Available online www.jsaer.com

Journal of Scientific and Engineering Research, 2024, 11(11):38-44

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Efficacy of *Beauveria bassiana* (Bals.) Vuill. Isolates on Colorado Potato Beetle [*Leptinotarsa decemlineata* (Say.) (Coleoptera: Chrysomelidae)]

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Abstract: The aim of the present study was to determine the effects of entomopathogenic fungus *Beauveria bassiana* (Bals.) Vull. isolates (Bb-1, Bb-18, ET-101, ET-10, BMAUM M6-4, and BMAUM LD 2016) isolated from various hosts and regions on Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say, 1824) larvae in their third instar under laboratory conditions. The isolates of *B. bassiana* were applied as a single dose (10^8 conidia mL^{-1}) by spraying using a hand sprayer. Counts were conducted 1, 3, 5, and 7 days later, and percentage mortality data were calculated after the application. The experiments were carried out in a randomized plot design with 10 replications, in climate chambers conditions (25±1°C, 60±5% relative humidity, and 16 h Light: 8 h Dark photoperiod). According to the obtained data, it was observed that the mortality rates increased with the increase in dose on the 1st, 3rd, 5th, and 7th days after the application. As a result of the study, mortality rates in isolates of *B. bassiana* isolated were recorded as 72-96%. The LT50 values of the applied Bb-1, Bb-18, ET-101, ET-10, BMAUM M6-4, and BMAUM LD 2016 isolates were determined as 3.80, 4.13, 4.06, 5.03, 4.20, and 4.33 days, respectively. Finally, isolates of the entomopathogenic fungus *B. bassiana*, Bb-1, Bb-18, and ET-101 isolates were found promising for the biological control of Colarado potato beetle.

Keywords: *Beauveria bassiana*, *Leptinotarsa decemlineata*, Entomopatojen fungus, Mortality, Biological control

1. Introduction

Potato (*Solanum tuberosum* L.) is the most cultivated plant species in the world after cereals such as corn (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) [1]. Potato is an important agricultural crop not only because of its great importance in human nutrition but also because it is an input in the food industry [2]. Various processed forms of potato, such as canned, frozen, chips, puree, granules, and powder are marketed in developed countries. Additionally, potatoes are used as raw materials in the production of alcohol, starch and animal feed [3, 4]. According to FAO 2022 data, total potato production in the world as of 2020 is 359 million tons and the average yield is 2176 kg da-1. In the world potato production, China ranks first with 78 million tons, and India ranks second with 51 million tons [5]. Türkiye ranks 16th with a production of 5.2 million tons, accounting for 1.4% of the world's potato production. Niğde, Afyonkarahisar, and Konya are the provinces with the largest potato cultivation areas in Turkey [6].

There are many diseases and pests that negatively affect yield and quality in potato production. One of the most important pests in potato plants is the Colorado potato beetle (CPB) [*Leptinotarsa decemlineata* (Say.) (Coleoptera: Chrysomelidae)]. The CPB was first detected in 1963 in the Bosna and Karaağaç villages of Edirne, Turkey. Later, this pest spread to the inner regions of our country, starting from the Thrace Region [7].

The CPB adults and larvae feed on the leaves of their hosts, and in both the adult and larva stages, they usually gnaw at the leaves of their hosts starting from the outside and working inwards, or they feed by opening a hole in the leaf and widening this hole. *L. decemlineata* first feeds by leaving the main veins of the leaves and then eats them, turning the plants into just trunks [8]. The CPB not only feeds on leaves but also acts as a carrier for diseases like potato brown rot, spindle tuber viroid, and potato ring rot [9].

There is a 100% yield loss in potato production if the CPB is not controlled [10]. Insecticides are generally used in our country to control CPB. However, due to high levels of insecticide use, pest resistance and phytotoxicity problems arise [11]. This situation increases the possibility of using entomopathogens alone or in integrated pest management programs in the control of CPB [12].

Entomopathogenic fungi (EPF) such as *Beauveria bassiana* have emerged as a promising alternative. EPF penetrates directly into the insect cuticle, eliminating the need for the pest to ingest it. Over 700 species of entomopathogenic fungi have been found so far, belonging to at least 90 genera [13]. Entomopathogenic fungi belonging to the genera Metarhizium, Beauveria, Paecilomyces, Isaria, and Lecanicillium have been reported to infect a wide variety of Arthropoda species due to their wide spectrum of action [14-16]. Their advantages are that they can be relatively easily produced, are able to penetrate the host cuticle so they do not need to be ingested, and there is no risk of resistance development in target pests and none or few side effects on non-target organisms [17, 18]. The entomopathoegenic fungus *B. bassiana* has been successfully used in our country studies to control a variety of pests, including whitefly [19], Colarado potato beetle [20], pine processionary moth [21], rice weevil [22], and aphid [23]. The aim of the present study was to evaluate the efficacy six isolates of *B. bassiana* using spraying method against third instar larvae of the Colorado potato beetle under laboratory conditions and to determine the most effective isolate of used entomopathogenic fungi.

2. Materials and Methods

The hosts, isolated regions and references of the entomopathogenic fungus *Beauveria bassiana* isolates used in the study are listed in Table 1.

Table 1: Details of entomopathogenic fungus *Beauveria bassiana* isolates used in the study

***Beauveria bassiana* isolates**	**Host**	**Location (City-Country)**	**References**
Bb-1	Forest soil	Düzce, Türkiye	Erdoğan and Sağlan [24]
Bb-18	Field soil	Düzce, Türkiye	Erdoğan and Sağlan [24]
ET-101	Coleoptera larvae	Erzurum, Türkiye	Erdoğan and Sağlan [24]
ET 10	*Sphenoptera antiqua*	Erzurum, Türkiye	Tozlu et al. [25]
BMAUM M6-4	Field soil	Isparta, Türkiye	Baydar et al. [26]
BMAUM LD 2016	*L. decemlineata*	Isparta, Türkiye	Gök et al. [21]

Preparation of fungal suspension

Six local isolates of *B. bassiana* were grown within the dark at 25±1°C for 7-15 days and after that subcultured on potato dextrose agar (PDA-Difco, 39 g L^{-1}). Firstly, the prepared PDA medium was transferred to glass Erlenmeyer bottles (500 mL) and sterilized in an autoclave at 121°C for 15 minutes. PDA medium cooled at room temperature was poured into each plastic petri dish (90 mm) as 20 mL and spore discs (0.5 cm) of *B. bassiana* Bb-1, Bb-18, ET-101, ET-10, BMAUM M6-4 and BMAUM LD 2016 isolates taken from the stock culture were transferred to the middle of the petri dishes containing PDA medium. Petri dishes covered with parafilm were incubated within the dark at 25±1°C for 14 days in a cooled incubator. The conidia were harvested by scraping them into a sterile solution of 0.05% (v/v) Tween 80 (Sigma-Aldrich, Munich, Germany). The conidial suspension was filtered through sterile gauze to separate the mycelium and clusters of conidia. In the uniform suspension, the spores were counted under a light microscope using a Thoma slide, and subsequently, the suspension was adjusted to a concentration of 1×10^8 conidia per mL [28, 29].

Rearing of third instar larvae of *Leptinotarsa decemlineata*

Adult individuals of CPB were collected from the terminal shoots of potato plants in the Çevrepınar neighborhood of Sandıklı district of Afyon province and brought to the laboratory. No pesticide was applied in the field during the potato cultivation. Adult individuals brought to the laboratory were allowed to multiply by placing them on previously grown potato plants. In this way, the development of this insect was ensured and the third instar larvae of the Colorado potato beetle were obtained. New potato plants were placed in order to increase the population of the CPB and ensure the continuity of mass production. Plant maintenance and watering were carried out regularly.

Potato cultivation

Sterilized soil mix (soil:peat/1:1) was filled into plastic pots (1.5 L), and potato tubers were planted. The pots were then moved into 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.

Application of *Beauveria bassiana* isolates on larvae of *Leptinotarsa decemlineata*

Third instar larvae of *L. decemlineata* were used in the experiments. This larvae were transferred to petri dishes (90 mm) containing blotting papers impregnated with 1 mL of sterile water using a fine sable brush. A total of 50 larvae were used for each EPF isolate application, five 3rd instar larvae in each replicate. *B. bassiana* isolates were sprayed onto the larvae three times with a hand sprayer at a concentration of 1 x 10^8 conidia mL^{-1}. Control petri dishes were sprayed with sterile distilled water containing 0.05% Tween 80. Fresh potato leaf shoots were added to petri dishes to feed the larvae during the experiments. The experiments were conducted in climatic chambers conditions (25±1°C, 60±5% relative humidity, and 16-hour Light and 8-hour Dark photoperiod) and in a randomized plot design with 10 replications. The number of live and dead individuals was recorded 1, 3, 5 and 7 days after application.

Statistical analysis

Mortality rates were adjusted for control mortality using Abbott's formula [29]. Angle transformation was applied to the percentage values, then one-way analysis of variance (One-Way ANOVA) and the differences between the means were compared according to Tukey's HSD multiple comparison test at a significance level of $P < 0.05$ [30]. Data analyses were performed using the SPSS® 20.0 (SPSS Inc., Chicago, Illinois, USA) package program. In addition, the estimated time (LT_{50}) to kill 50% of the insects was determined by the Probit analysis program [31].

3. Results and Discussion

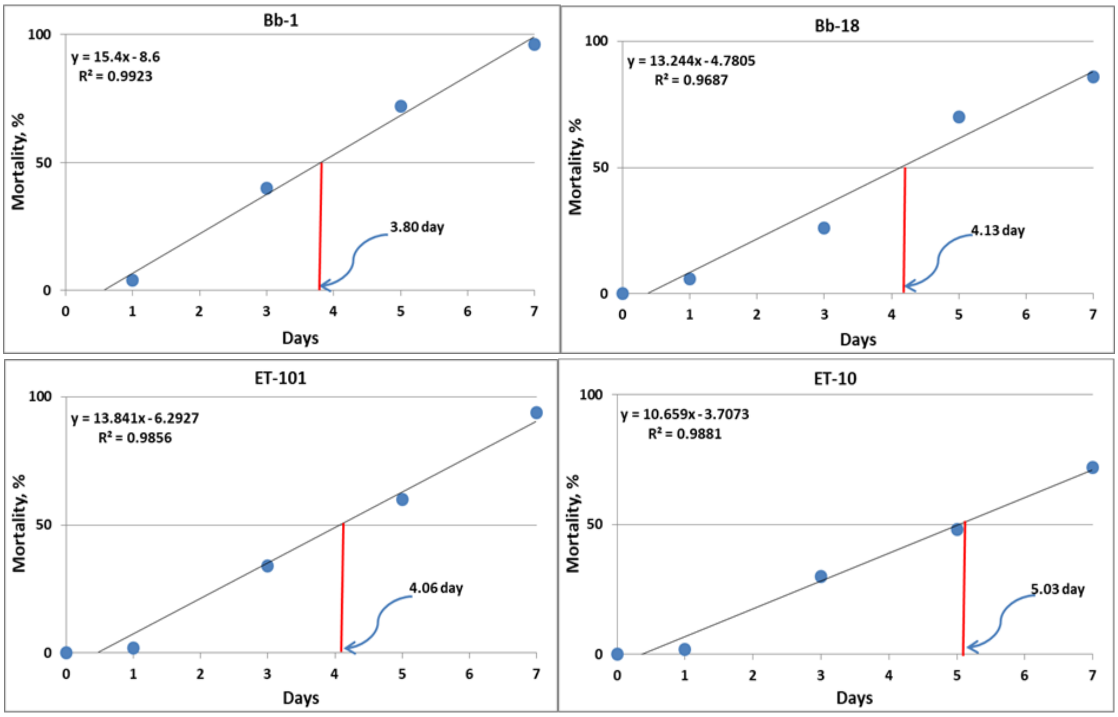
The results in Table 2 show the mortality of third instar larvae of *L. decemlineata* treated with 1 x 10^8 conidia mL^{-1} of *B. bassiana* isolate (Bb-1, Bb-18, ET-101, ET-10, BMAUM M6-4, and BMAUM LD-2016). The mortality rate in Colorado potato beetle larvae increased with increasing time in all applied *B. bassiana* isolates. *B. bassiana* isolates were statistically in the same group in the 1st and 3rd day counts. In the fifth day count, the highest mortality rate was detected in the Bb-1 isolate at 72.00%. Other *B. bassiana* isolates were statistically included in the same group. On the seventh day count, the highest mortality rate was determined as 96% in the Bb-1 isolate, followed by Bb-18 (86.00%) and BMAUM LD 2016 (82.00%) isolates. The lowest mortality rate was recorded in the ET-10 isolate (72.00%).

Table 2: Mortality (%) of *Leptinotarsa decemlineata* larvae treated with *Beauveria bassiana* isolates

***Beauveria bassiana* isolates**	**Mortality ± SDM*(%)**			
	1 DAT**	**3 DAT**	**5 DAT**	**7 DAT**
Bb-1	4.00 ± 2.66 a	40.00 ± 5.96 a	72.00 ± 5.33 a	96.00 ± 2.66 a***
Bb-18	6.00 ± 3.05 a	26.00 ± 4.26 a	70.00 ± 6.83 ab	86.00 ± 5.20 abc
ET-101	2.00 ± 2.00 a	34.00 ± 6.69 a	60.00 ± 5.16 ab	94.00 ± 6.11 ab
ET-10	2.00 ± 2.00 a	30.00 ± 4.47 a	48.00 ± 5.33 ab	72.00 ± 6.11 c
BMAUM M6-4	6.00 ± 3.05 a	48.00 ± 7.42 a	62.00 ± 4.66 ab	76.00 ± 5.81 bc
BMAUM LD 2016	2.00 ± 2.00 a	46.00 ± 6.69 a	52.00 ± 6.11 ab	82.00 ± 3.59 abc

*SDM, Standard deviation of the mean. **DAT, days after treatment. ***Means followed by the same letter within columns are not significantly different (Tukey's HSD test, P < 0.05).

LT50 values for *B. bassiana* isolates are shown in Figure 1. LT_{50} values for *B. bassiana* isolates Bb-1, Bb-18, ET-101, ET-10, BMAUM M6-4, and BMAUM LD 2016 were recorded as 3.80, 4.13, 4.06, 5.03, 4.20 and 4.33 days, respectively. Among the applied *B. bassiana* isolates, the most effective isolate on Colarado potato beetle was determined Bb-1 and LT_{50} value was recorded as 3.80 days (Figure 1).
Similar to our data, Todorova et al. [32] evaluated 10 isolates of *B. bassiana* under laboratory conditions at a concentration of 1 x 10^7 conidia mL^{-1} adults of *L. decemlineata* and reported that 6 isolates were caused mortalities 86.7 to 100%. In another study, Çam et al. [29] conducted a study to determine the effect of applying the *B. bassiana* isolate at a dose of 1 x 10^8 spores mL^{-1} on the third instar larvae of the Colarado potato beetle and reported that 89% of the deaths due to fungal infection occurred after 6 days. Güven et al. [20] applied *B. bassiana* isolates isolated from different hosts and regions to the 3rd instar larvae of Colarado potato beetle by spraying, dipping and residue methods. As a result of the applications, 72.7%, 64.5%, 67.7% mortality rates were recorded for BMAUM-001 isolate, 83.6%, 92.9%, 90.8% for BMAUM-002 isolate and 83.6%, 59.7%, 79.2% for BMAUM-003 isolate, respectively. Wraight and Ramos [33] reported significant reductions of Colarado potato beetle populations after *B. bassiana* foliar treatments. As a result of the application of *B. bassiana* BIM-001 at a concentration of 1 x 10^8 spores mL^{-1} to the larvae of the Colarado potato beetle, 94% mortality rates were detected in the 1st and 3rd larvae stages, 80% in the 2nd instar larvae and 90% in the 4th instar larvae [34]. *B. bassiana* four isolates (BbDm-1, BbDs-2, BbMg-2, and BbMp-1) were against L. decemlineata, causing mortalities 96.7 and 100% in the 1st and 2nd instar larvae when they were sprayed with a suspension of 1 x 10^7 conidia mL^{-1} [35]. Zemek et al. [36] applied the *B. bassiana* isolate to the adults of the Colarado potato beetle at a concentration of 1 x 10^8 spores mL^{-1}, resulting in 100% mortality and reported the LT_{50} value as the 7th day. In another study, the application of *B. bassiana* GOPT-552 and GOPT-562 isolates to the larvae and adults of the Colarado potato beetle resulted in 100% and 93% mortality rates [37]. Konopická et al. [38] recorded a 100% decrease in pot experiments and a 30% decrease in field experiments as a result of the application of *B. bassiana* (Bb8) isolate to Colarado potato beetle. *B. bassiana* LdA-1 isolate showed corrected mortality of 80% with an LT_{50} of 5.09 days when larvae were treated with a concentration of 10^7 conidia mL^{-1} [39]. Yüceer [9] determined a mortality rate of 85% at the the seventh day after the application of the *B. bassiana* isolate at a dose of 10^8 conidia mL^{-1} to the Colarado potato beetle.



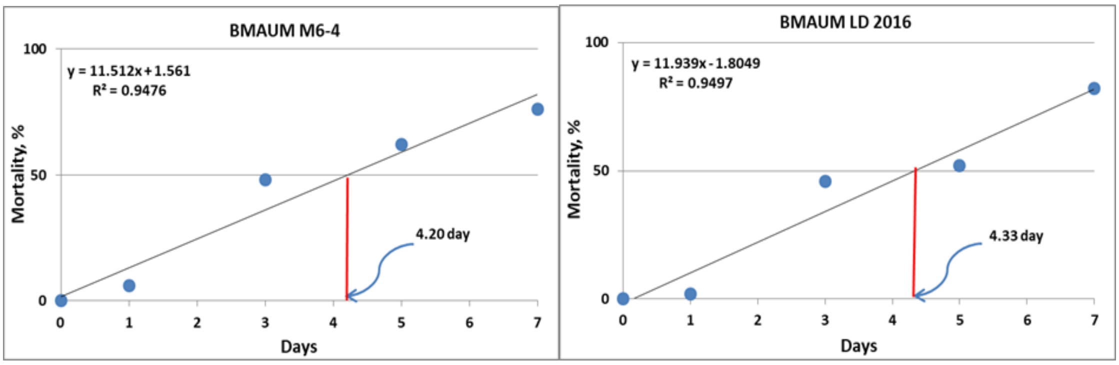


Figure 1. The mean LT_{50} values of Leptinotarsa decemlineata larvae treated with Beauveria bassiana isolates

4. Conclusions

In this study, Bb-1, Bb-18, ET-101 isolates of the entomopathogenic fungus *B. bassiana* were found to be more effective against the 3rd instar larvae of the Colorado potato beetle than other *B. bassiana* isolates (ET-10, BMAUM M6-4, and BMAUM LD 2016). Meanwhile, different biological periods of the Colorado potato beetle can be found in the same time period. Therefore, the effectiveness of the *B. bassiana* isolates used should be determined by applying them to other periods of the CPB. Further studies should be conducted to confirm these results under field conditions. In addition, developing the use of EPF isolates in the control against Colorado potato beetle will be beneficial in terms of organic agriculture, good agricultural practices and integrated management.

Acknowledgements

The authors thank to Prof. Dr. Elif Tozlu (Atatürk University, Erzurum, Türkiye), Prof. Dr. Salih Karabörklü (Sakarya Applied Sciences University, Sakarya, Türkiye), and Prof. Dr. İsmail Karaca (Isparta Applied Sciences University, Isparta, Türkiye) for kindly providing local isolates of *B. bassiana*

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