Bitki Koruma Bülteni / Plant Protection Bulletin

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Original article

Investigation of the effect of *Beauveria bassiana* (Balsamo) Vuillemin against potato beetle (*Leptinotarsa decemlineata* Say.) (Coleoptera: *Chrysomelidae*)

Beauveria bassiana (Balsamo) Vuillemin'in Patates böceği (*Leptinotarsa decemlineata* Say.) (Coleoptera: *Chrysomelidae*)'ne karşı etkisinin araştırılması

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ARTICLE INFO

Article history:
DOI: 10.16955/bitkorb.1426255
Received : 26-01-2024
Accepted : 05-03-2024

Keywords:
potato, potato beetle, biological control, mortality

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ABSTRACT

In this study, the objective was to determine the efficacy of entomopathogenic fungi on the Colorado potato beetle [*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae)]. To reach this goal, surveys were conducted in potato fields, and dead *L. decemlineata* and soil samples were collected to isolate entomopathogenic fungi. Pathogenicity tests were carried out using 30 entomopathogenic fungi that were obtained as a result of the analyses. According to the biological activity results, EP-1 isolate was identified and diagnosed as having 75% efficacy against *L. decemlineata*. *Beauveria bassiana* (EP-1), was identified as the most effective isolate. In the dose determination studies, 10^6, 10^7, and 10^8 conidia ml/l doses of *B. bassiana*'s most effective isolate were used. The experiments were set up with five replications for each dose and control group. The spore suspension of the entomopathogenic fungus was sprayed on fully-grown potato plants cultivated in pots, targeting mature and 2nd or 3rd instar *L. decemlineata*. The number of live individuals was recorded on the 1st, 3rd, 5th, 7th, and 9th days after application to calculate the percentage of mortality. The most effective dose of *B. bassiana* isolate was determined to be 85% mortality on the 7th day after application with a dose of 10^8 conidia ml/l. According to the obtained data, it was observed that the mortality rates increased with the increase in dose on the 1st, 3rd, 5th, 7th, and 9th days after application. The highest impact was observed in applications with a dose of 108 conidia ml/l. In conclusion, the entomopathogenic fungus *B. bassiana* isolate, which is less harmful to humans and the environment, is considered suitable for use as a biological control agent against *L. decemlineata*.

INTRODUCTION

Potatoes are cultivated in 79% of countries worldwide, ranking 4th in production after wheat, corn, and rice (TUİK 2022). Various processed forms of potatoes, such as canned, frozen, chips, puree, granules, and powder, are marketed in developed countries. Additionally, potatoes are used as raw materials in alcohol, starch, and animal feed production

(Alisdair et al. 2001, Yüceer 2011). Potato cultivation is widespread in almost every province in Türkiye, with key production areas including Niğde, Nevşehir, İzmir, Bolu, and Afyonkarahisar, contributing to 57.9% of the national production (TUİK 2022). The Colorado potato beetle [(*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae)] is a significant pest affecting potato productivity, causing 70%-80% product loss. This beetle not only feeds on leaves but also acts as a carrier for diseases like potato brown rot, spindle tuber viroid, and potato ring rot (Oerke et al. 1994, Yüceer 2011).

Traditionally, chemical pesticides have been used to control the Colorado potato beetle; however, due to the associated risks to human health, the environment, and pest resistance, there is a growing interest in biological control methods (Erdoğan 2015). Entomopathogenic fungi, such as *Beauveria bassiana*, have emerged as a promising alternative. These fungi directly penetrate the insect cuticle, eliminating the need for ingestion by the pest. To date, over 700 entomopathogenic fungi species from at least 90 genera have been identified (Rath 2000). Commercially produced *B. bassiana* and *Metarhizium anisopliae* isolates have effectively controlled plant-feeding insects without harming bees (Uzuner et al. 2017).

Despite the potential of *B. bassiana* for biological control, there is considerable variation in pathogenicity among isolates. Selection criteria often focus on observed insect mortality rates, neglecting considerations of environmental suitability and the ability to persist in the intended application environment (Meyling and Eilenberg 2007). Recent evidence suggests that *B. bassiana* may exhibit an opportunistic endophytic strategy, prompting further investigation into isolate variation in plant tissue colonization (Kia et al. 2017, McKinnon et al. 2018, Vidal and Jaber 2015). Understanding whether different *B. bassiana* isolates vary in their ability to colonize leaf and root tissues is crucial, as this can affect the effectiveness of biological control strategies.

In addition to insect pathogenicity, some entomopathogenic fungi, particularly *Metarhizium* species, form associations with plant roots in the rhizosphere. These fungi may contribute to nutrient cycling by translocating nitrogen from insect cadavers to plants (Behie et al. 2012). However, the potential effects of entomopathogenic fungi on soil microbial communities, especially in terms of carbon utilization, remain poorly understood. Following the application of entomopathogenic fungi to the rhizosphere, an assessment of community-level physiological profiles (CLPPs) using techniques such as BiologTM and MicroRespTM can shed light on microbial functional diversity and soil functioning (Calbrix et al. 2005).

The purpose of this study was to assess the impact of different concentrations (10^6, 10^7, and 10^8 conidia ml/l) of the most effective *B. bassiana* isolates on the Colorado potato beetle. The study aimed to determine if entomopathogenic fungi could be utilized as part of pest management strategies.

MATERIALS AND METHODS

Production of potato beetle

The cultivation of potato plants utilized Marabel variety seeds and took place in the climate rooms of the Adana Biological Control Research Institute. Sterilized soil was filled halfway into pots, and potato tubers were planted. The pots were then moved into hygienic climate chambers, provided with water, and subjected to regular irrigation at 2-3 day intervals. No fertilizers or pesticides were applied during the potato cultivation process.

The production of potato beetle individuals for the experiment involved rearing adults and larvae on potato plants in climate chambers, maintaining conditions of 25±1 °C and 60±5% relative humidity. On potato seedlings, potato beetle larvae and egg packets were transferred from stock culture.

Isolation and culture of entomopathogenic fungi and preparation of spore suspensions

The primary focus of this study was on the potato plant (*Solanum tuberosum*) and the adults of *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae). Additionally, soil samples were collected from potato cultivation fields in the Niğde province. *Beauveria bassiana* isolates were acquired from these soils using the "Galleria trap method" (Zimmermann 1986, 2007). Isolations of *B. bassiana* were also conducted from deceased potato beetle adults found in these agricultural fields.

To evaluate the activity of entomopathogenic fungi on the potato beetle (*L. decemlineata*), experiments were conducted in a climate chamber at 25±2 °C and 60±5% relative humidity. The entomopathogenic fungal isolates used in the experiment were cultured on a Potato Dextrose Agar (PDA) medium. PDA (39 g/l, Merck) was prepared with distilled water, sterilized in an autoclave at 121 °C for 20 minutes, and then poured into Petri dishes (9 cm) to establish a new fungal culture. The entomopathogenic fungi obtained were identified under a stereomicroscope. The spores of the entomopathogenic fungi were collected from pure cultures and spread on Petri dishes containing 12-15 ml of medium

to initiate a fresh culture under aseptic conditions. Petri dishes were incubated at 20-25 oC with 75% humidity in the dark. Spores collected from 14-day-old fungal cultures incubated in the dark at 25±1 °C and 60±5% humidity in Petri dishes containing PDA were gently scraped into 50 ml sterile distilled water containing 0.05% Tween 80, and spore suspensions were prepared.

Spore suspensions of entomopathogenic fungi were then sprayed on the leaves of potato plants grown in climate chamber against the 2nd, 3rd, and 4th stage larvae and adults of the potato beetle, with 10 individuals per treatment, and on the control group with sterile distilled water. In plastic containers weighing 1 kg, two layers of sterile blotting paper were placed and moistened with sterile pure water. Ten *L. decemlineata* 2nd, 3rd, and 4th instar larvae and adults were transferred to the leaves of potato plants grown in pots using a brush, and the pots were covered to prevent the escape of the pests. In the spraying method, spore suspensions of entomopathogenic fungi were sprayed three times (2 ml) as fine particles with a hand sprayer at a distance of 20 cm from the larvae and adults placed on the leaves. The pots were kept in four replicates and exposed to 16 hours of light and 8 hours of darkness in a laboratory setting, with a temperature of 25±2 °C throughout the experiment (Saruhan et al. 2015). The first count was performed 24 hours after the start of the experiment, and subsequent counts were performed every 24 hours for 7 days. The study was monitored daily and deceased individuals were recorded and re-incubated again at 25±2 °C to promote fungal growth. To confirm that mortality was caused by the fungus, dead individuals were transferred to Petri dishes with moist blotting paper and incubated to allow for spore development.

Following the experiment, trials were conducted to optimize the dosage using the most effective isolate. The concentrations of the suspensions prepared for this purpose were calibrated as 10^6, 10^7, and 10^8 conidia/ml for application to *L. decemlineata*, utilizing a Thoma slide and light microscopy. For all three doses of the two most effective isolates of *B. bassiana* (10^6, 10^7, and 10^8), sterile distilled water containing 0.05% Tween 80 was applied as the control treatment. Consequently, 40 individuals were allocated to each treatment, and the experiment was structured with four replications, using one pot for each replication. The number of surviving individuals on the 3rd, 5th, 7th, and 9th day after treatment was recorded separately for each treatment. To observe the growth of entomopathogenic fungi, deceased potato beetles were transferred to slides in Petri dishes containing moistened blotting paper, and fungal growth was examined under a binocular stereo microscope. The experiment was conducted in climatic chambers at 25±1 °C and 60±10% humidity under a 16-hour light and 8-hour dark cycle.

Data analysis

The experiment followed a single-factorial randomized experimental design with a total of four replications, with each pot representing one replication. Percent mortality values, calculated by enumerating the number of live individuals in the entomopathogenic and control groups each day, underwent homogeneity and Shapiro-Wilk normality tests. A one-way analysis of variance (One-Way ANOVA) was applied following Arc Sin angle transformation, identified as nonparametric. Subsequently, to identify similar and distinct groups, Duncan's multiple comparison tests was conducted at a 5% significance level. All statistical analyses were performed using the SPSS (Version 23) software package.

RESULTS

The "Galleria trap method" was employed to extract entomopathogenic fungi from soils collected from potato cultivation areas in Niğde. Additionally, deceased adult potato beetles collected from agricultural fields were sterilized and then cultured on Potato Dextrose Agar (PDA) to isolate entomopathogens (Figure 1). Entomopathogenic fungi isolates that developed on deceased potato beetles were purified and cultured. The isolated fungi code and source material were detailed in Table 1. While numerous fungi were obtained through the isolations, only 30 were confirmed to be entomopathogenic. These fungi exhibited efficacy against the potato beetle, as indicated in Table 2. The fungi were further identified, revealing them to be distinct isolates of *Beauveria bassiana, Simplicillium lamellicola, Lecanicillium muscarium* and *Fusarium subglutinans*.

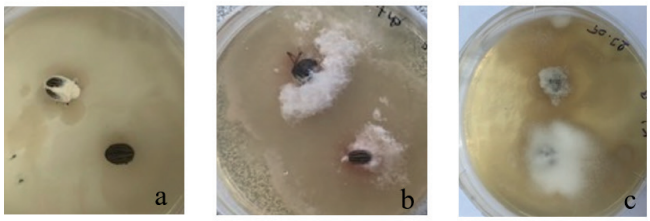

Figure 1. Entomopathogenic fungus development observed in dead potato beetle collected from agricultural areas (*a-Beauveria bassiana b-Fusarium subglutinans c-Lecanicillium muscarium*)

Table 1. The isolate code of entomopathogenic fungi obtained as a result of surveys and the material isolated in the study

Fungal isolate No	Isolated material
EP-1	Dead potato beetle
EP-2	Soil
EP-3	Dead potato beetle
EP-4	Soil
EP-5	Dead potato beetle
EP-6	Dead potato beetle
EP-7	Soil
EP-8	Soil
EP-9	Soil
EP-10	Dead potato beetle
EP-11	Soil
EP-12	Dead potato beetle
EP-13	Dead potato beetle
EP-14	Soil
EP-15	Dead potato beetle
EP-16	Soil
EP-17	Dead potato beetle
EP-18	Soil
EP-19	Dead potato beetle
EP-20	Soil
EP-21	Soil
EP-22	Soil
EP-23	Dead potato beetle
EP-24	Soil
EP-25	Soil
EP-26	Soil
EP-27	Dead potato beetle
EP-28	Soil
EP-29	Dead potato beetle
EP-30	Dead potato beetle

Experiments were conducted to assess the efficacy of the obtained isolates. In each experiment, 10 live insect adults and larvae were introduced, and a 200 ml spore suspension solution of fungi was prepared and sprayed onto them. The experiment was set up with four replications (Table 2, Figure 2).

Table 2. Efficacy rates of various entomopathogen

Isolate	Average Number of Dead Insects ±SS*	Average Impact Rate ±SS*
EP-1	7.50±1.29 g	75.00±12.91 g
EP-2	5.75±1.71 f	57.50±17.08 f
EP-3	5.00±0.82 f	50.00±8.16 f
EP-4	2.00±0.82 bcd	20.00± 8.16 bcd
EP-5	2.75±0.96 cde	27.50± 9.57 cde
EP-6	3.00±1.41 de	30.00±14.14 de
EP-7	5.00±0.82 f	50.00± 8.16 f
EP-8	5.00±0.82 f	50.00± 8.16 f
EP-9	3.00±0.82 de	30.00± 8.16 de
EP-10	2.75±1.71 cde	27.50± 17.08 cde
EP-11	1.25±0.50 ab	12.50± 5.00 ab
EP-12	0.50±0.58 ab	5.00± 5.77 ab
EP-13	0.75±0,96 ab	7.50± 9.57 ab
EP-14	3.00±1.83 de	30.00± 18.26 de
EP-15	1.25±0.50 ab	12.50± 5.00 ab
EP-16	0.50±0.58 ab	5.00± 5.77 ab
EP-17	0.75±0.50 ab	7.50± 5.00 ab
EP-18	3.50±1.29 e	35.00± 12.91 e
EP-19	1.50±0.58 abc	15.00± 5.77 abc
EP-20	0.00 a	0.00 a
EP-21	0.25±0.50 a	2.50±5.00 a
EP-22	0.00 a	0.00 a
EP-23	0.00 a	0.00 a
EP-24	0.00 a	0.00 a
EP-25	0.50±0.58 a	5.00± 5.77 a
EP-26	0.75± 0.50 ab	7.50± 5.00 ab
EP-27	0.50± 1 a	5.00± 10.00 a
EP-28	0.00 a	0.00 a
EP-29	0.75± 0.50 ab	7.50± 5.00 ab
EP-30	0.00 a	0.00 a
Mean	1.92± 2.16	19.17±21.60

* Values marked with different letters are in different groups
**SS – Standard Deviation

Pathogenicity trials were conducted to determine the entomopathogenic nature of the obtained fungi, and the impact of the isolates on insects was measured by calculating effect values (Figure 2).

Figure 2. Pathogenicity trial of the obtained entomopathogenic fungi

The efficacy rates of 30 different entomopathogenic fungi used against the potato beetle exhibited a range from 75% to 20%. To assess the significance of differences between the entomopathogenic fungal isolates, One-way ANOVA and the Duncan test were applied to the data. The statistical analyses resulted in the categorization of entomopathogenic isolates into three distinct groups based on their impact levels. These groups were designated as highly effective (a), moderately effective (b), and low (c), as outlined in Table 2.

Subsequently, the most highly effective isolate against the potato beetle was identified as isolate number 1, characterized as *Beauveria bassiana*.

Efficiency trials of the isolates of *B. bassiana* were established and the efficacy of the most effective isolate on potato beetle in different doses was determined. For this purpose, 10^6-10^7-10^8 is set as conidia/ml. The obtained application results were found to be statistically different compared to the control (Table 3, Figure 3).

The disparities in death rates among *L. decemlineata* individuals on the 1st, 3rd, 5th, 7th, and 9th days following administration, in comparison to the death rates observed in the control group, were determined to be statistically significant. Analysis showed a 70% mortality rate for individuals exposed to 10^8 conidia/ml of entomopathogenic fungus isolates on the 5th day. Subsequently, the mortality rates on the 7th and 9th days were calculated to be 85%, and these values showed a statistically significant contrast to the death rates in the control group (Figure 3). The percentage mortality values for *L. decemlineata* Say. individuals (Coleoptera: Chrysomelidae) are graphically depicted in Figure 3.

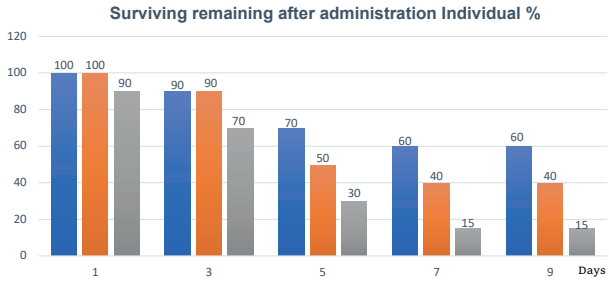

Figure 3. Percent survival rate (%) in *Leptinotarsa decemlineata* individuals exposed to different doses of EP-1 isolates of Beauveria bassiana

→10^6 → 10^7 → 10^8 different doses

DISCUSSION

In the 1980s, there were significant advancements in these studies, particularly in France around 1970 and in the United States of America. In Türkiye, Çam et al. (2002) conducted the first-ever test of an entomopathogenic fungus (*B. bassiana*) isolated from the potato beetle against the same insect. The study reported up to 100% mortality in 2nd, 3rd, and 4th instar larvae treated with entomopathogenic fungi by the end of the seventh day. While adult beetles are generally less susceptible to these fungi, the isolate BMAUM-LDE-001 caused 86.2% mortality. A study has revealed that

Table 3. Mortality rates occurring on different days as a result of application of different doses of entomopathogen (*Beauveria bassiana*) p≤0.05

Application	1. Day	3. Day	5. Day	7. Day	9. Day
10^6	10.00±0.00 b	9.25±0.24 b	6.50±0.33b	6.00±0.49 c	6.00±0.49 c
10^7	10.00±0.24 b	9.00±0.00 ab	5.75±0.31 b	4.50±0.39 b	4.50±0.39 b
10^8	9.50±0.33 a	8.50±0.29 a	3.00±1.62 a	1.50±0.68 a	1.50±0.68 a

* The differences between the means (± standard errors) carrying different letters in the same column, separately for each isolate, are statistically significant (SPSS (Version 23) package program, p> 0.05; each application was conducted on 40 individuals)

distinct entomopathogenic fungal species and isolates may demonstrate different levels of pathogenicity in a range hosts (Butt et al. 1994). Todorova et al. (2000) assessed 10 different *B. bassiana* isolates against *L. decemlineata, Myzus persicae,* and their predator *Coleomegilla maculata lengi*. Six isolates demonstrated high efficacy against all three insect species, while four isolates exhibited high pathogenicity against the two pest species but low pathogenicity against predators. One advantage of employing entomopathogenic fungi for pest control is their compatibility with insecticide spraying equipment. Direct spraying onto pests enhances the mortality rate (Boucias et al. 1998, Fernandez et al. 2001), ensuring rapid adhesion and germination of spores on the insect cuticle (Fernandez et al. 2001). Our study confirmed these findings, showing that directly spraying entomopathogenic fungus spore solutions was highly effective against both larvae and adults of the pest. Our study confirmed these findings, showing that directly spraying entomopathogenic fungus spore solutions was highly effective against both larvae and adults of the pest. Applying entomopathogens on plant leaves significantly influences pest control (Fernandez et al. 2001), depending on the spores' ability to withstand environmental conditions until they penetrate the pest. Wraight and Ramos (2015) examined the *B. bassiana* GHA strain against *L. decemlineata* larvae using two methods. In the first, *B. bassiana* conidia were directly applied, resulting in approximately 58% mortality. In the second, conidia were applied to the leaves, resulting in less than 10% mortality of potato beetle larvae. In summary, direct applications proved more effective against the potato beetle, aligning with the findings of other researchers.

Recent studies have focused on the extraction of entomopathogenic fungi and their application against pests, with specific attention given to investigating the lethal effect of *F. subglutinans* 12A. Uysal et al. conducted a study in 2022, exploring the potential effectiveness of this fungus against aphids and thrips. Notably, studies have revealed the lethal impact of *F. subglutinans* 12A on Coleoptera species. In the case of *L. decemlineata*, the proportion of *F. subglutinans* 12A was determined to be 8% in adults, and 16% and 18% in the 1st and 2nd instar larvae, respectively. Furthermore, the application of *F. subglutinans* 12A on the 3rd and 4th larval stages led to mortality rates of 64% and 84%, respectively. However, our study revealed that the majority of *Fusarium* species obtained exhibited saprophytic characteristics or demonstrated low pathogenicity.

The trial results obtained through this study are very hopeful, especially for the larval stage, and it has been concluded that it will serve as the basis for more comprehensive studies in the future. In the future, it is necessary to investigate the interactions of this isolate with other pesticides used in potato farming and to expand it to include direct soil application of fungi. In addition, the development of the use of entomopathogenic fungus isolates in the fight against potato beetle will be beneficial in terms of organic agriculture, good agricultural practices and integrated control. Wraight and Ramos (2015) tested *B. bassiana* GHA strain against *L. decemlineat*a larvae using two different methods. In the first method, *B. bassiana* conidia were applied directly against potato beetle larvae and approximately 58% mortality occurred. In the second method, conidia were applied to the leaf and less than 10% mortality was observed in potato beetle larvae. As a result, direct applications against potato beetle were more effective. Several inferences can be made in light of the current findings. This study unequivocally demonstrates the efficacy of entomopathogenic fungi. The research establishes that *L. decemlineata* exhibits highly lethal effects on both larval stages and adults, a phenomenon attributed to the intensive and indiscriminate use of broad-spectrum drugs. Recognizing the drawbacks associated with conventional methods, this study emphasizes the potential of entomopathogenic fungal isolates as effective alternatives for managing *L. decemlineata* in agricultural settings. The remarkably successful results obtained in the development of methods utilizing these entomopathogenic fungal isolates highlight their potential as effective tools in biocontrol strategies. This research not only contributes valuable insights but also serves as a foundation for future studies investigating the use of these isolates as biocontrol agents in agriculture.

ACKNOWLEDGEMENTS

The study was supported by the Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies (TAGEM).

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Entomopatojen fungusların Patates böceği (*Leptinotarsa decemlineata*) üzerindeki etkinliğini belirlemek amacıyla bu çalışma yapılmıştır. Bu amaçla patates ekim alanlarında sürveyler yapılarak entomopatojen fungusların izolasyonu için ölü patates böcekleri ile toprak örnekleri toplanmıştır. Yapılan analizler sonucunda elde edilen 30 adet entomopatojen fungus ile patajonisite testleri

gerçekleştirilmiştir. Biyolojik etkinlik sonuçlarına göre *L. decemlineata*'ya karşı %75 etki oranında EP-1 izolatı belirlenmiş ve tanısı yapılmıştır. *Beauveria bassiana*'nın (EP-1) en etkili izolat olduğu belirlenmiştir. Doz belirleme çalışmalarında ise, *B. bassiana* izolatından 10^6, 10^7 ve 10^8 konidi ml/l dozları kullanılmıştır. Yapılan çalışmalarda, her doz ve kontrol grubu için beş tekrarlı deneme kurulmuştur. Kontrollü koşullarda saksılarda yetiştirilen patates bitkileri üzerindeki ergin, 2. ve 3. dönem Patates böceği larvalarına entomopatojen fungusların spor süspansiyonu püskürtülmüştür. Canlı bireylerin sayısı, uygulamadan sonraki 1., 3., 5., 7. ve 9. günlerde kaydedilmiş ve ölüm yüzdesini hesaplamak için kullanılmış, *B. bassiana* izolatının en etkili dozu, 10^8 konidi ml/l dozuyla uygulamanın 7. gününde %85 ölüm olarak belirlenmiştir. Elde edilen verilere göre, ölüm oranlarının dozun artmasıyla 1., 3., 5., 7. ve 9. günlerde arttığı gözlemlenmiştir. En yüksek etki, 108 konidi ml/l dozuyla yapılan uygulamalarda gözlenmiştir. Sonuç olarak, insanlar ve çevre için daha az zararlı olan entomopatojen fungus izolatı *B. bassiana*'nın, *L. decemlineata*'ya karşı biyolojik kontrol ajanı olarak kullanılması uygun görülmektedir.

Anahtar kelimeler: patates, patates böceği, biyolojik kontrol, ölüm oranı

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Cite this article: Yüceer, D. S. (2024). Investigation of the effect of *Beauveria bassiana* (Balsamo) Vuillemin against potato beetle (*Leptinotarsa decemlineata* Say.) (Coleoptera: *Chrysomelidae*). Plant Protection Bulletin, 64-3. DOI: 10.16955/bitkorb.1426255

Atıf için: Yüceer, D. S. (2024). *Beauveria bassiana* (Balsamo) Vuillemin'in Patates böceği (*Leptinotarsa decemlineata* Say.) (Coleoptera: *Chrysomelidae*)'ne karşı etkisinin araştırılması. Bitki Koruma Bülteni, 64-3. DOI: 10.16955/bitkorb.1426255