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Short communication

EFFECT OF THE ENDOPHYTIC COLONIZATION OF *BEAUVERIA BASSIANA* ON THE POPULATION DENSITY OF PEACH APHID (*MYZUS PERSICAE*) AND THE GROWTH PARAMETERS OF PLANTS

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Endophytic properties of entomopathogenic fungi currently receive considerable attention from the scientific community. In the present work, it was shown that the fungus *Beauveria bassiana* (strain BBK-1) is able to successfully colonize broad bean and sweet pepper plants under laboratory conditions. The green peach aphid actively bred on both plant species. The density of aphids developing on plants colonized by *B. bassiana* was significantly lower as compared to the control, both on peppers and beans. The growth-stimulating effect of endophytic colonization by *B. bassiana* was less pronounced on beans, while on sweet pepper plants, a significant increase in plant height and an earlier onset of the budding were found. The observed effects indicate that *B. bassiana* has a potential to be used as a polyfunctional biocontrol agent in agricultural practice.

Keywords: entomopathogenic fungi, broad bean, sweet pepper, growth stimulation

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Introduction

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a cosmopolitan fungus, primarily known as a necrotrophic pathogen of arthropods with an extensive host range (Mascarin, Jaronski, 2016). Saprotrophic properties of this species, on one hand, facilitate its adaptation to diverse ecosystems while on the other hand, allow it to be isolated and cultivated easily on various substrates and to be used for production of a broad spectrum of microbial insecticides to suppress phyto- and hematophagous arthropods (Faria, Wraight, 2007). During the last three decades, *B. bassiana* also started drawing attention of many researchers as an endophyte, able to colonize the rhizoplane and to develop within plant tissues (Vega et al. 2009). Endophytic activity and pathogenicity towards insects favor the establishment of a complex mutualistic association between the fungus and the plant. Interactions between plants, entomophagous insects and entomopathogenic fungi can be thus considered as a tritrophic consortium (Bamisile et al., 2018). Endophytic fungi also can serve as antagonists of phytopathogenic fungi (Sasan and Bidochka, 2013; Gothandapani et al., 2015; Lozano-Tovar et al., 2017; Barra-Bucarei et al., 2020), as plant growth stimulants (Lopez, Sword, 2015; Jaber, Enkerli, 2017; Jaber, Araj, 2018) and as immunity modulators (Ownley et al., 2010; Maksimov et al., 2015). In turn, plants protect the fungi from environmental stress, such as insolation, provide them with additional nutrients, and serve as a platform to parasitize insects (Ownley et al., 2010; Keyser et al., 2014).

The fungus *B. bassiana* is able to colonize over hundred plant species from different families (McKinnon et al., 2017; Vega, 2018; etc). In contrast to the fungi in the genus *Metarhizium*, which are predominately concentrated in plant rhizosphere, *Beauveria* spp. colonize both roots and aboveground plant parts (Ownley et al., 2010; Behie et al., 2015). Extensive experimental evidence has been accumulated to demonstrate endophytic colonization of Fabaceae (Akello, Sikora, 2012; Parsa et al., 2013; Akutse et al., 2013) and Solanaceae (Ownley et al., 2008; Barra-Bucarei et al., 2020; Tomilova et al., 2020) by *Beauveria*.

Numerous studies indicate a decrease in disease development caused by viruses (Jaber and Salem, 2014), bacteria (Ownley et al., 2008), and fungi (Ownley et al., 2008; Jaber, 2015; Rivas-Franco et al., 2019) in *Beauveria*-colonized plants. Notably, the results of laboratory experiments were reproducible under field conditions. In particular, seed treatment with *B. bassiana* caused a significant decrease in powdery mildew, root rots, chocolate and other spot diseases in broad beans (Ashmarina et al., 2021). Similarly, treatment of potato tubers with *B. bassiana* impeded the development of Rhizoctonia disease during vegetation and increased weight and quality of new yiled tubers (Tomilova et al., 2020).

Growth stimulation observed in plants under influence of *Beauveria* is explained by the provision of supplementary nitrogen due to the mycelium development on roots (Behie et al., 2012; Behie and Bidochka, 2014), induction of protein synthesis involved in photosynthesis and energy metabolism of plants (Gomez-Vidal et al., 2009), activation of their phytohormone production (Raad et al., 2019), or by synthesis of the hormone-like substances, such as indolyl acetic acid, by the fungi (Liao et al., 2017).

Numerous reports are published concerning the influence of plant colonization by *B. bassiana* on various phytophagous insects in the orders Homoptera, Thysanoptera, Coleoptera, Hymenoptera, Lepidoptera, etc. However, available data are somewhat contradictory when different parasite-host systems are compared. The observed effects may vary between the elevated mortality due to fungal infection (Gurulingappa et al., 2010; Lopez and Sword, 2015; Garrido-Jurado et al., 2017; Sánchez-Rodríguez et al., 2018) and the increase in pest numbers (Clifton et al., 2018; Raad et al., 2019). These contradictions can result from the differences in biological properties of plants and insects, as well as from the fungal ability to colonize the plants. It should be also noted that plants are usually inhabited by several species of endophytes

(Hartley, Gange, 2009) and naturally occurring ones may affect plant interactions with their pests and cause variations in the observed effects.

The green peach aphid *Myzus persicae* Sulzer (Homoptera: Aphididae) is a typical polyphagous pest, feeding on over 900 species of plants from 50 families, including Solanaceae, Fabaceae, Brassicaceae, Cucurbitaceae, Rutaceae, etc. (Holman, 2009). It is considered to be one of the most important sucking pests of agriculture worldwide (Blackman, Eastop, 1984). Moreover, *M. persicae* displays resistance to numerous synthetic insecticides (Devonshire et al., 1998), which requires development of complex approaches to control this pest. The abundance of economically important aphid species, such as *Aphis gossypii* Glover, *Acyrthosiphon pisum* Harris, *Aphis fabae* Scopoli and *M. persicae* (Akello, Sikora, 2012; Castillo-Lopez et al., 2014; Jaber, Araj, 2018) has been found to be negatively affected by the entomopathogenic fungi which colonized the infested plants. Thus, in the present study these effects were tested against the single pest species in two model plants belonging to Fabaceae and Solanaceae. Both species are damaged by the green peach aphid, while certain aspects of endophyte colonization have been already assessed in these plants, including the stimulation of their growth and suppression of their pathogens.

The goal of the present study was to compare effects of endophytic colonization of the broad bean and the sweet pepper by the fungus *B. bassiana* (strain BBK-1) on the green peach aphid abundance and the growth of the colonized plants.

Materials and Methods

The fungal strain *B. bassiana* BBK-1 from the collection of All-Russian Institute of Plant Protection, originally isolated from a cadaver of *Calliptamus italicus* L. (Orthoptera: Acrididae) in Novosibirsk Province in 2000, was used in this study. Species identification was confirmed by sequencing the intergenic locus B (Rehner et a., 2011). Fungal conidia were grown in Petri dishes on the modified Sabouraud medium containing 1 % peptone, 1 % glucose, 1 % maltose, 0.5 % yeast extract, and 2 % agar.

Two species of plants were used: the broad bean *Vicia faba* L. (Fabaceae), 'Russkiy chernyy' variety, and the sweet pepper *Capsicum annuum* L. (Solanaceae), 'Podarok Moldovy' variety. The plants were grown in sterile soil-sand mixture (1:1 ratio) from seeds sterilized by the consequent surface treatment with 1 % sodium hypochlorite (2 min) and 70 % ethanol (2 min), followed by three washes with distilled water (Parsa et al., 2013). Each plant was grown individually in plastic plant pot using 25 pots per treatment at 22–25 °C, 12 hrs light, relative humidity 40–60 %.

The fungus was applied one week after appearance of the broad bean shoots or after the picking of the seedlings of the sweet pepper (the picking performed at the stage of 2–3 true leaves). For the application, the soil was watered with fungal suspension containing 10^8 conidia/mL in water with 0.01 % Tween-80 (10 mL/plant). Control plants were treated with water containing Tween only. The endophyte colonization was evaluated using the method of Posad et al. (2007) in two stages: at day 15 post treatment prior to aphid introduction (see below), and at day 30 post treatment when the experiment was finalized. The leaves were detached from the middle tier, sterilized as above and cut into fragments (1 cm long) using a sterile scalpel. To control the quality of the surface sterilization, the leaf fragments were pressed against the sterile medium (McKinnon et al., 2017) and then placed in Petri dishes (10 fragments per plant) on the Sabouraud medium as above with addition of 0.035 % cetrimonium bromide, 0.005 cycloheximide, 0.005 % tetracycline and 0.06 % streptomycin to suppress saprotrophic microorganisms. The dishes were incubated for 10 days at 24 °C and the number of *B. bassiana* colonies per plant was counted, with the exception of the specimens showing fungal growth in the surface sterilization test.

Two weeks after fungal conidia application, both control and treated plants were artificially infested with the green peach aphid, using 10 insects per plant. In the broad beans, larvae were used while in the sweet pepper, where aphid colonization is a more complicated process which needs more time (as found in a preliminary experiment), parthenogenetic females were used. The pots were placed on trays each covered with a gauze hood, 12–13 pots per tray. The total aphid number per plant was scored on days 15 and 20 post introduction.

Plant height (10, 15, 20 days post treatment with the fungus) was measured and the proportion of plants with flower buds was estimated after its onset. For statistical analysis, the software package Statistica 8.0 (StatSoft Inc., Tusla, USA) was used. The percentage of plant colonization by the fungus and the proportion of plants with buds were evaluated using non-parametric statistics (Fisher exact test) while aphid numbers and plant height were assessed by t-test.

Results and Discussion

The endophytic colonization percentage by *B. bassiana* was quite high in both plant species, reaching 73 % in the sweet pepper and 56 % in the broad beans on day 30 after the fungal application. Though the colonization rate was numerically higher in the former species (Fig. 1), no significant differences were found between the two plant species neither at the time of aphid introduction (χ^2=0.033, Fisher exact p=0.177) nor at the end of the experiment (χ^2=0.031, Fisher exact p=0.188). During experiment, the percent of the plants colonized by the fungus significantly increased. In the pepper, the increase was 1.8-fold (χ^2=0.103, Fisher exact p=0.033), while in the bean, it was 2.3-fold (χ^2=0.107, Fisher exact p=0.021). None of the control plants were colonized by *B. bassiana*. Thus, the application of BBK-1 strain to the soil provided a reliable level of plant colonization in both species, which was consistent with the previous observations in Fabaceae (Akello and Sikora, 2012; Parsa et al., 2013; Akutse et al., 2013) and Solanaceae (Ownley et al., 2008; Jaber and Araj, 2018; Barra-Bucarei et al., 2019).

The green peach aphid infested and multiplied in both plant species. On day 20 after aphid introduction to the control plants, their number has significantly increased as compared to the initial number on the bean (10-fold, $p<0.001$) and on the pepper (11.5-fold, p=0.017). Meanwhile, for the *B. bassiana*-colonized plants (Fig. 2), the increase in the aphid numbers at that date was significantly lower as compared to the control both on the pepper (4.2-fold, $p<0.045$) and on the bean (1.5-fold, $p<0.045$). The aphid mortality could not be estimated. Therefore, it is not completely clear whether the observed effect was caused by the suppression of aphid reproduction or by the increase in mortality due to the fungal infection. In previous studies, detrimental effects of endophytic fungi on both ontogenetic and reproductive parameters of different aphid species have been demonstrated (Akello, Sikora, 2012; Jaber and Araj, 2018).

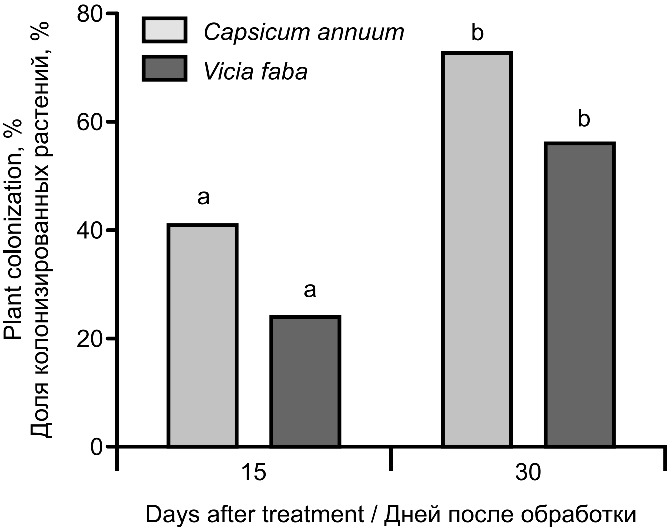

Figure 1. Plant colonization by *Beauveria bassiana* (1×10^8 conidia/ml, 10 ml per plant) of the sweet pepper (‘Podarok Moldovy’ variety) and the broad bean (‘Russkiy chernyy’ variety) after treatment with the fungus. Different letters above the bars indicate the presence of significant differences (Fisher exact $p<0.05$)

Рисунок 1. Уровень колонизации *Beauveria bassiana* (1×10^8 конидий/мл, 10 мл на растение) растений сладкого перца (сорт «Подарок Молдовы») и кормовых бобов (сорт «Русский черный») после обработки грибом. Различными буквами над столбцами отмечено наличие существенных различий (Fisher exact $p<0.05$)

The sweet pepper plants colonized by *B. bassiana* displayed a significant increase ($p\leq0.004$) in height on days 10 and 20 after fungal application (Fig. 3). The percentage of the colonized pepper producing flower buds on day 20 was 1.4-fold higher as compared to the control (Fig. 4) but the difference was not significant ($\chi^2=0.069$, Fisher exact $p=0.081$). In the broad beans the height of the colonized plants was similar to the control, with the only exception for day 15 after fungal application (Fig. 4) when a statistically significant increase was observed ($p=0.013$). In both control and treated plants, the onset of flower budding was observed on days 18–20 after fungal introduction. On day 20, the percent of plants with flower buds was not significantly different between the control and the treatment groups ($\chi^2=0.002$, Fisher exact $p=0.500$, Fig. 4). This can be explained by a more rapid growth of the bean plants compared to the pepper plants, as well as by a lower percentage of the colonized bean plants.

In several previous studies, plant growth was also found to be augmented by entomopathogenic fungi (Lopez, Sword, 2015; Jaber, Enkerli, 2017). Moreover, Jaber and Araj (2018) could demonstrate the ability of these fungi to positively affect sweet pepper growth in the presence of biotic stressors, such as consequent introduction of two generations of the green peach aphids. One of the explanations provided by the authors was that a decrease due to the fungal infection in damage caused by the pest may stimulate plant growth.

It can be therefore inferred that the colonization percentage by the fungus *B. bassiana* depends upon the plant species. At the 56–73 % level of plant colonization, a significant retardation of aphid population growth was demonstrated as compared

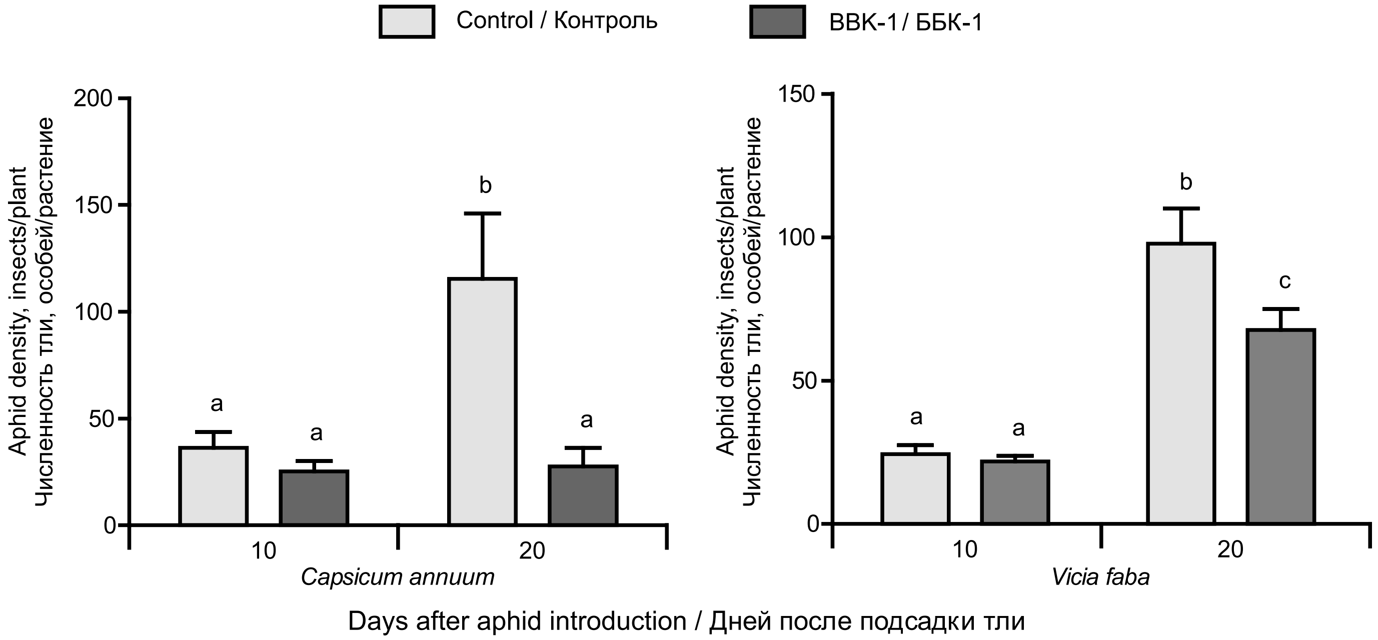

Figure 2. Effect of colonization by *Beauveria bassiana* (1×10^8 conidia/ml, 10 ml per plant) on the number of *Myzus persicae* developing on the sweet pepper (left) and the broad beans (right). Different letters above the bars indicate significantly different values (t-test, $p<0.05$)

Рисунок 2. Влияние колонизации *Beauveria bassiana* (1×10^8 конидий/мл, 10 мл на растение) на численность *Myzus persicae*, развивавшейся на растениях сладкого перца (слева) и кормовых бобов (справа). Разными буквами над столбцами отмечены существенно различающиеся значения (t-test, $p<0.05$)

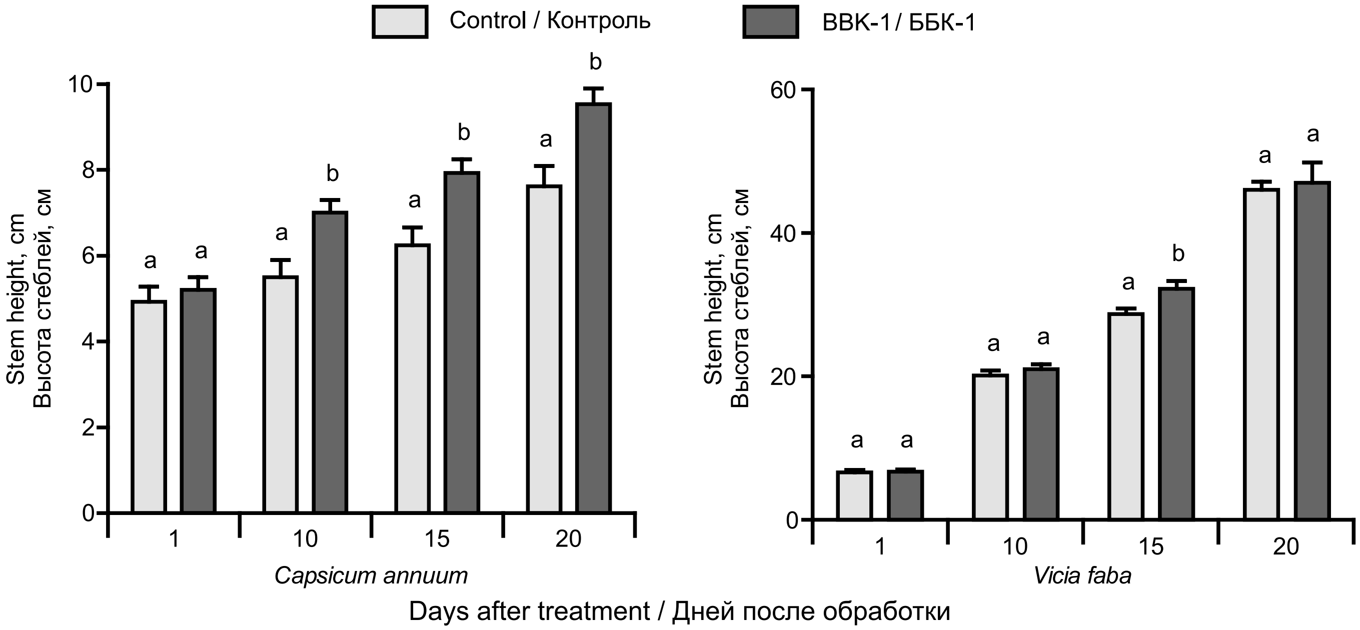

Figure 3. Effect of *Beauveria bassiana* colonization (1×10^8 conidia/ml, 10 ml per plant) on stem height of the sweet pepper (left) and the broad beans (right). Different letters above the bars indicate the presence of significant differences, comparison by day (t-test, $p<0.05$)

Рисунок 3. Влияние колонизации *Beauveria bassiana* (1×10^8 конидий/мл, 10 мл на растение) на высоту стеблей сладкого перца (слева) и кормовых бобов (справа). Разными буквами над столбцами отмечено наличие существенных различий, сравнение по суткам (t-test, $p<0.05$)

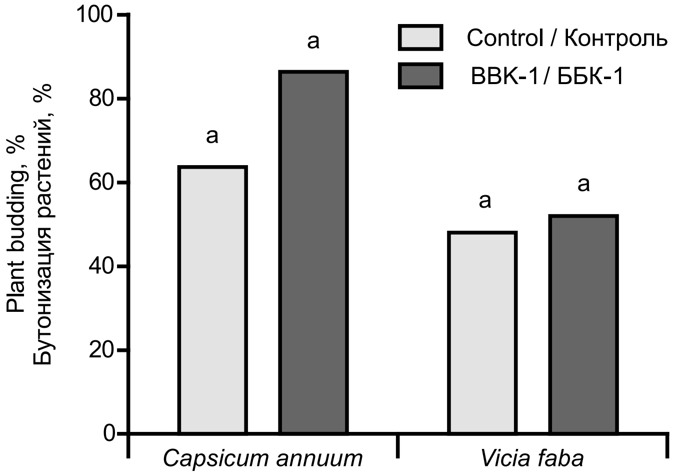

Figure 4. Effect of colonization by *Beauveria bassiana* (1×10^8 conidia/ml, 10 ml per plant) on the budding percentage of the sweet pepper and the broad beans 20 days after treatment with the fungus. Different letters above the bars indicate the presence of significant differences, comparison by day (t-test, $p<0.05$)

Рисунок 4. Влияние колонизации *Beauveria bassiana* (1×10^8 конидий/мл, 10 мл на растение) на уровень бутонизации сладкого перца и кормовых бобов (20 суток после обработки грибом). Разными буквами над столбцами отмечено наличие существенных различий, сравнение по суткам (t-test, $p<0.05$)

to the control. Further laboratory experiments are needed to determine survival and longevity of phytophagous insects on fungus-colonized plants. Similarly, plant growth stimulation deserves special attention to study positive side-effects on plants through fungal application against pests. At the next phase, studies under field conditions are required to prove efficacy of these approaches for their practical applications.

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Краткое сообщение

ВЛИЯНИЕ ЭНДОФИТНОЙ КОЛОНИЗАЦИИ *BEAUVERIA BASSIANA* НА ЧИСЛЕННОСТЬ ПЕРСИКОВОЙ ТЛИ (*MYZUS PERSICAE*) И РОСТ РАСТЕНИЙ

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В последнее время активно изучаются эндофитные свойства энтомопатогенных грибов. В работе показано, что гриб *Beauveria bassiana* (штамм ББК-1) способен успешно колонизировать растения кормовых бобов и сладкого перца в лабораторных условиях. Персиковая тля активно размножалась на обоих видах растений. Численность тли, развивавшейся на колонизированных *B. bassiana* растениях, была достоверно ниже контрольных значений, как на перцах, так и на бобах. Ростостимулирующий эффект эндофитной колонизации *B. bassiana* был менее выраженным на бобах, тогда как на растениях сладкого перца установлено достоверное увеличение высоты растений и более раннее наступление фазы бутонизации. Полученные эффекты свидетельствуют о перспективности дальнейших исследований возможности использования *B. bassiana* в качестве полифункционального агента биоконтроля в сельскохозяйственной практике.

Ключевые слова: энтомопатогенные грибы, кормовые бобы, сладкий перец, ростостимуляция

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