. conidial suspension for each strain separately. The conidial suspension has a concentration of 10^6 conidia/ml. Three replicates were set up for each isolate, and the control was treated with water. The room was illuminated only by daylight. The temperature in the room was 22–25 °C. The number of alive individuals was counted on the 5th, 10th, and 15th day after treatment. Efficacy was calculated using Abbott's formula [13]:

$$E(\%) = (T - t)100/T,$$

where T is the number of living grasshoppers in control; t is the number of living grasshoppers after the treatment. Green grasshoppers which did not react when touched were counted as dead.

The fungal strains 214 (Accession number in NCBI – OM366244), 644 (Accession number in NCBI – OM366261), and 733 (Accession number NCBI – OM366259) were provided by Prof. Slavimira Draganova from Agricultural Academy – Bulgaria, Institute of Soil Science, Agrotechnologies and Plant Protection (ISSAPP). The strains were isolated from various hosts and areas. Strain 214 was isolated from *Agrilus mokrzeckii* (Coleoptera, *Buprestidae, Agrilus*) on red oil roses (*Rosa damascena* Mill.), strain 644 from *Tanymecus dilaticollis* Gyll. (Coleoptera, Curculionidae, *Tanymecus*), and strain 733 was isolated from *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae, *Leptinotarsa*). The dry conidia of fungal cultures were grown on Sabouraud's dextrose agar in the dark at 27 °C.

For the preparation of the conidial suspension, conidia were taken from cultures grown on yeast extract agar (HiMedia, Mumbai, India) after incubation for 10 days at 27 °C in darkness. Conidia were harvested with glass cell scrapers and placed in test tubes containing 0.01% (v/v) Tween 80 (polyoxyethylene sorbitan monolaurate) (Merck® KGaA, USA). Suspensions were vortexed for 2 min, filtered through four layers of sterile muslin, and adjusted to 1×10^6 conidia ml^{-1} [14] after cell counting by Thoma chamber.

The oriental tobacco plants were treated using foliar spray inoculation techniques. The leaves treatment was conducted with conidia suspension with concentration of 1×10^6.

The data was statistically processed by using one-way ANOVA in Excel at $P \leq 0.05$.

Results and discussion. The bio-insecticidal efficacy of *B. bassiana* strains 214, 644, and 733 is shown in Fig. 1.

All tested strains of *B. bassiana* showed bio-insecticidal efficacy against *T. viridissima.* The efficacy against the green grasshopper increased as the period term rose. Applying the method of [13], the linear trendline results $y = 50x - 44.433$ $R^2 = 0.9641$ for strain 644 and $y = 50x - 55.567$ $R^2 = 0.9641$ for strains 214 and 733 showed 100% mortality against *T. viridissima* after the 15th day. After the 10th day strain 644 showed 66.7% efficacy, while strains 214 and 733 showed 33.3%. Strain 644 had a little bit higher virulence in this period compared to strains 214 and 733, while the last ones showed similar results. No efficacy against grasshopper was established after the 5th day (Fig. 1). No *T. viridissima* mortality was detected in the control group. Although, strain 644 had higher virulence after the 10th day compared to strains 214 and 733, while the last ones

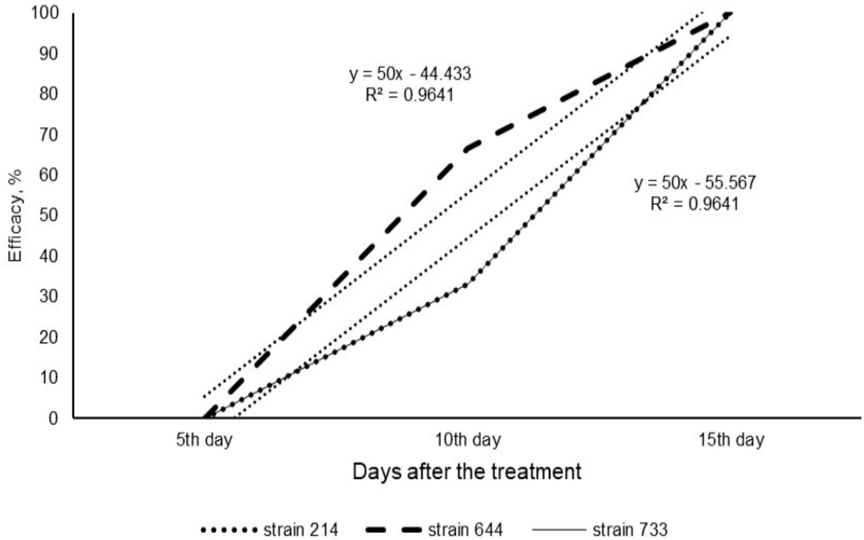

Fig. 1. Bio-insecticidal efficacy of *B. bassiana* strains 214, 644 and 733 against *T. viridissima*

showed similar results, there were no significant differences found when comparing results with different strains ($F \leq F_{\text{crit}}$, $P = 0.8$ and $P = 1$).

A number of studies have proven the negative effect and the possibility of *B. bassiana* to reduce the population on other tobacco pests, such as *Myzus persicae* Sulzer [15], *Thrips tabaci* Lindeman [16], *Spodoptera litura* Fabricius [17], *Tetranychus evansi* Baker & Pritchard [18].

No phytotoxicity was detected of the tobacco plants after spraying with any of the tested strains, and it could be said that tobacco plants are stable to *B. bassiana* strains 214, 644, and 733.

Other studies have found that *B. bassiana* can colonize endophytically tobacco plants and exhibit no negative effect on tobacco development [19, 20].

Conclusions. The use of microorganisms for biological control against pests, as an alternative to conventional pesticides is a promising opportunity to reduce the negative effect of chemical preparations on soil, ecosystems, human and animal health. In this study bio-insecticidal efficacy of *B. bassiana* strains 214, 644, and 733 against *T. viridissima* was detected. The results of the study showed no phytotoxicity of tobacco plants under laboratory conditions. *Beauveria* strains studied in laboratory conditions show a high potential for application as biocontrol agents in tobacco production. Due to the specificity of the tobacco culture, the formation of the final product and the diverse microflora of the fields, it is necessary to study their effect on various indicators in field conditions.

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