

RESEARCH

Open Access



# Efficacy of indigenous isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)

Derya Baki<sup>1</sup>, Hilal Sule Tosun<sup>2</sup> and Fedai Erler<sup>2\*</sup>

## Abstract

**Background:** The Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is one of the most damaging pests of potatoes all over the world as well as in Turkey. Both adults and larvae of this pest feed on the foliage of potatoes, and often cause complete defoliation of potato plants, with considerable yield losses of up to 60%. Chemical control of this pest has induced the selection of resistant CPB populations and negative environmental impact. The aim of the present study was to evaluate the pathogenicity of 14 Turkish isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) against different stages of CPB under laboratory conditions.

**Results:** All isolates were tested as conidial suspensions of ( $1 \times 10^7$  conidia/ml), using spray method. The results from pathogenicity assays showed that virulence of tested isolates was variable, and mortality increased with the time of exposure. The highest mortality was seen in larvae of CPB; however, young larvae (1st and 2nd instars) were more susceptible to fungal isolates than older ones (3rd and 4th instars). Four isolates (BbDm-1, BbDs-2, BbMg-2, and BbMp-1) were more pathogenic than others against *L. decemlineata*, causing mortalities between 96.7 and 100% in the 2 youngest larval instars, respectively, between 91.7 and 96.7% in the 2 oldest larval instars, respectively, and between 93.3 and 96.7% in 0–48-h-old adults, respectively, 9 days post treatment. Furthermore, these 4 isolates had the most potent egg-hatching inhibitory effects (66.7–90%). The results of molecular phylogenetic analyses based on the ITS region sequence indicated that all tested *B. bassiana* isolates showed a high similarity (99%) with other *B. bassiana* isolates in GenBank.

**Conclusions:** Results suggested that the 4 *B. bassiana* isolates were highly virulence and had the potential for biological control of CPB. Further tests under field conditions are recommended to validate the laboratory results.

**Keywords:** Entomopathogenic fungi, *Beauveria bassiana*, Biological control, Colorado potato beetle, *Leptinotarsa decemlineata*

\* Correspondence: [erler@akdeniz.edu.tr](mailto:erler@akdeniz.edu.tr)

<sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Akdeniz University, 07070 Antalya, Turkey

Full list of author information is available at the end of the article



© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## Background

Potato (*Solanum tuberosum* L.) is a tuber crop belonging to the family Solanaceae. After wheat and rice, potato is the third largest crop in the world in terms of human consumption (FAOSTAT 2019). As a horticultural crop only, potato is the second most grown crop in Turkey after tomato. There are many insect pest species attacking potato crop and affect its production as well as quality in Turkey. One of the most damaging pests is the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). The pest is native to Mexico and was first described by Thomas Say in 1824 (Casagrande 1987). It spreads across most of the USA and was introduced into France in the 1920s from where it spread further reaching also parts of China (CABI 2017). In Turkey, the pest was detected for the first time in 1963 in Edirne (in the Thrace Region of Turkey), which borders with Bulgaria and Greece, and spread towards the inner regions of the country (Atak 1973). Adults and larvae of the pest feed on the foliage of potatoes and can cause complete defoliation of potato plants, with considerable yield losses of up to 60% (Jacques and Fasulo 2015).

In Turkey, control of this pest is usually based on the use of synthetic insecticides. Some populations of the beetle have already developed a resistance to many groups of insecticides (Keskin and Yorulmaz-Salman 2020). As more insecticides are becoming ineffective due to resistance development or becoming phased out due to environmental concerns, the number of options available to potato growers dwindles. As an alternative method, biological control is often considered as the most environmentally friendly tool to control many pests. One of the main factors in biological control is the use of entomopathogenic fungi (EPF) (Mahr et al. 2008). Lately, attracting attention worldwide as a potential biological control agent for many insect pest species was noticed. The EPF *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) is the most widely used fungal agent that has a potential to protect a range of crops (Goettel and Inglis 1997). Some previous studies have proved the potential effectiveness of *B. bassiana* against the insecticide resistant CPB strains (Roberts et al. 1981; Campbell et al. 1985).

The aim of the present study was to evaluate the efficacy of 14 indigenous isolates of *B. bassiana* isolated from cultivated and uncultivated soils in Antalya province, Turkey against different stages of CPB under laboratory conditions.

## Methods

### Insect material and maintenance

Insects used in the experiments were taken from a laboratory colony of CPB that originated with field-

collected adults from potato production areas in Cameli (Denizli, Turkey). Rearing was carried out on the foliage of potted potato (*S. tuberosum* var. *nahita*) plants in a climate room set to  $25\pm 1^\circ\text{C}$ ,  $60\pm 5$  R.H., and 16:8 (L/D) hours photoperiod. Subsequent cultures were maintained in screen cages (90 × 90 × 90 cm) to obtain a fresh (known age) insect material. In the experiments, 0–48-h-old eggs and adults, young (1st and 2nd instars), and old (3rd and 4th instars) larvae of *L. decemlineata* were used. Young and old instars were selected based on their head capsule width and pronotum color (Niezgodzinski and Koroluk 1976; Boiteau and LeBlanc 1992).

### Indigenous *B. bassiana* isolates

Fourteen *B. bassiana* isolates were used in this evaluation. Isolate names, sampling locations, isolation areas, and geographic coordinates are listed in Table 1.

### Viability of fungal isolates

The viability of conidia of each fungal isolate was determined before any bioassay by the method described by Goettel and Inglis (1997). For this purpose, conidia of each fungal isolate were harvested from 3-week-old cultures with a sterile spatula and suspended in a 20-ml sterile distilled water with a 0.03% Tween 80 and counted using a hemocytometer. Conidial suspensions (1 ml) adjusted to  $1 \times 10^7$  conidia/ml were spread on Petri dishes containing SDA (Sabouraud Dextrose Agar) medium. The dishes were then incubated in complete darkness at  $25\pm 2^\circ\text{C}$  and examined after 24 h. Percentage germination of conidia of each isolate was determined by counting the number of germinated conidia (a germ tube two times the diameter of the propagule) from 100 spores counted randomly on the surface area under the light microscope (400 ×). Three replicate plates per isolate were used. The viability of all tested fungal isolates was above 95%.

### Preparation of conidial suspension

To determine the pathogenicity of indigenous *B. bassiana* isolates against CPB, the experiments were carried out under the controlled conditions of ( $25\pm 2^\circ\text{C}$  and  $65\pm 5$  RH). For this purpose, conidia from 2 to 3-week-old cultures of each fungal isolate, grown on PDA medium at ( $25\pm 2^\circ\text{C}$ ,  $65\pm 5$  RH), were collected by a sterile loop and suspended in a 20-ml sterile distilled water with a 0.03% Tween 80. Then, all suspensions were filtered through 4 layers of sterile cheesecloth in order to remove mycelium and agar pieces. Later, the conidial suspensions were vortexed for 3 min for homogenization. Conidial concentrations were determined using a hemocytometer and final concentration of each fungal isolate was adjusted to  $1 \times 10^7$  conidia/ml (Fancelli et al. 2013). In all bioassays, all isolates were tested at a

**Table 1** Indigenous isolates of *Beauveria bassiana* used in the experiments

Isolate name	Sampling site	Vegetation	Geographic coordinates
BbKm-1	Kumluca/Antalya	Olive	N 36° 19' 17.1" E 30° 20' 23.0"
BbKm-2	Kumluca/Antalya	Orange	N 36° 22' 18.8" E 30° 16' 29.1"
BbDm-1	Demre/Antalya	Orange	N 36° 14' 39.7" E 29° 58' 45.0"
BbFn-3	Finike/Antalya	Orange	N 36° 19' 53.7" E 30° 08' 40.6"
BbDs-2	Döşemaltı/Antalya	Pomegranate	N 37° 00' 02.4" E 30° 38' 16.1"
BbAk-1	Aksu/Antalya	Grassland	N 36° 56' 03.3" E 30° 52' 35.1"
BbSr-1	Serik/Antalya	Orange	N 36° 55' 33.8" E 31° 07' 20.7"
BbKr-1	Kemer/Antalya	Forest	N 36° 35' 51.0" E 30° 33' 22.7"
BbKp-1	Kepez/Antalya	Forest	N 36° 54' 50.4" E 30° 37' 48.4"
BbMg-1	Manavgat/Antalya	Olive	N 36° 49' 40.8" E 31° 20' 35.3"
BbMg-2	Manavgat/Antalya	Wheat	N 36° 58' 58.8" E 31° 14' 48.5"
BbMp-1	Muratpaşa/Antalya	Fig	N 36° 53' 07.2" E 30° 44' 30.4"
BbGp-1	Gazipaşa/Antalya	Forest	N 36° 12' 52.0" E 32° 23' 45.0"
BbKl-1	Korkuteli/Antalya	Pear	N 37° 03' 21.3" E 30° 10' 33.8"

conidial suspension of  $1 \times 10^7$  conidia/ml using the spray method.

### Experimental bioassays

#### *Bioassay against L. decemlineata* eggs

This experiment was conducted, using CPB egg masses (0–48-h-old eggs) on potato leaves. The egg masses were treated in 3 replications (10 eggs each) with one of the tested *B. bassiana* isolates through direct spray, using a handheld sprayer with a tank capacity of 0.5 l. However, separate sprayers were used for each fungal isolate to prevent cross-contamination. The control group was treated by distilled water + 0.03% Tween 80. Treated eggs were then removed and placed in clean Petri dishes (9 cm in diameter) at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 5$  RH till egg hatching. Each Petri dish was counted as one replicate. All egg masses were checked daily for hatching and a small potato leaf was placed within each Petri dish as food for the larvae when hatching day approached. Number of hatched larvae was recorded to determine a percentage of hatching for each replicate within a treatment. During data collection, infected (unhatched) eggs conspicuously shrunk, usually turned orange-brown and had more or less fungal outgrowths (with a white fuzzy appearance) on the surface, that could be easily distinguished from those killed by factor(s) that had a shriveled and light brownish appearance, were counted as dead and included in the count. Moreover, the rate of mortality in surviving 1st instar larvae was also recorded for each replicate within a treatment. After hatching, 1st instar larvae were then reared under the abovementioned conditions on clean potato leaves until developed to the 2nd instar. This procedure allowed detecting

latent effect of the exposure of egg masses to the tested *B. bassiana* isolates on 1st instar larvae.

#### *Bioassay against L. decemlineata* larvae

Larval bioassay was conducted using young (1st and 2nd instars) and old (3rd and 4th instars) larvae of CPB. Each treatment, using spray method, was replicated 3 times with 10 larvae each. For each treatment, 10 young (five 1st and five 2nd instars) or 10 old (five 3rd and five 4th instars) larvae were placed in each Petri dish (9 cm in diameter) lined with 3 layers of filter paper, and they were then sprayed from 30-cm distance with a handheld sprayer, using 2.0 ml of conidial suspension of any fungal isolate. The control group was treated by sterile distilled water + 0.03% Tween 80. After air drying, the treated larvae were carefully transferred to new Petri dishes containing clean potato leaves using a fine camel-hair brush. The lid of each Petri dish was closed and perforated 20 times using a hot needle for ventilation. All dishes were kept under the abovementioned laboratory conditions. Surviving larvae were fed on untreated leaves for the rest of the experimental period. To record mortality, Petri dishes were daily examined, and mortality rates were recorded 3, 5, 7, and 9 days after treatment. At each observation, larvae were touched using a fine-forceps and if the insect did not move, it was recorded as dead. To assess the growth of fungal mycelium on the insects, which would indicate insect mortality caused by the EPF agents, all dead larvae were removed from the dishes and placed in new dishes lined with moistened filter paper, incubated at  $25 \pm 2^\circ\text{C}$  and  $65 \pm 5$  RH in complete darkness, and evaluated for up to 14 days under a stereomicroscope to observe fungal growth

on larval cadavers. Dead larvae showed no fungal outgrowths of similar characteristics of the treated ones were excluded.

#### **Bioassay against *L. decemlineata* adults**

The bioassay was conducted using 0–48-h-old adults of *L. decemlineata*. Adult CPB was treated by each of *B. bassiana* isolates as previously mentioned in larval bioassay. Each treatment was replicated 3 times (10 adults each). The control group was treated by sterile distilled water + 0.03% Tween 80. Treated adults (10 per dish) were transferred into new Petri dishes containing fresh potato foliage. The dishes were then closed and kept under the aforementioned laboratory conditions. Treatments were checked at daily basis and the rate of adult mortality was recorded 3, 5, 7, and 9 days post treatment. Final mortality counts were performed using the same procedure mentioned in larval mortality counts.

#### **Phylogenetic placement of tested *B. bassiana* isolates**

Genomic DNA of tested *B. bassiana* isolates were extracted, following the modified CTAB method described by Doyle and Doyle (1990). The PCR was performed in a Thermal Cycler by using two different primers based on ITS-rDNA region gene sequences, which included, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). Each PCR reaction was conducted in a final volume of 50.0 µl containing 3.0 µl 2.5 mM MgCl<sub>2</sub>, 5.0 µl 10× Taq buffer with KCl, 8.0 µl 100 mM dNTPs, 0.25 µl 5 Uµl<sup>-1</sup> Taq-DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 1 µl of each primer, 27.8 µl sterile ddH<sub>2</sub>O, and 4.0 µl fungal DNA suspension. The cycling conditions included an initial denaturation for 60 s at 95°C, followed by 35 cycles of 60 s at 94°C, 120 s at 58°C, 60 s at 72°C, and a final extension 300 s at 72°C. The amplified PCR products (10 µl) and 1 kb DNA Ladder (Thermo Fisher Scientific) were separated by gel electrophoresis in 1% agarose gel in 0.5× TAE (dH<sub>2</sub>O 1000 ml, Tris-Base 4.84 g, glacial acetic acid 1.02 ml, 0.5 M. EDTA (pH: 8.0) 2 ml) buffer after staining with jel red (1 mg/l) and run at 80 V/cm for 90 min. The gel was then visualized with an ultraviolet light imaging system (Vilber Lourmat SR 12 575 UV transilluminator, France). The amplified PCR products were sequenced using the ABI 3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA) and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) in the Macrogen Netherlands laboratory. The DNA sequences of isolates were performed using the ClustalW algorithm in the Bioedit program (Thompson et al. 1994; Hall 1999). The nucleotide sequence of tested isolates was compared with that of the other isolates of the related species using a Blast

Bioinformatics search of sequences from the National Center for Biotechnology Information (NCBI) GenBank (Altschul et al. 1997).

Molecular phylogenetic analyses were conducted with MEGA5 software (Biodesign Institute, Tempe, Arizona) using the maximum likelihood method based on the Tamura 3-parameter model (Kimura 1980; Tamura et al. 2011). The phylogenetic analysis was done based on the ITS region sequence of tested isolates and the nucleotide sequence of the other fungi retrieved from GenBank, the accession numbers of which are presented in Table 6.

#### **Statistical analysis**

In larval and adult bioassays, no control mortality was observed; therefore, no correction was necessary for the mortality data. All values were arcsine transformed prior to analysis. Data were then analyzed by one-way ANOVA using the general linear model of the SPSS 23.0 Windows (IBM Corp. 2015, New York, USA). Differences among the treatment means were compared using the Tukey's HSD test at a significance level of  $P < 0.05$ .

## **Results**

#### **Effectiveness of tested *B. bassiana* isolates against *L. decemlineata* eggs**

Data presented in Table 2 showed that the tested *B. bassiana* isolates generally had low pathogenic activity against 0–48-h-old eggs. There were significant differences among some of the *B. bassiana* isolates tested at  $1 \times 10^7$  conidia/ml in terms of hatching rate of *L. decemlineata* eggs ( $P < 0.05$ ). The lowest hatching rate (6.7%) was recorded in eggs treated with isolate BbMg-2, which showed the most potent egg-hatching inhibitory effect (93.3%) (Table 2). This isolate was followed the most strongly by the isolates BbMp-1, BbDs-2, and BbDm-1, all the three of which exhibited the same rate of inhibitory effect (90%). The lowest inhibitory effect (6.7%) was exhibited by isolate BbKm-2, recording (93.3%) hatching rate in eggs.

For the mortality rates of the fed 1st instar larvae during molting to 2nd instar (usually 4 days), there were significant differences among some of the isolates tested ( $P < 0.05$ ). The highest mortality rate (63.3%) in 1st instar larvae hatched from the eggs treated with *B. bassiana* was caused by the isolates BbDs-2 and BbMp-1. These 2 isolates were followed most strongly by the isolates BbMg-2 and BbDm-1, both of which exhibited the same rate of mortality (60%).

#### **Effectiveness of tested *B. bassiana* isolates against *L. decemlineata* larvae**

All tested *B. bassiana* isolates at  $1 \times 10^7$  conidia/ml showed pathogenic activity against both young (1st and 2nd instars) and old (3rd and 4th instars) larvae of *L.*

**Table 2** Egg-hatching rates of *Leptinotarsa decemlineata* eggs treated with fourteen indigenous *Beauveria bassiana* isolates, egg-hatching inhibitory effects of tested fungal isolates, and mortality rates of fed 1st larval instar during molting to 2nd instar\*

Isolate name	Egg-hatching (%)	Inhibition of egg-hatching (%)	First instar mortality (%)
BbKm-1	70.0±0.0 <sup>bc**</sup>	30.0	20.0±0.0 <sup>d</sup>
BbKm-2	93.3±3.3 <sup>a</sup>	6.7	6.7±3.3 <sup>f</sup>
BbDm-1	10.0±0.0 <sup>e</sup>	90.0	60.0±0.0 <sup>a</sup>
BbFn-3	66.7±3.3 <sup>bc</sup>	33.3	23.3±3.3 <sup>cd</sup>
BbKp-1	43.3±3.3 <sup>d</sup>	56.7	40.0±0.0 <sup>b</sup>
BbAk-1	80.0±0.0 <sup>ab</sup>	20.0	10.0±0.0 <sup>ef</sup>
BbSr-1	76.7±6.7 <sup>b</sup>	23.3	16.7±3.3 <sup>e</sup>
BbKr-1	60.0±5.8 <sup>c</sup>	40.0	33.3±3.3 <sup>bc</sup>
BbDs-2	10.0±0.0 <sup>e</sup>	90.0	63.3±3.3 <sup>a</sup>
BbMg-1	60.0±0.0 <sup>c</sup>	40.0	30.0±0.0 <sup>c</sup>
BbMg-2	6.7±3.3 <sup>e</sup>	93.3	60.0±5.8 <sup>a</sup>
BbMp-1	10.0±0.0 <sup>e</sup>	90.0	63.3±3.3 <sup>a</sup>
BbGp-1	43.3±3.3 <sup>d</sup>	56.7	40.0±0.0 <sup>b</sup>
BbKl-1	66.7±3.3 <sup>bc</sup>	33.3	26.7±3.3 <sup>c</sup>
df	41	-	41
F	85.0	-	49.7
P	0.000	-	0.000

\*In a single-dose trial, all the isolates were tested at a concentration of  $1 \times 10^7$  conidia/ml

\*\*Means in a column followed by the same letter are not significantly different (Tukey's HSD test;  $P < 0.05$ )

*decemlineata*. Mortality rates decreased with increasing age of *L. decemlineata* larvae but increased with the elapsed time after treatment (Tables 3 and 4). There were significant differences among the tested *B. bassiana* isolates in their mortality rates in young larvae 3, 5, 7, and 9 days after treatment ( $P < 0.05$ ) (Table 3). Based on the mortality rates of young larvae on the 9th day, 4 isolates (BbMp-1, BbMg-2, BbDm-1, and BbDs-2) of the 14 *B. bassiana* isolates tested were more virulent than others, and caused 100, 100, 98.3, and 96.7% mortalities, respectively. On the 3rd and 5th days of treatment, the highest mortality (53.3 and 85%, respectively) was exhibited by the isolate, BbKl-1; however, efficacy of this isolate remained stable on the 7th and 9th days. As for the oldest larvae, data presented in Table 4 indicated that mortality rate increased with the elapsed time after treatment. As in the young larvae, 4 isolates (BbMp-1, BbMg-2, BbDm-1, and BbDs-2) were significantly more pathogenic than others against the oldest larvae and exhibited 96.7, 93.3, 95.0, and 91.7% mortalities, respectively, 9 day post treatment ( $P < 0.05$ ).

#### Effectiveness of tested *B. bassiana* isolates against *L. decemlineata* adults

As shown in Table 5, all tested *B. bassiana* isolates had different efficacy rates against 0–48-h-old adults of *L. decemlineata*. Mortality was isolate- and elapsed time-dependent, and differences in mortality at each count

date were generally significant among the different isolates tested ( $P < 0.05$ ). As in the larvae of *L. decemlineata*, 4 isolates (BbMp-1, BbMg-2, BbDm-1, and BbDs-2) were significantly more virulent than the others against *L. decemlineata* adults ( $P < 0.05$ ). Of these 4 isolates, BbDs-2 caused the highest adult mortality by 96.7%. The remaining 3 isolates had the same mortality rate (93.3%) 9 day post treatment. Isolates BbFn-3 and BbAk-1 caused the lowest adult mortalities (0 and 3.3% on the 3rd and 5th days of treatment, respectively) that were non-significantly different from each other ( $P < 0.05$ ).

#### Phylogenetic placement of tested *B. bassiana* isolates

The DNA sequences of the tested *B. bassiana* isolates were loaded into GenBank, and their accession numbers were obtained and used for comparison in phylogenetic analysis. The accession numbers of the isolates are presented in Table 6. After alignment, the ITS region sequence data set consisted of 487 aligned positions for *Beauveria* isolates. All the *B. bassiana* isolates from Turkey and GenBank were clustered together. All the *B. bassiana* isolates tested had 99% evolutionary homology with other *B. bassiana* isolates from the NCBI (Fig. 1).

#### Discussion

The present study demonstrated that some of the tested indigenous isolates of *B. bassiana* can be used for the

**Table 3** Percentage mortalities of young larvae (1st and 2nd instars) of *Leptinotarsa decemlineata* in response to the tested *Beauveria bassiana* isolates at 3, 5, 7, and 9 days after treatment under laboratory conditions

Isolate name	Percent mortality ( $\pm$ SE) from a single-dose trial*				df	F	P
	3 days	5 days	7 days	9 days			
BbKm-1	8.3 $\pm$ 1.7 <sup>cdC**</sup>	30.0 $\pm$ 5.7 <sup>efB</sup>	41.7 $\pm$ 1.7 <sup>deAB</sup>	56.7 $\pm$ 6.7 <sup>cA</sup>	11	20.0	0.000
BbKm-2	0.0 $\pm$ 0.0 <sup>dC</sup>	15.0 $\pm$ 5.0 <sup>fbC</sup>	26.7 $\pm$ 6.7 <sup>ebB</sup>	53.3 $\pm$ 3.3 <sup>cA</sup>	11	25.2	0.000
BbDm-1	46.7 $\pm$ 4.4 <sup>abC</sup>	78.3 $\pm$ 4.4 <sup>abcB</sup>	95.0 $\pm$ 5.0 <sup>aAB</sup>	98.3 $\pm$ 1.7 <sup>aA</sup>	11	33.4	0.000
BbFn-3	6.7 $\pm$ 4.4 <sup>dB</sup>	25.0 $\pm$ 8.7 <sup>fb</sup>	61.7 $\pm$ 6.0 <sup>cdA</sup>	70.0 $\pm$ 5.0 <sup>bcA</sup>	11	23.2	0.000
BbKp-1	3.3 $\pm$ 3.3 <sup>dC</sup>	53.3 $\pm$ 3.3 <sup>deB</sup>	88.3 $\pm$ 6.0 <sup>abA</sup>	91.7 $\pm$ 8.9 <sup>abA</sup>	11	52.8	0.000
BbAk-1	0.0 $\pm$ 0.0 <sup>dC</sup>	13.3 $\pm$ 3.3 <sup>fbC</sup>	30.0 $\pm$ 5.0 <sup>eaB</sup>	46.7 $\pm$ 6.0 <sup>cA</sup>	11	22.7	0.000
BbSr-1	30.0 $\pm$ 2.9 <sup>bcB</sup>	81.7 $\pm$ 1.7 <sup>abA</sup>	85.0 $\pm$ 2.9 <sup>abA</sup>	86.7 $\pm$ 3.3 <sup>abA</sup>	11	97.6	0.000
BbKr-1	48.3 $\pm$ 1.7 <sup>abB</sup>	58.3 $\pm$ 4.4 <sup>bcdAB</sup>	80.0 $\pm$ 7.6 <sup>abcA</sup>	85.0 $\pm$ 7.6 <sup>abA</sup>	11	13.7	0.002
BbDs-2	50.0 $\pm$ 5.0 <sup>abC</sup>	78.3 $\pm$ 1.7 <sup>abcb</sup>	83.3 $\pm$ 3.3 <sup>abcAB</sup>	96.7 $\pm$ 1.7 <sup>aA</sup>	11	37.0	0.000
BbMg-1	36.7 $\pm$ 10.1 <sup>abb</sup>	58.3 $\pm$ 4.4 <sup>bcdAB</sup>	71.7 $\pm$ 6.0 <sup>bcA</sup>	86.7 $\pm$ 3.3 <sup>abA</sup>	11	10.7	0.004
BbMg-2	46.7 $\pm$ 3.3 <sup>abC</sup>	56.7 $\pm$ 1.7 <sup>cdC</sup>	75.0 $\pm$ 2.9 <sup>abcB</sup>	100.0 $\pm$ 0.0 <sup>aA</sup>	11	98.8	0.000
BbMp-1	38.3 $\pm$ 1.7 <sup>abD</sup>	78.3 $\pm$ 1.7 <sup>abcb</sup>	91.7 $\pm$ 1.7 <sup>abB</sup>	100.0 $\pm$ 0.0 <sup>aA</sup>	11	358.6	0.000
BbGp-1	5.0 $\pm$ 2.9 <sup>dC</sup>	50.0 $\pm$ 7.6 <sup>deB</sup>	71.7 $\pm$ 1.7 <sup>bcA</sup>	86.7 $\pm$ 4.4 <sup>abA</sup>	11	57.0	0.000
BbKl-1	53.3 $\pm$ 6.7 <sup>abB</sup>	85.0 $\pm$ 2.9 <sup>aA</sup>	88.3 $\pm$ 6.0 <sup>abA</sup>	88.3 $\pm$ 6.0 <sup>abA</sup>	11	9.3	0.006
df	41	41	41	41			
F	23.0	30.5	25.4	15.3			
P	0.000	0.000	0.000	0.000			

\*In a single-dose trial, all the isolates were tested at a concentration of  $1 \times 10^7$  conidia/ml

\*\*Means in a column followed by the same lower-case letter are not significantly different, and means in a row followed by the same upper-case letter are not significantly different (Tukey's HSD test;  $P < 0.05$ )

management of CPB. However, successive subcultures of indigenous *B. bassiana* isolates on solid media differed significantly in their virulence. Moreover, different stages of CPB had different susceptibilities to the isolates tested. Eggs were not so much susceptible to infection. Adults showed susceptibility much lower than larvae. In larval bioassay, the tested *B. bassiana* isolates significantly differed in their pathogenicity to *L. decemlineata* larval instars. High mortality was obtained in younger larvae than the oldest ones. The findings of this research are in accordance with the previous studies that compared efficacy of different *B. bassiana* isolates on different stages of CPB (Ignoffo et al. 1983; Watt and LeBrun 1984; Fargues 1991; Long et al. 1998).

A review of the related literature indicated that *B. bassiana* isolates obtained from different parts of the world varied in their pathogenic activity against different stages of CPB, and there was a high variability among the fungal isolates in terms of mortality rates. For instance, Timonin (1939) tested the efficacy of *B. bassiana* against *L. decemlineata* eggs and newly hatched larvae and reported that there was no effect of *B. bassiana* on egg mortality, but he observed 1st instar mortality on the 4th day after egg hatch. He also reported that 1st instar mortality was due to ingestion of fresh spores still on the egg chorion by the larvae. In a similar study,

Long et al. (1998) evaluated effects of the *B. bassiana*-based commercial product (Mycotrol®, Mycotech Corporation, Butte, Montana) at 5 concentrations (ranging from  $1 \times 10^4$  to  $1 \times 10^8$  conidia/ml) on egg hatching and subsequent 1st instar larval survival of *L. decemlineata* by spraying egg masses on potato leaves. They reported that there was no effect of *B. bassiana* on egg and 1st instar mortality, even at the highest concentration. Obtained findings on 1st instar mortality are in accordance with those of Timonin (1939), but in contrast with those of Long et al. (1998). In another study, Todorova et al. (2000) evaluated 10 isolates of *B. bassiana* from different sources and geographical sites under laboratory conditions at a concentration of  $1 \times 10^7$  conidia/ml for their pathogenicity against adults of *L. decemlineata* and reported that 6 isolates were highly virulent and caused mortalities ranging from 86.7 to 100% by 8 day post-treatment. In a more recent study, Ozturk et al. (2015) tested pathogenicity of 3 local *B. bassiana* isolates at  $1 \times 10^8$  conidia/ml, using spray method against different stages of *L. decemlineata* in Isparta province of Turkey and reported that tested isolates caused mortalities between 57.1 and 100% in young (2nd and 3rd instars) larvae, between 36.7 and 100% in old (4th instar) larvae, and between 23.3 and 86.2% in 1-week adults; 3 and 7 days post treatment. As well, obtained results on adult

**Table 4** Percentage mortalities of old larvae (3rd and 4th instars) of *Leptinotarsa decemlineata* in response to the tested *Beauveria bassiana* isolates at 3, 5, 7, and 9 days after treatment under laboratory conditions

Isolate name	Percent mortality ( $\pm$ SE) from a single-dose trial*				df	F	P
	3 days	5 days	7 days	9 days			
BbKm-1	3.3 $\pm$ 3.3 <sup>deC**</sup>	15.0 $\pm$ 2.9 <sup>cdBC</sup>	26.7 $\pm$ 3.3 <sup>cdeB</sup>	50.0 $\pm$ 2.9 <sup>defA</sup>	11.0	40.8	0.000
BbKm-2	3.3 $\pm$ 1.7 <sup>deC</sup>	11.7 $\pm$ 1.7 <sup>dBC</sup>	20.0 $\pm$ 2.9 <sup>eB</sup>	45.0 $\pm$ 2.9 <sup>efA</sup>	11.0	58.3	0.000
BbDm-1	18.3 $\pm$ 1.7 <sup>abcdC</sup>	45.0 $\pm$ 2.9 <sup>aB</sup>	61.7 $\pm$ 6.0 <sup>abB</sup>	95.0 $\pm$ 2.9 <sup>aA</sup>	11.0	74.1	0.000
BbFn-3	0.0 $\pm$ 0.0 <sup>eB</sup>	10.0 $\pm$ 2.9 <sup>dB</sup>	41.7 $\pm$ 3.3 <sup>abcdeA</sup>	51.7 $\pm$ 1.7 <sup>cdefA</sup>	11.0	110.2	0.000
BbKp-1	1.7 $\pm$ 1.7 <sup>deC</sup>	21.7 $\pm$ 4.4 <sup>bcdB</sup>	53.3 $\pm$ 1.7 <sup>abcA</sup>	61.7 $\pm$ 4.4 <sup>cdeA</sup>	11.0	70.1	0.000
BbAk-1	3.3 $\pm$ 3.3 <sup>deB</sup>	8.3 $\pm$ 1.7 <sup>dB</sup>	25.0 $\pm$ 2.9 <sup>deA</sup>	38.3 $\pm$ 4.4 <sup>fA</sup>	11.0	24.6	0.000
BbSr-1	10.0 $\pm$ 2.9 <sup>bcdeC</sup>	30.0 $\pm$ 2.9 <sup>abcBC</sup>	48.3 $\pm$ 7.3 <sup>abcdB</sup>	81.7 $\pm$ 3.8 <sup>aBA</sup>	11.0	46.0	0.000
BbKr-1	18.3 $\pm$ 1.7 <sup>abcdC</sup>	31.7 $\pm$ 3.3 <sup>abcC</sup>	46.7 $\pm$ 3.3 <sup>abcdeB</sup>	68.3 $\pm$ 3.3 <sup>bcdA</sup>	11.0	50.9	0.000
BbDs-2	28.3 $\pm$ 4.4 <sup>aC</sup>	46.7 $\pm$ 3.3 <sup>aBC</sup>	58.3 $\pm$ 6.7 <sup>abB</sup>	91.7 $\pm$ 6.0 <sup>aA</sup>	11.0	25.6	0.000
BbMg-1	16.7 $\pm$ 4.4 <sup>abcdeC</sup>	30.0 $\pm$ 2.9 <sup>abcBC</sup>	40.0 $\pm$ 5.8 <sup>bcdeB</sup>	70.0 $\pm$ 2.9 <sup>bcA</sup>	11.0	29.6	0.000
BbMg-2	26.7 $\pm$ 3.3 <sup>abD</sup>	41.7 $\pm$ 1.7 <sup>aC</sup>	61.7 $\pm$ 1.7 <sup>abB</sup>	93.3 $\pm$ 4.4 <sup>aA</sup>	11.0	92.0	0.000
BbMp-1	21.7 $\pm$ 6.0 <sup>abcdD</sup>	45.0 $\pm$ 5.8 <sup>aC</sup>	68.3 $\pm$ 4.4 <sup>aB</sup>	96.7 $\pm$ 1.7 <sup>aA</sup>	11.0	45.0	0.000
BbGp-1	5.0 $\pm$ 2.9 <sup>cdeC</sup>	18.3 $\pm$ 1.7 <sup>bcdBC</sup>	45.0 $\pm$ 10.0 <sup>abcdeB</sup>	81.7 $\pm$ 6.7 <sup>abA</sup>	11.0	29.4	0.000
BbKl-1	16.7 $\pm$ 3.3 <sup>abcdeC</sup>	33.3 $\pm$ 4.4 <sup>abC</sup>	55.0 $\pm$ 5.8 <sup>abB</sup>	85.0 $\pm$ 2.9 <sup>abA</sup>	11.0	48.3	0.000
df	41	41	41	41			
F	9.0	17.9	8.1	27.0			
P	0.000	0.000	0.000	0.000			

\*In a single-dose trial, all the isolates were tested at a concentration of  $1 \times 10^7$  conidia/ml

\*\*Means in a column followed by the same lowercase letter are not significantly different, and means in a row followed by the same uppercase letter are not significantly different (Tukey's HSD test;  $P < 0.05$ )

**Table 5** Percentage mortalities of 0–48-h-old adults of *Leptinotarsa decemlineata* in response to the tested *Beauveria bassiana* isolates at 3, 5, 7, and 9 days after treatment under laboratory conditions

Isolate name	Percent mortality ( $\pm$ SE) from a single-dose trial*				df	F	P
	3 days	5 days	7 days	9 days			
BbKm-1	0.0 $\pm$ 0.0 <sup>aB**</sup>	6.7 $\pm$ 3.3 <sup>cdB</sup>	13.3 $\pm$ 3.3 <sup>bB</sup>	36.7 $\pm$ 8.8 <sup>eA</sup>	11	10.2	0.004
BbKm-2	0.0 $\pm$ 0.0 <sup>aC</sup>	16.7 $\pm$ 3.3 <sup>abcdBC</sup>	26.7 $\pm$ 6.7 <sup>abAB</sup>	40.0 $\pm$ 0.0 <sup>deA</sup>	11	20.5	0.000
BbDm-1	6.7 $\pm$ 3.3 <sup>aC</sup>	33.3 $\pm$ 3.3 <sup>aB</sup>	43.3 $\pm$ 6.7 <sup>abB</sup>	93.3 $\pm$ 3.3 <sup>abA</sup>	11	67.6	0.000
BbFn-3	0.0 $\pm$ 0.0 <sup>aC</sup>	3.3 $\pm$ 3.3 <sup>dBC</sup>	13.3 $\pm$ 3.3 <sup>bB</sup>	46.7 $\pm$ 3.3 <sup>deA</sup>	11	54.6	0.000
BbKp-1	3.3 $\pm$ 3.3 <sup>aB</sup>	10.0 $\pm$ 5.8 <sup>bcdB</sup>	23.3 $\pm$ 8.8 <sup>abAB</sup>	53.3 $\pm$ 8.8 <sup>cdeA</sup>	11	9.8	0.005
BbAk-1	0.0 $\pm$ 0.0 <sup>aC</sup>	3.3 $\pm$ 3.3 <sup>dC</sup>	26.7 $\pm$ 3.3 <sup>abB</sup>	56.7 $\pm$ 6.7 <sup>cdeA</sup>	11	41.1	0.000
BbSr-1	10.0 $\pm$ 5.8 <sup>aB</sup>	13.3 $\pm$ 8.8 <sup>abcdB</sup>	30.0 $\pm$ 10.0 <sup>abB</sup>	76.7 $\pm$ 6.7 <sup>abcA</sup>	11	14.8	0.001
BbKr-1	3.3 $\pm$ 3.3 <sup>aB</sup>	10.0 $\pm$ 5.8 <sup>bcdB</sup>	20.0 $\pm$ 5.8 <sup>abB</sup>	80.0 $\pm$ 0.0 <sup>abcA</sup>	11	63.4	0.000
BbDs-2	10.0 $\pm$ 5.8 <sup>aC</sup>	26.7 $\pm$ 3.3 <sup>abcBC</sup>	46.7 $\pm$ 8.8 <sup>aB</sup>	96.7 $\pm$ 3.3 <sup>aA</sup>	11	42.3	0.000
BbMg-1	3.3 $\pm$ 3.3 <sup>aB</sup>	16.7 $\pm$ 3.3 <sup>abcdB</sup>	20.0 $\pm$ 0.0 <sup>abAB</sup>	36.7 $\pm$ 6.7 <sup>eA</sup>	11	11.3	0.003
BbMg-2	16.7 $\pm$ 3.3 <sup>aB</sup>	30.0 $\pm$ 0.0 <sup>abB</sup>	36.7 $\pm$ 8.8 <sup>abB</sup>	93.3 $\pm$ 3.3 <sup>abA</sup>	11	45.7	0.000
BbMp-1	10.0 $\pm$ 5.8 <sup>aB</sup>	26.7 $\pm$ 3.3 <sup>abcC</sup>	50.0 $\pm$ 0.0 <sup>aB</sup>	93.3 $\pm$ 6.7 <sup>abA</sup>	11	58.8	0.000
BbGp-1	3.3 $\pm$ 3.3 <sup>aB</sup>	10.0 $\pm$ 5.8 <sup>bcdB</sup>	23.3 $\pm$ 6.7 <sup>abB</sup>	66.7 $\pm$ 8.8 <sup>bcdA</sup>	11	19.4	0.000
BbKl-1	0.0 $\pm$ 0.0 <sup>aC</sup>	6.7 $\pm$ 3.3 <sup>cdC</sup>	30.0 $\pm$ 5.8 <sup>abB</sup>	80.0 $\pm$ 0.0 <sup>abcA</sup>	11	118.3	0.000
df	41	41	41	41			
F	2.2	5.1	3.3	15.7			
P	0.035	0.000	0.004	0.000			

\*In a single-dose trial, all the isolates were tested at a concentration of  $1 \times 10^7$  conidia/ml

\*\*Means in a column followed by the same lowercase letter are not significantly different, and means in a row followed by the same uppercase letter are not significantly different (Tukey's HSD test;  $P < 0.05$ )

**Table 6** GenBank nucleotide accessions of tested *Beauveria bassiana* isolates along with other *Beauveria* species based on ITS region used for phylogenetic analysis

Strain/isolates	Accession no.	Strain/isolates	Species	Accession no.
BbKm-1	MT441868	TF6-1B	<i>B. bassiana</i>	JX122736.1
BbKm-2	MT441869	EABb 04/01	<i>B. bassiana</i>	DQ364698.1
BbDm-1	MT441872	CGAIPFBS-012	<i>B. bassiana</i>	KY495188.1
BbFn-3	MT441875	IISR-EPF-04	<i>B. bassiana</i>	KU363833.1
BbKp-1	MT441877	2718	<i>B. bassiana</i>	KU364353.1
BbAk-1	MT441881	HHWG1	<i>B. brongniartii</i>	JX110385.1
BbSr-1	MT441882	SASRI	<i>B. brongniartii</i>	JX110388.1
BbKr-1	MT441871	ARSEF 2641	<i>B. amorpha</i>	HQ880808.1
BbDs-2	MT441879	B518a	<i>B. amorpha</i>	HQ880806.1
BbMg-1	MT441883	BYYC-05	<i>B. asiatica</i>	MG345071.1
BbMg-2	MT441884	BUB824	<i>B. asiatica</i>	MG642836.1
BbMp-1	MT441880	FUM03	<i>B. varroae</i>	MF667767.1
BbGp-1	MT441886	B5	<i>B. varroae</i>	MH374536.1
BbKl-1	MT441885	ARSEF 4622	<i>B. australis</i>	HQ880790.1
F19-N	MG640376.1	ARSEF 4598	<i>B. australis</i>	HQ880789.1
MG562497	MG562497.1	F585	<i>B. caledonica</i>	DQ529233.1
SHU.M.161	KU158472.1	BG47	<i>B. caledonica</i>	MT180427.1
SHU.M.131	KU158461.1	1717	<i>B. vermiconia</i>	FJ973063.1
EABb04	KC753382.1	ARSEF 7281	<i>B. sungii</i>	HQ880815.1
SASRI BB444	JX110368.1	EFCC 5657	<i>B. sungii</i>	JX463219.1

and larval mortalities are in agreement with those reported by Todorova et al. (2000) and Ozturk et al. (2015).

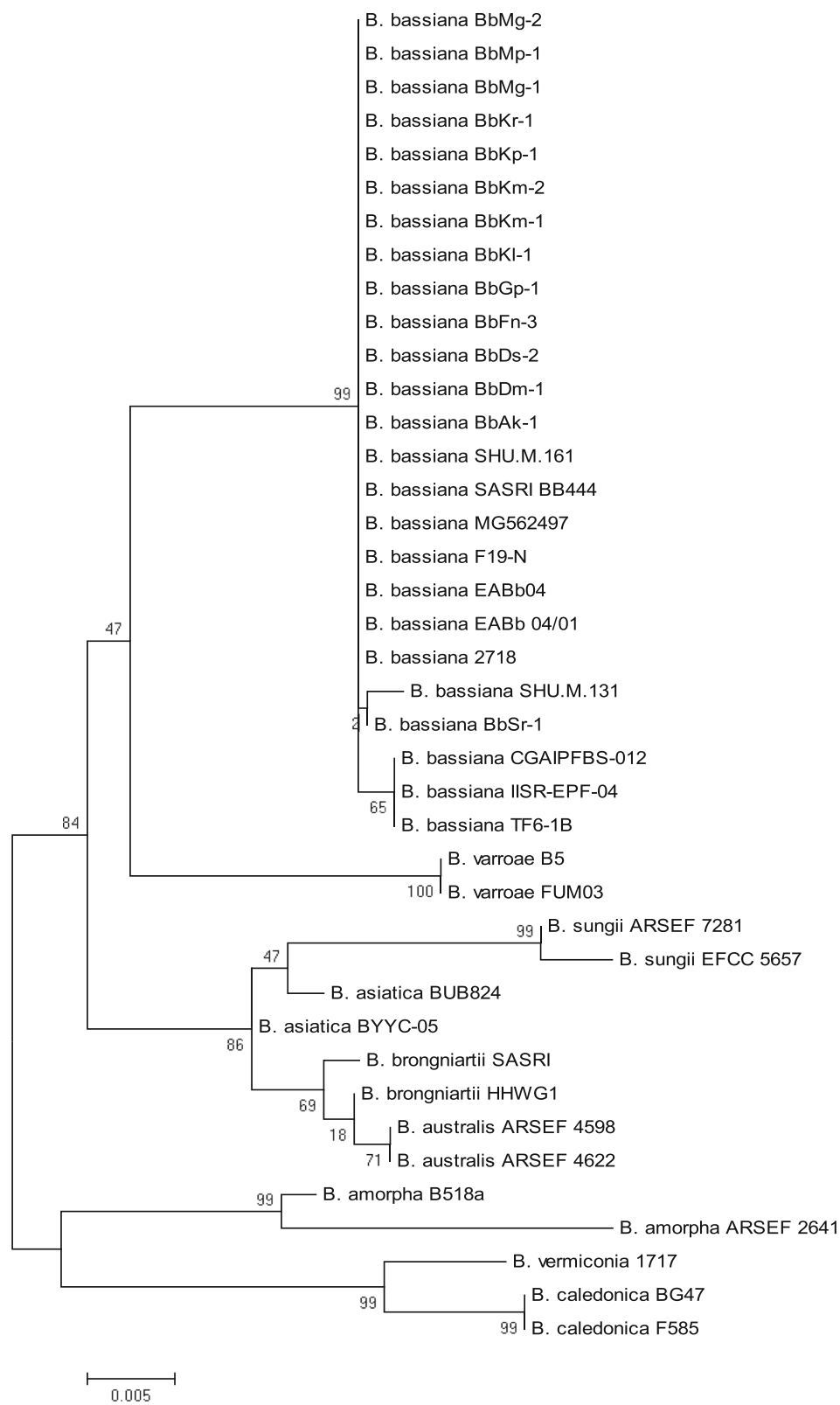
As for the phylogenetic placement of tested *B. bassiana* isolates, the results demonstrated that all the *B. bassiana* isolates tested had 99% evolutionary homology with other *B. bassiana* isolates from the NCBI database. In a previous study, Sayed et al. (2018) investigated the genetic difference of 11 *B. bassiana* isolates from the Taif region of Saudi Arabia by sequencing based on the ITS and COI regions and found genetic differences among the 4 isolates tested. In another study, Gürlek et al. (2018) defined and characterized the molecular phylogenetic diversity of 40 fungus isolates including *Beauveria* spp. and *Metarhizium* spp. using Bloc and B-tubulin gene regions, and they determined that there was a close relationship among the isolates examined. Dhar et al. (2019) performed phylogenetic analysis of 13 *B. bassiana* isolates from India by comparative RAPD-PCR amplification with 10 different RAPD primers. They found that 3 of them were closely related each other. Zhang et al. (2020) examined the genetic difference among *B. bassiana* isolates from different hosts by phylogenetic analysis and determined genetic differences between the virulent isolates. In a more recent study, Gasmi et al. (2021) characterized the variety of genes involved in diverse mechanisms of the infective cycle of 42

isolates that have different thermo-tolerance, growth rates, and virulence. The analyzed genes showed general genetic diversity measured as and copy number variation (CNV) and non-synonymous changes (NSC) with most of them being subjected to positive episodic diversifying selection. Correlation analyses between NSC or CNV and the isolate growth rate, thermo-tolerance, and virulence revealed that various genes shaped the biological features of the fungus. ITS1/ITS4 primers, which are used to reproduce the ITS region that is one of the general diagnosis primers in fungi, are widely used in the diagnosis of EPF in the world (Fergani and Yehia 2020). Therefore, it was used in molecular identification of fungi isolated in this study. The similarity value in the dendrogram drawn at the highest Boot Strap value (Select distribute test) (1000 replicates), using ITS gene regions in GenBank with all *B. bassiana* isolates tested in this study varied around 99%.

## Conclusion

This study demonstrated that indigenous isolates of *B. bassiana* were pathogenic to different stages of CPB under laboratory conditions. However, 4 isolates (BbDm-1, BbDs-2, BbMg-2, and BbMp-1) were more pathogenic than others. Further studies are however needed to evaluate the efficacy of *B. bassiana* isolates on CPB under field conditions.





**Fig. 1** Maximum likelihood tree based on the Tamura 3-parameter model showing the phylogenetic relationship between the tested *Beauveria bassiana* isolates and other *Beauveria* isolates from GenBank based on ITS region sequence

## Abbreviations

IPM: Integrated pest management; RH: Relative humidity; EPF: Entomopathogenic fungi; PCR: Polymerase chain reaction; CTAB: Cetyltrimethylammonium bromide; NCBI: National Center for Biotechnology Information

## Acknowledgements

The authors thank to the Scientific Projects Coordination Unit of Akdeniz University (Antalya, Turkey) for financially supporting the study (Project no.: BAP FDK-2019-4859).

## Authors' contributions

DB and HST substantially contributed to the conception and design of the article. Data curation and analysis were maintained by HST. Writing the entire manuscript was done by FE. The authors have read, revised, and approved the manuscript.

## Funding

This study was funded by the Scientific Projects Coordination Unit (SPCU) of Akdeniz University (Antalya, Turkey) under grant number BAP FDK-2019-4859. The SPCU provided fund for collecting samples and buying chemicals for experiments and supported experimental facilities to carry out experiments.

## Availability of data and materials

All data generated or analyzed in this study are available in this published manuscript.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no conflict of interest.

### Author details

<sup>1</sup>Antalya Directorate of Agricultural Quarantine, Ministry of Agriculture and Forestry, Antalya, Turkey. <sup>2</sup>Faculty of Agriculture, Department of Plant Protection, Akdeniz University, 07070 Antalya, Turkey.

Received: 14 January 2021 Accepted: 17 March 2021

Published online: 22 March 2021

## References

- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25(17):3389–3402. <https://doi.org/10.1093/nar/25.17.3389>
- Atak U (1973) Studies on the morphology, bio-ecology and control methods of potato beetle (*Leptinotarsa decemlineata* Say.) in the Thrace Region. Turkish Ministry of Agriculture General Directorate of Plant Protection and Agricultural Quarantine Publications. Tech Bull 6:63
- Boiteau G, LeBlanc J-PR (1992) Colorado potato beetle life stages. Agriculture Canada Publication, Ottawa
- CABI (2017) *Leptinotarsa decemlineata* (Colorado potato beetle) data sheet. In: Invasive species compendium. CAB International, Wallingford <http://www.cabi.org/isc/datasheet/30380>. Accessed 25 Nov 2020
- Campbell RK, Anderson TE, Semel M, Roberts DW (1985) Management of the Colorado potato beetle using the entomogenous fungus *Beauveria bassiana*. *Am Potato J* 62(1):29–37. <https://doi.org/10.1007/BF02871297>
- Casagrande RA (1987) The Colorado potato beetle: 125 years of mismanagement. *Bull Entomol Soc Am* 33(3):142–150. <https://doi.org/10.1093/besa/33.3.142>
- Dhar S, Jindal V, Jariyal M, Gupta V (2019) Molecular characterization of new isolates of the entomopathogenic fungus *Beauveria bassiana* and their efficacy against the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Egypt J Biol Pest Control* 29(1):8. <https://doi.org/10.1186/s41938-019-0110-3>
- Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15
- Fancelli M, Dias AB, Delalibera IJ, Cerqueira De Jesus S, Souza Do Nascimento A, Oliveira e Silva S (2013) *Beauveria bassiana* strains for biological control of *Cosmopolites sordidus* (Germ.) (Coleoptera: Curculionidae) in plantain. *BioMed Res Int*. <https://doi.org/10.1155/2013/184756>
- FAOSTAT (2019) Food and agriculture data. <http://www.fao.org/faostat/en>. Accessed 22 Nov 2020.
- Fargues J (1991) Fecundity and egg fertility in the adult Colorado beetle (*Leptinotarsa decemlineata*) surviving larval infection by the fungus *Beauveria bassiana*. *Entomol Exp Appl* 61(1):45–51. <https://doi.org/10.1111/j.1570-7458.1991.tb02394.x>
- Fergani YA, Yehia RS (2020) Isolation, molecular characterization of indigenous *Beauveria bassiana* isolate, using ITS-5.8 s rDNA region and its efficacy against the greatest wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a model insect. *Egypt J Biol Pest Control* 30:96
- Gasmi L, Baek S, Kim JC, Kim S, Lee MR, Park SE, Shin TY, Lee SJ, Parker BL, Kim JS (2021) Gene diversity explains variation in biological features of insect killing fungus, *Beauveria bassiana*. *Sci Rep* 11(1):91. <https://doi.org/10.1038/s41598-020-78910-1>
- Goettel MS, Inglis GD (1997) Fungi. In: Fungi hyphomycetes: manual of techniques in insect pathology. Academic Press, San Diego, pp 213–248
- Gürlek S, Sevim A, Sezgin FM, Sevim E (2018) Isolation and characterization of *Beauveria* and *Metarhizium* spp. from walnut fields and their pathogenicity against the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Egypt J Biol Pest Control* 28:50
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. *Nucleic Acids Symp Ser* 41:95–98
- Ignoffo CM, Garcia C, Kroha M, Samisinakova A, Kalalova S (1983) A leaf surface treatment bioassay for determining the activity of conidia of *Beauveria bassiana* against *Leptinotarsa decemlineata*. *J Invertebr Pathol* 41(3):385–386. [https://doi.org/10.1016/0022-2011\(83\)90257-4](https://doi.org/10.1016/0022-2011(83)90257-4)
- Jacques RL, Fasulo TR (2015) Colorado potato beetle *Leptinotarsa decemlineata*. DPM Entomology Circular 271. Florida Department of Agriculture and Consumer Services, Division of Plant Industry and University of Florida. <http://entnemdept.ufl.edu/creatures/veg/leaf/potato>. Accessed 26 Nov 2020.
- Keskin C, Yorulmaz-Salman S (2020) Deltamethrin and imidacloprid resistance levels of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) populations collected from Afyonkarahisar, Turkey. *Turkish J Eng* 2(1):1–5
- Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16(2):111–120. <https://doi.org/10.1007/BF01731581>
- Long DW, Drummond FA, Groden E (1998) Susceptibility of Colorado potato beetle (*Leptinotarsa decemlineata*) eggs to *Beauveria bassiana*. *J Invertebr Pathol* 71(2):182–183. <https://doi.org/10.1006/jjpa.1997.4714>
- Mahr DL, Whitaker P, Ridgway NM (2008) Biological control of insects and mites: an introduction to beneficial natural enemies and their use in pest management. University of Wisconsin Cooperative Extension, No. A3842.
- Niezgodzinski P, Koroluk L (1976) Biometric measurements of the width of the head capsule of different developmental phases of the Colorado beetle larvae *Leptinotarsa decemlineata* (Say) [Coleoptera: Chrysomelidae]. *Bull Entomol Pologne* 46:559–561
- Ozturk HE, Guven O, Karaca I (2015) Effects of some bioinsecticides and entomopathogenic fungi on Colorado potato beetle (*Leptinotarsa decemlineata* L.). *Comm Appl Biol Sci Ghent Univ* 80(2):205–211
- Roberts DW, LeBrun RA, Semel M (1981) Control of the Colorado potato beetle with fungi. In: Lashomb JH, Casagrande RA (eds) *Advances in Potato Pest Management*. Hutchinson Ross Publ. Co., Stroudsburg, pp 119–137
- Sayed SM, Ali EF, El-Arnaouty SA, Samy FM, Sayed AA (2018) Isolation, identification, and molecular diversity of indigenous isolates of *Beauveria bassiana* from Taif region, Saudi Arabia. *Egypt J Biol Pest Control* 28(1):47. <https://doi.org/10.1186/s41938-018-0054-z>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28(10):2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22(22):4673–4680. <https://doi.org/10.1093/nar/22.22.4673>
- Timonin MI (1939) Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. on Colorado potato beetle larvae. *Can J Res* 17:103–107

- Todorova SI, Coderre D, Côté JC (2000) Pathogenicity of *Beauveria bassiana* isolates toward *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), *Myzus persicae* (Homoptera: Aphididae) and their predator *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae). *Phytoprotection* 81:15–22
- Watt BA, Lebrun RA (1984) Soil effects of *Beauveria bassiana* on pupal populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Environ Entomol* 13(1):15–18. <https://doi.org/10.1093/ee/13.1.15>
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*. Academic Press, New York, pp 315–322
- Zhang Z, Lu Y, Xu W, Sui L, Du Q, Wang Y, Zhao Y, Li Q (2020) Influence of genetic diversity of seventeen *Beauveria bassiana* isolates from different hosts on virulence by comparative genomics. *BMC Genomics* 21(1):451. <https://doi.org/10.1186/s12864-020-06791-9>

### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen<sup>®</sup> journal and benefit from:

- ▶ Convenient online submission
- ▶ Rigorous peer review
- ▶ Open access: articles freely available online
- ▶ High visibility within the field
- ▶ Retaining the copyright to your article

---

Submit your next manuscript at ▶ [springeropen.com](https://www.springeropen.com)

---