BioControl (2022) 67:593–603
https://doi.org/10.1007/s10526-022-10156-4

ORIGINAL ARTICLE

Soil isolation, identification, and virulence testing of Turkish entomopathogenic fungal strains: a potential native isolate of *Beauveria bassiana* for the control of *Leptinotarsa decemlineata*

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Received: 15 February 2022 / Accepted: 21 July 2022 / Published online: 30 July 2022
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Abstract The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is one of the most important pests of potatoes and causes great losses in potato production worldwide. Chemical insecticides are primarily used to control this pest, but this has rapidly caused insecticide resistance. In this study, 24 entomopathogenic fungi were obtained from 43 soil samples in potato fields and identified by ITS gene sequencing. Nine of the isolates were identified as *Beauveria bassiana* (Bals.) Vuill and 15 as *Metarhizium* sp. All fungal isolates were first tested against the adults and larvae of CPB under laboratory conditions. The most effective isolate was determined as *B. bassiana* SK-8 with 86% mortality and mycosis against adults, and 100% mortality and 80% mycosis against larvae. Therefore, isolate SK-8 was further characterized by phylogenetic analysis using *bloc*, *rpb1* and *tef* gene sequences and this also confirmed that the isolate SK-8 was *B. bassiana*. *B. bassiana* SK-8 was finally tested against adults and larvae of CPB under field conditions. LC_{50} values were estimated as 3.42×10^6 and 1.15×10^7 conidia ml^{-1} for adults and larvae, respectively. LC_{90} values were estimated as 1.12×10^9 and 4.08×10^{10} conidia ml^{-1} for adults and larvae, respectively. Consequently, *B. bassiana* SK-8 seems to be a promising biocontrol control agent against CPB.

Keywords Colorado potato beetle · *Beauveria bassiana* · Phylogeny · Biological control

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), causes significant economic damage to many agriculturally important plants such as potato, eggplant, and tomato. This insect has attracted a great attention in the scientific community, as it appeared as a major problem in the mid-nineteenth century. While CPB is already a great danger in potato-producing areas, it is increasingly expanding its geographical spread to other new regions of the world (Alyokhin et al. 2013). This insect pest is now present in many parts of the world including Canada, Europe, Central Asia, Russia, Kazakhstan, and China (Jacques 1988; Wilde and Hsiao 1981; Wang et al. 2017). Both larvae and adults cause damage to the potato plant and can cause complete defoliation (Balasko et al. 2020).

Handling Editor: Éverton Kort Kamp Fernandes

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10526-022-10156-4.

Handling Editor: Éverton Kort Kamp Fernandes

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It has also been reported to be effective in spreading several viruses that cause disease in potato (Sorokan et al. 2020). A diverse and flexible life cycle, ecological mobility, symbiotic relationship with various types of bacteria and its extraordinary adaptability to various stressors increase the spread of this pest and make it a very difficult pest to control (Alyokhin et al. 2013; Sorokan et al. 2019, 2020).

Biological, biotechnological, cultural, and chemical control methods are used in the current CPB management. Among these methods, the chemical control is historically the most used and preferred method (Alyokhin et al. 2008; Grafius and Douches 2008; Balasko et al. 2020). However, although the use of various chemical products suppressed and significantly reduced the pest population, CPB has developed resistance against the active substances over time. This pest has been developed resistance to 56 compounds from different insecticide classes which is available on the market (Scott et al. 2015; Grafius 1997; Balasko et al. 2020). Due to this pesticide resistance problem and the effects of chemicals on human and environmental health, different control methods are needed to be developed.

Although many natural enemies of CPB are identified, these are often inadequate in reducing the pest population to the required level (Capinera 2001). Besides these natural enemies, bacteria, viruses, fungi, nematodes, and protozoa cause disease in CPB and have the potential to be used in microbial control (Lacey 2008). Among these entomopathogenic microorganisms, B*eauveria bassiana* (Bals.) Vull. is the first used entomopathogenic fungus against CPB and successful results against both adults and larvae were obtained in the trials (Lacey et al. 2009).

Mycoinsecticides based on entomopathogenic fungi are environmentally friendly and have many advantages in biocontrol such as not having toxic effects on mammals, not developing resistance in insects, being suitable for development with biotechnological and genetic engineering, being able to infect all developmental stages of their hosts and staying in the environment for a long time after application (Rajula et al. 2021; Wan 2003). Today, there are an increasing number of bioinsecticides mostly containing anamorphic genera such as *Beauveria*, *Metarhizium* and *Isaria*. Approximately 80% of the commercial products based on entomopathogenic fungi consist of *Metarhizium* and *Beauveria* species (Butt et al. 2016; de Faria and Wraight 2007; Mascarin et al. 2016). In the control of CPB, there have been many studies related to entomopathogenic fungi (especially *B. bassiana*) and successful results have been obtained (Poprawski et al. 1997; Wraight and Ramos 2002, 2005, 2015). Currently, although a lot of work has been done on isolation and characterization of various entomopathogenic fungi for the target insect, it is still desirable to search for new and more potentially effective local entomopathogenic fungi strains since the choice and application of native isolates can reduce future environmental impacts and may be better adapted to survive local conditions (Bilgo et al. 2018).

In this study, various entomopathogenic fungi were isolated from soil samples in potato fields. Gene sequencing (ITS, *bloc*, *rpb1* and *tef*) and phylogenetic analysis were mainly used for species identification. The identified species were tested against adults and larvae of CPB under laboratory conditions to find out the most virulent isolate. Finally, spray application of several concentrations onto infested plants in the field were performed, LC_{50} and LC_{90} values were calculated.

Materials and methods

Collection of soil samples

43 soil samples were collected from potato fields in the vicinities of Konya, Muğla and Denizli in Turkey, 2020. Soil samples were collected as described in the study of Sevim et al. (2010a). Soil samples were used for fungal isolation within a week.

Insect bait method

Entomopathogenic fungi were isolated from soil samples according to the *Galleria* bait method with minor modifications (Zimmermann 1986). The fourth-fifth instar yellow mealworm larvae [(*Tenebrio molitor* L.) (Coleoptera: Tenebrionidae)] were used as bait insect. Infected larvae obtained from each soil sample were considered as one isolate (Sevim et al. 2010a). All fungal isolates were propagated from single conidium. To do this, 100 µl conidial suspensions of 1×10^6 conidia ml^{-1} were spread on PDAY (Potato dextrose agar + 1% yeast extract) and incubated at

25 °C for 2–3 days in the dark. After that, a single colony for each isolate was transferred onto another PDAY and incubated at 25 °C for two weeks. Finally, they were cryopreserved at −20 °C with 15% (v/v) glycerol for further studies in the Microbiology Laboratory, Department of Plant Protection, Kırşehir Ahi Evran University.

Fungal identification

Morphological identification of the isolated fungi was performed according to the identification key of Humber (1997). Morphological identification of the isolated fungi was also confirmed by gene sequencing and phylogenic analysis. Genomic DNAs were extracted from fungi using Powersoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's recommendations.

ITS1-5.8S-ITS2 gene region between the 18S and 23S rRNA sub-units were first amplified for all fungal isolates. The primer pairs of ITS5: 5′-GGAAGT AAAAGTCGTAACAAGG- 3′ as forward and ITS4: 5′TCCTCCGCTTATTGATATCG- 3′ as reverse were used for PCR amplification (White et al. 1990). The reaction and cycling conditions were adapted and performed according to the study of Sevim et al. (2010a). In addition, the nuclear intergenic region (*bloc*), translation elongation factor-1 alpha (*tef*) and RNA polymerase II largest subunit (*rbp1*) gene sequences were carried out for further characterization of the most effective isolate (SK-8) used in field trial. The primer pairs of B5.1F (5′-CGACCCGGCCAACTA CTTTGA-3′) as forward and B3.1R (5′-GTCTTC CAGTACCACTACGCC-3′) as reverse primers were used to amplify *bloc* gene region and PCR conditions were adapted as described in the study of Rehner et al. (2006). The partial sequence of *tef* gene region was amplified with primer pairs of EF1T (5′-ATGGGT AAGGARGACAAGAC-3′) and 1567R (5′-ACHGTR CCRATACCACCSATCTT-3′) and PCR, cycling conditions were adapted according to the study of Rehner and Buckley (2005). Finally, *rpb1* gene region were amplified with the degenerate primers of RPB1Af (5′-GARTGYCCDGGDCAYTTYGG-3′) and RPB1C (5′- CCNGCDATNTCRTTRTCCATRTA-3′) and PCR conditions were described in the study of Stiller and Hall (1997). After performing PCRs, all products were sent to Macrogen for sequencing. The resulting DNA sequences were compared with DNA sequences at NCBI GenBank by Blast search to confirm species identification, and then phylogenetic analysis was performed. Also, the sequences were used to compare the isolate SK-8 with reference strains in the study of Rehner et al. (2011). All sequences were deposited in GenBank under the accession numbers given in Supplementary Table S1.

Laboratory screening tests

Fifty ml of stock solutions of fungal isolates (1×10^6 conidia ml^{-1}) were separately spread on PDAY and incubated at 25 °C for 2–3 days. At the end of the incubation period, single colonies were selected and transferred to another PDAY and incubated at 25 °C for four weeks. After that, 10 ml of sterile 0.01% Tween 80 were added to each Petri dish and spores were obtained by scraping them with glass rod. Spore suspensions were filtered into 50 ml sterile conical centrifuge tubes through two-layers of sterile cheese cloth to remove mycelium and agar pieces. The resulting suspensions were vortexed for 5 min for homogenization. Spore suspensions were adjusted to the desired concentrations based on Neubauer hemocytometer derived counts. The viability of spores was tested by spreading 100 µl spore suspension on the PDAY agar and determining the germination rate after a 24 h incubation. Spores which produced germ tubes longer than their diameter were considered to have germinated. Isolates with 95% germination rate or over were used in virulence tests (Sevim et al. 2010b).

A total of 24 fungal isolates were tested against both larvae and adults of CPB. Adults and larvae were collected from potato fields in Konya, Turkey. They were fed in the laboratory for three days to eliminate the injured and diseased individuals and the selected healthy insects were used in bioassays. Ten larvae (3rd and 4th instars) and adults were separately used for each repetition and all bioassays were repeated three times. Ten healthy larvae and adults were separately placed in plastic boxes (20×20×20 cm) and a conidial concentration of 1×10^7 conidia ml^{-1} of each isolate were sprayed on insects using an aerosol type sprayer (airbrush). Freshly collected potato leaves were used as food and were changed daily. The control group was inoculated with only sterile 0.01% Tween 80. After inoculation, all boxes were left to incubate at 28 °C for 15 days under a L:D 12:12

photoperiod. All boxes were examined for 15 days, dead larvae were counted, and percentage mortality values were calculated. The percent mycosis values were also calculated. For this, dead larvae and adults were washed with 1% sodium hypochlorite solution for 3 min for surface sterilization. Afterwards, they were washed with sterile distilled water three times and taken into sterile Petri dishes including moist filter paper and left to incubate at 28 °C and in the dark (Sevim et al. 2010c).

Outdoor tests

Based on the initial screening tests, the isolate SK-8 was selected and used in small scale field trials. The conidial suspensions of the isolate SK-8 were prepared as described in the screening tests. In the field trials, a total of thirty larvae (3rd and 4th instars) and adults were separately used for each conidial concentration and repetition. All experiments were repeated three times. Cultural conditions were uniform for all plants of the trial and conformed to local agricultural practice. 25 m^2 field was used as the trial area. Two adjacent potato plants in the area were separately selected for both larvae and adults and used separately for each conidial concentration. Before applying conidial suspensions, 30 larvae and adults were released into each of the two potato plants for each concentration. Therefore, different experiment groups for each concentration were set. Before insects were placed on plants, weeds surrounding potato plants was removed to restrict larval movement between plants (Petek et al. 2020). After that, 200 ml of each concentration (1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1}) belonging to *B. bassiana* SK-8 were applied to the larvae and adults for 10–15 s using the aerosol type sprayer. The control group was inoculated with only sterile 0.01% Tween 80 for both larvae and adults. After inoculation, the upper part of each plant to ground were covered with a wooden cage with plastic holes which are too small for larvae and adults to escape. Then all plants were left to incubate for 15 days under field conditions. All plants were examined during 15 days of incubation, the dead larvae and adults were counted, and the percentage mortality were calculated for each concentration. The average temperature and RH values for the date range in which field trials were performed were obtained from https://tr.freemeteo.com/. The average temperature and RH values were 27.19 ± 0.4 °C (24–31 °C) and 66.81 ± 1.64% (58–79%), respectively.

Data analysis

All DNA sequences were edited with the BioEdit 7.09 (Hall 1999) and their percentage similarities with other known DNA sequences in GenBank were determined by Blast search (Benson et al. 2012; Altschul et al. 1990). Cluster analysis of DNA sequences were done with the ClustalW packed in the BioEdit and the obtained data were used in neighbor-joining (NJ) analysis in MEGA 11.0.10 (Tamura et al. 2021). The phylogenetic tree was constructed using the concatenated sequences of *bloc*, *rpb1* and *tef* gene regions. Alignment gaps were considered as missing data. The reliability of the generated phylogram was tested with 1.000 replicates by bootstrap analysis using the MEGA 11.0.10.

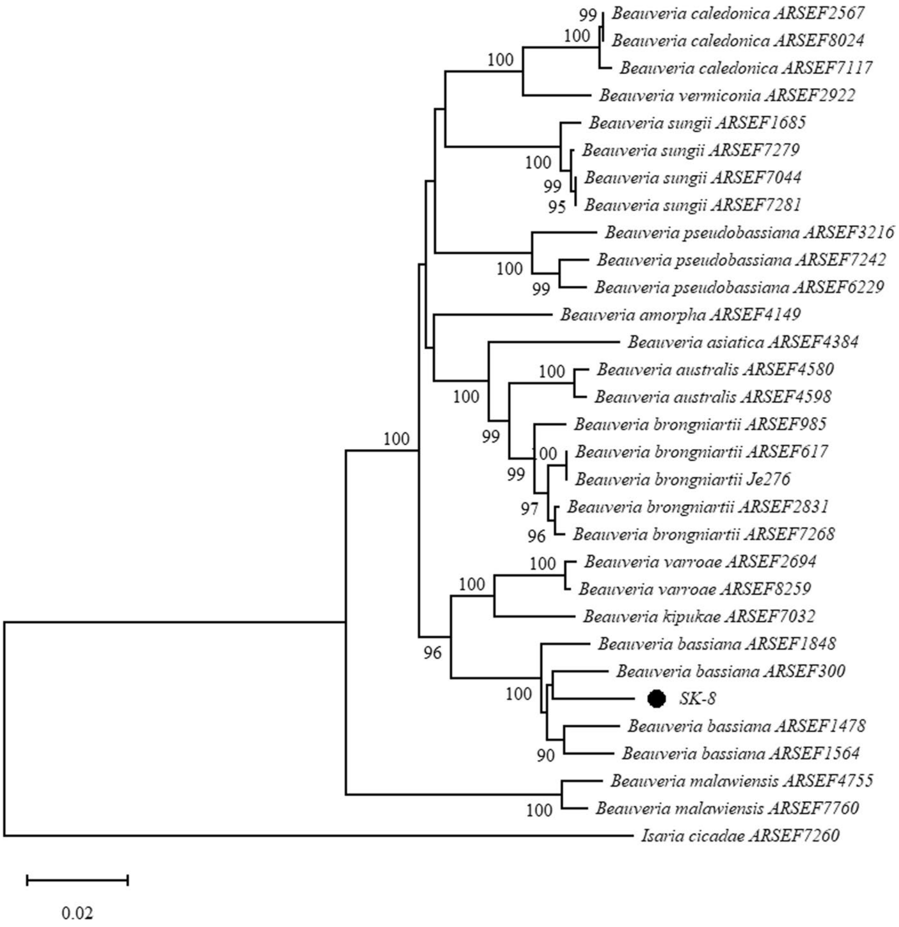
The data from the virulence tests were corrected using the Abbott formula and percent mortalities were calculated (Abbott 1925). In addition, the percent mycosis values were calculated as described above. One-way ANOVA followed by LSD post-hoc test was used to compare fungal isolates with each other in terms of mortality and mycoses. Before performing ANOVA, all data were evaluated in terms of variance homogeneity using Levene statistics, and all percentage data were subjected to arcsin transformation. Calculation of LC_{50} and LC_{90} values were performed by probit analysis. Pearson's χ^2 statistic for goodness-of-fit test was then calculated to evaluate a significant fit between the observed and expected regression models. All data obtained were analyzed using SPSS 16.0 statistical software.

Results

Twenty-four fungal isolates were obtained from 43 soil samples and 55.8% of soil samples were positive with respect to the presence of entomopathogenic fungi. Localities, geographic coordinates and GenBank accession numbers for all isolates are given in Supplementary Table S1. Based on their colony morphologies and macroscopic characters, all isolates were placed in two genera as *Beauveria* and *Metarhizium*. ITS gene sequence analysis also confirmed the morphological characterization and the isolates were identified as *Beauveria bassiana* (SK-1, SK-5, SK-8, SK-14, SK-16, SK-17, SK-28, SK-40, and SK-45) and *Metarhizium* sp. (SK-3, SK-9, SK-10, SK-12, SK-15, SK-21, SK-22, SK-24, SK-27, SK-29, SK-37, SK-42, SK-47, SK-49, and SK-50) (Supplementary Table S2). Recent phylogenetic studies on *Beauveria* and *Metarhizium* genera showed that some species in these genera are morphologically similar but phylogenetically distinct (Rehner et al. 2011; Bischoff et al. 2009). To differentiate these similar species, different gene regions other than ITS should be used in phylogenetic analysis. Therefore, *bloc*, *rpb1* and *tef* gene sequences were used for further characterization of the isolate SK-8 which was the most effective isolate in the screening tests and used in the field trial. Isolate SK-8 was compared with the reference strains in the study of Rehner et al. (2011) and the concatenated tree generated using *bloc*, *rpb1* and *tef* gene sequences showed that the isolate SK-8 was identical to *B. bassiana* (Fig. 1).

In the screening test against adults, significant differences were found amongst isolates and the highest mortalities were obtained from *B. bassiana* SK-1, SK-5, SK-8, SK-14, SK-16, SK-17, SK-28, SK-45 and *Metarhizium* sp. SK-9, SK-10, SK-15, SK-21, SK-22, SK-24, SK-27, SK-29, SK-47, SK-49 (F24,50 = 6.85, $p < 0.001$). Among these, nine isolates (*B. bassiana* SK-1, SK-8, SK-16, SK-17, SK-28 and *Metarhizium* sp. SK-10, SK-22, SK-24, and SK-49) caused mortalities ranging from 96 to 83% and they were significantly different from the control (F24,50 = 6.85, $p < 0.001$). The other isolates caused different mortalities (F24,50 = 6.85, $p < 0.001$) and they were not different from the control. In terms of mycosis, significant differences were found amongst isolates (F24,50 = 11.11, $p < 0.001$). The highest mycosis values were

Fig. 1 The concatenated tree showing the phylogenetic position of the isolate SK-8 and the reference strains in the study of Rehner et al. (2011) based on the concatenated sequences of *bloc*, *rpb1* and *tef* gene regions. The tree was constructed using neighbor-joining (N-J) analysis with p-distance correction. The bootstrap analysis was based on 1.000 pseudoreplicates and bootstrap values with > 70% are indicated. The solid black circle indicates isolate SK-8. The scale at the bottom represents genetic distances in nucleotide substitutions per site



obtained from *B. bassiana* SK-5, SK-8, SK-16, SK-17, and SK-28 (F24,50 = 11.11, $p < 0.001$) and they were significantly different from the control. The other isolates caused different mycosis values (F24,50 = 11.11, $p < 0.001$) and they were not different from the control (Fig. 2).

In the screening test against 3rd and 4th instar larvae, significant differences were found amongst isolates. The highest mortalities were obtained from *B. bassiana* SK-1, SK-5, SK-8, SK-14, SK-16, SK-17, SK-28, SK-40, SK-45 and *Metarhizium* sp. SK-3, SK-10, SK-12, SK-15, SK-24, SK-27, SK-29, SK-37, SK-42, SK-47, SK-49, SK-50 (F24,50 = 5.82, $p < 0.001$), ranging from 100 to 63% and all of them were different from the control, except for SK-5, SK-9, SK-21 and SK-22. The other isolates caused different mycosis values (F24,50 = 5.82, $p < 0.001$) and they were not different from the control. In terms of mycosis, significant differences were found amongst isolates (F24,50 = 7.11, $p < 0.001$). Nine isolates (*B. bassiana* SK-1, SK-8, SK-16, SK-17, SK-28, SK-40, SK-45 and *Metarhizium* sp. SK-3, SK-27) caused the highest mycosis values and four of them (SK-8, SK-17, SK-28, and SK-45) were significantly different from the control (F24,50 = 7.11, $p < 0.001$). The other isolates caused different mycosis values (F24,50 7.11, $p < 0.001$) and they were not different from the control (Fig. 3).

LC_{50} values for isolate SK-8 in the outdoor tests were estimated as 3.42×10^6 and 1.15×10^7 conidia ml^{-1} for adults and larvae, respectively. LC_{90} values were estimated as 1.12×10^9 and 4.08×10^{10} conidia ml^{-1} for adults and larvae, respectively (Table 1).

Discussion

All fungal isolates examined had some degree of pathogenicity for both larvae and adult CPB, with considerable variability in response to the one dose used (1×10^7 conidia ml^{-1}). Several isolates caused 80% or greater mortality under the conditions of the assays. Fungal outgrowth and sporulation were also variable among the isolates. The isolate selected for outdoor evaluation on sprayed, insect infested plants, demonstrated good efficacy. Storch and Dill (1987) tested *B. bassiana* (5×10^{12} and 5×10^{13} CFU ha^{-1}) against CPB in the field and concluded that adequate control of *L. decemlineata* in Maine using *B. bassiana* may be possible considering defoliation rate, average yield of tubers and the number of Colorado

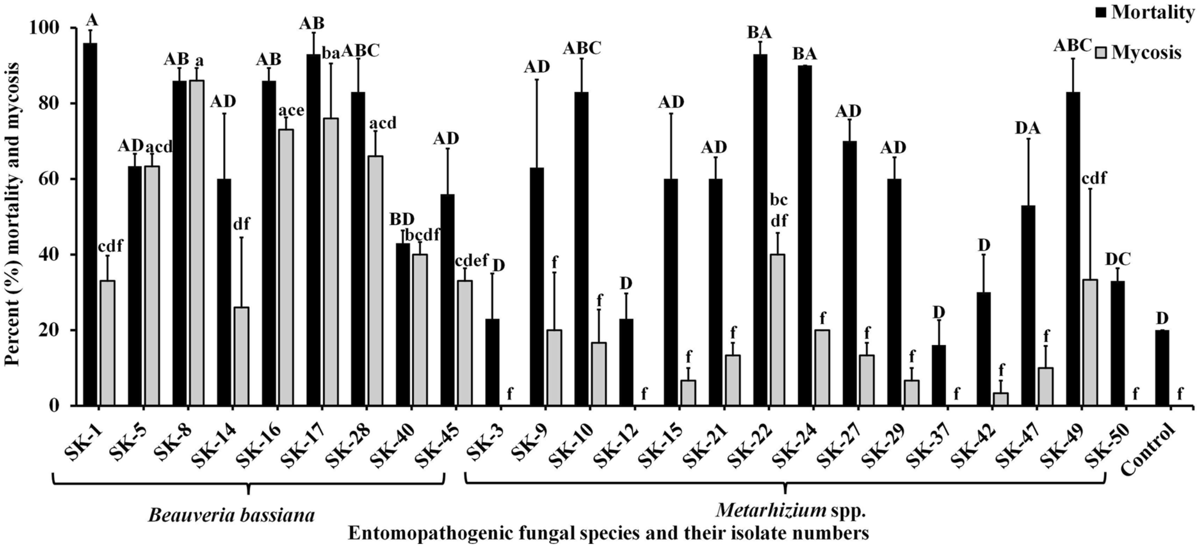


Fig. 2 Percent mortality (+ SE) and mycosis of CPB adults after exposure of different fungal isolates obtained from soil samples in potato fields within 15 days. 1×10^7 conidia ml^{-1} spore suspensions were applied to adults. Mortality values was calculated using the Abbott's formula (Abbott 1925). The different uppercase and lowercase letters represent the statistical difference among isolates with respect to mortality and mycosis, respectively, according to LSD multiple comparison test ($p < 0.001$). 0.01% Tween 80 was used as the control group

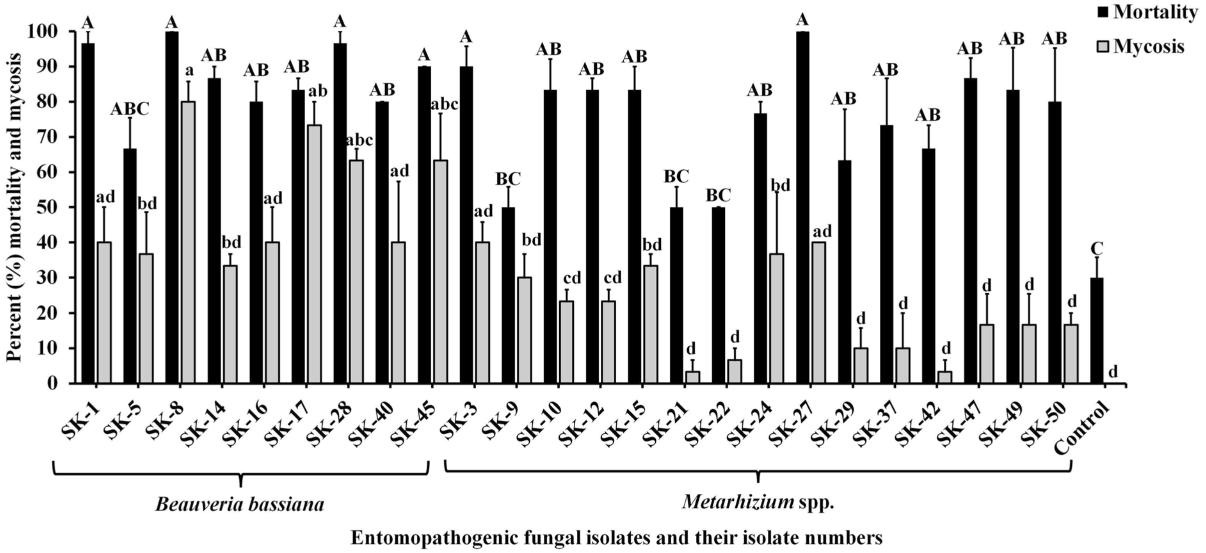


Fig. 3 Percent mortality (+ SE) and mycosis of CPB larvae after exposure of different fungal isolates obtained from soil samples in potato fields within 15 days. 1 × 10^7 conidia ml^{-1} spore suspensions were applied to larvae. Mortality values was calculated using the Abbott's formula (Abbott 1925). The different uppercase and lowercase letters represent the statistical difference among isolates with respect to mortality and mycosis, respectively, according to LSD multiple comparison test ($p<0.001$). 0.01% Tween 80 was used as the control group

Table 1 Summary of probit analysis parameters from the virulence bioassays performed with different doses of *Beauveria bassiana* isolate SK-8 against adult and larvae of CPB under field conditions

Development stage	Intercept ± SE	Slope ± SE[a]	LC_{50} (95% fiducial limits)	LC_{90} (95% fiducial limits)	Pearson goodness of fit test[b]		
					χ2	*df*	p
Adult	− 3.326 ± 0.541	0.509 ± 0.086	3.42×10^6 (1.25×10^6–1.15×10^7)	1.12×10^9 (1.73×10^8–3.74×10^{10})	1.045	3	> 0.05
Larvae	− 2.550 ± 0.509	0.361 ± 0.080	1.15×10^7 (2.8×10^6–1.21×10^8)	4.08×10^{10} (1.49×10^9–1.36×10^{14})	0.665	3	> 0.05

[a] Slope of the concentration response of adult and larvae of CPB to *B. bassiana* isolate SK-8

[b] Pearson χ2 goodness-of-fit test on the probit model. There is no significant difference between the observed and expected regression models ($p>0.05$)

potato beetle egg masses and adults. Poprawski et al. (1997) applied the unformulated conidia of *B. bassiana* (5 × 10^{13} viable conidia ha^{-1}) as four rapid (at three -to four day intervals) and early season (at the green row and touch in row growth stages of potato) foliar applications. They determined the rate of mycosis in larval populations at > 90% two days after the last *B. bassiana* application. Öztürk et al. (2015) tested four different entomopathogenic fungi, three of which were *B. bassiana,* on 2nd and 3rd instar larvae, 4th instar larvae and adults of CPB by spray and leaf dipping methods using the conidial concentration of 1 × 10^8 conidia ml^{-1}. In both application methods, all three isolates reached 100% mortality against 2nd, 3rd and 4th larval instars within seven days while the highest mortalities were 86.2% for spray method and 69% for leaf dipping method against adults within the same time. Puza et al. (2021) applied *B. bassiana* (1.72 × 10^{11} spores per plot (25.2 m^2)) against CPB in the field and they determined that the fungus reduced the number of emerging adults by 30% compared to the control sites within 14 days. Baki et al. (2021)

tested 14 different indigenous isolates of *B. bassiana* (1×10^7 conidia ml^{-1}) against different developmental stages of CPB under laboratory conditions and stated that four isolates were highly virulent causing mortalities between 91.7 and 100% in larvae and between 93.3 and 96.7% in adults within nine days. In addition, these four isolates had the most egg hatching inhibitory effects. To improve the efficacy of *B. bassiana*, Anderson et al. (1989) tested *B. bassiana* on CPB with five insecticide formulations and found that the fungus and insecticides (abamectin, triflumuron, thuringiensin, carbaryl and fenvalerate) were more effective than their use alone when used together. Similarly, Furlong and Groden (2001) determined a synergy between *B. bassiana* and imidacloprid. Also, Wraight and Ramos (2005) combined *B. bassiana* with Btt (*Bacillus thuringiensis* subsp. *tenebrionis*) and showed that the combination of the fungus and bacterium provided a significant reduction in larval populations of CPB. All these studies show that *B. bassiana* can have good potential in the control of CPB. *B. bassiana* SK-8, which was evaluated in this study, and was shown to be effective on larvae and adults of CPB under both laboratory and field conditions. The choice and application of native isolates may reduce future environmental impacts formed by selection pressure when new species of an organism are introduced into an environment. Moreover, native isolates might be adapted to local climatic conditions and can survive local conditions (Bilgo et al. 2018). For this reason, Turkish isolate *B. bassiana* SK-8 seems to be a good candidate for further studies in the control of CPB.

It is interesting to mention that the isolate SK-8 scored promising LC$_{50}$ (1.15×10^7 and 3.42×10^6 conidia ml^{-1}) and LC$_{90}$ (4.08×10^{10} and 1.12×10^9 conidia ml^{-1}) values against both larvae and adults of CPB compared to commercially formulated *B. bassiana* [Botanigard® 22WP (Lam International Co., Butte, MT, USA)] which is registered for use in the USA and other regions on potatoes production. Botanigard® 22WP contains 4.4×10^{13} conidia kg^{-1}. The label of this product states maximum concentration of the *Beauveria* formulation 62.5 g per 100 l, which equals 2.75×10^7 conidia ml^{-1}, with a maximum application rate of 1.500 l spray ha^{-1}.

Entomopathogenic fungal isolates represent different genotypes under the different field conditions, and these genotypes can interact with each other, host populations, and their environment (Meyling and Eilenberg 2007). In this sense, it is possible to say that the populations of *B. bassiana* and *Metarhizium* spp. are affected by both abiotic and biotic factors in the habitat, separated into different genetic groups according to these factors and adapted to the particular environmental conditions. For instance, Bidochka et al. (2002) showed that a genetic group of *B. bassiana* was associated with agricultural areas, two groups were associated with forest habitats, and the last group was associated with Canadian Arctic. In the same study, certain relationships such as growth at different temperatures and UV resistance were found between different groups of *B. bassiana.* In addition, Bidochka et al. (2001) showed that the same relationship among *B. bassiana* populations was also found in *M. anisopliae* populations in Canada. A similar study was conducted between different species of *Metarhizium* spp. and a high genetic variability between *Metarhizium* spp. isolates was detected in terms of conidial thermotolerance. *M. anisopliae* var. *anisopliae* and *M. flavoviridae* isolates were shown to be more sensitive to heat than *M. anisopliae* var. *acridum* isolates. Conversely, in the same study, many *Metarhizium* spp. isolates were inactive at low temperatures (Fernandes et al. 2010). Maurer et al. (1997) studied the genetic diversity of 38 *B. bassiana* isolates obtained from different geographical regions and insect orders. They determined that *B. bassiana* isolates was divided into different groups according to their host range. Wang et al. (2005) showed a certain relationship among *B. bassiana*, obtained from different geographical regions with respect to their geographical origin. Fernandes et al. (2009) also showed a clear genomic difference between Brazilian and USA *B. bassiana* isolates and larger geographical distances were associated with higher genetic distances. More importantly, even local populations of entomopathogenic fungi can be separated into different genetic groups according to their local habitats. For example, Meyling et al. (2006) determined that only a certain group of *B. bassiana* was found in organic farming areas in Denmark, while the other five groups existed in hedgerows adjacent to these farmlands and were genetically separated. Considering all these studies, it might be important to obtain indigenous biological control agents adapted to a specific or local environment, and the use of indigenous isolates may be more effective than non-local isolates, cost considerations aside (Alfiky 2022; Klingen et al. 2015; Sevim et al. 2010a). In this study, entomopathogenic fungi (especially *B. bassiana* and *Metarhizium* spp.) were widely found in soils of potato fields (55.8%). Considering that the isolates obtained from this study could be adapted to both biotic and abiotic factors in the environment in which they survive, it should be advantageous to use them against potato pests, especially CPB, in the study region under both inoculative and conservation biological control strategies. Moreover, after application of the fungus on a large scale, the fungus may survive in the soil environment for a long time, and this should be advantageous against soil-dwelling larvae and overwintering adults. But it should be noted that fungal persistence in the soil is very variable and can be affected by many factors (Jaronski 2010).

Consequently, various entomopathogenic fungi were isolated from soil samples in potato fields and they were tested against adults and larvae of CPB under laboratory conditions. The most effective isolate was determined to be *B. bassiana* SK-8 and it was further characterized by multilocus phylogeny using *bloc*, *rpb1* and *tef* gene sequences and its efficacy was evaluated in field trials. As a result, the indigenous isolate *B. bassiana* SK-8 appears to be a promising agent in the control of CPB. However, further studies such as horizontal transmission, the susceptibility to certain environmental factors and predisposition to mass production should be performed.

Acknowledgements Not applicable

Author contributions AS conceived and supervised the study, designed experiments, and edited the manuscript; SK performed fungal isolation and preparation of stock cultures; SK and AB performed fungal identification and initial screening tests; SK, AB, AK, and TÇ performed the outdoor tests; SK collected the data; AS performed all analyses of the related data, wrote the manuscript, and revised it. All authors read and approved the final version of the manuscript.

Funding This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under the project application number of 1919B012001809.

Data availability Data and materials are available upon request.

Declarations

Conflict interest The authors have no conflict of interest to declare.

Ethical approval There are no ethical concerns about this publication.

Research involving human and animal rights This article does not contain any studies with human participants or animals (vertebrate) performed by any of the authors.

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Alfiky A (2022) Screening and identification of indigenous entomopathogenic fungal isolates from agricultural farmland soils in Nile Delta. Egypt J Fungi 8(1):54

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Alyokhin A, Baker M, Mota-Sanchez D, Dively G, Grafius E (2008) Colorado potato beetle resistance to insecticides. Am J Potato Res 85:395–413

Alyokhin A, Udalov M, Benkovskaya G (2013) The Colorado potato beetle. In: Alyokhin A, Vincent C, Giordanengo P (eds) Insect pests of potato: Global perspectives on biology and management. Academic Press, Oxford, pp 11–29

Anderson TE, Hajek AE, Roberts DW, Preisler HK, Robertson JL (1989) Colorado potato beetle (Coleoptera: Chrysomelidae): effects of combinations of *Beauveria bassiana* with insecticides. J Econ Entomol 82(1):83–89

Baki D, Tosun HS, Erler F (2021) Efficacy of indigenous isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Egypt J Biol Pest Control 31:56

Balaško KM, Mikac KM, Bažok R, Lemic D (2020) Modern techniques in Colorado potato beetle (*Leptinotarsa decemlineata* Say) control and resistance management: history review and future perspectives. Insects 11(9):581

Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW (2012) GenBank. Nucleic Acids Res 40(Database issue):D48–D53

Bidochka MJ, Kamp AM, Lavender TM, Dekoning J, De Croos JNA (2001) Habitat association in two genetic groups of the insect-pathogenic fungus *Metarhizium anisopliae*: uncovering cryptic species? Appl Environ Microbiol 67:1335–1342

Bidochka MJ, Menzies FV, Kamp AM (2002) Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. Arch Microbiol 178:531–537

Bilgo E, Lovett B, St Leger RJ, Sanon A, Dabire RK, Diabate A (2018) Native entomopathogenic *Metarhizium* spp. from Burkina Faso and their virulence against the malaria vector *Anopheles coluzzii* and non-target insects. Parasites Vectors. https://doi.org/10.1186/s13071-018-2796-6

Bischoff JF, Rehner SA, Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101(4):512–530

Butt TM, Coates CJ, Dubovskiy IM, Ratcliffe NA (2016) Entomopathogenic fungi: new insights into host-pathogen interactions. Adv Genet 94:307–364

Capinera JL (2001) Handbook of vegetable pests. Academic Press, San Diego

Correy GJ, Zaidman D, Harmelin A, Carvalho S, Mabbitt PD, Calaora V, James PJ, Kotze AC, Jackson CJ, London N (2019) Overcoming insecticide resistance through computational inhibitor design. Proc Natl Acad Sci USA 116(42):21012–21021

de Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Cont 43:237–256

De Wilde J, Hsiao TH (1981) Geographic diversity of the Colorado potato beetle and its infestation in Eurasia. In: Lashomb JH, Casagrande RA (eds) Advances in potato pest management. Hutchinson Ross Publishing Company, Pennsylvania, pp 47–68

Fernandes É, Moraes Á, Pacheco R, Rangel D, Miller M, Bittencourt V, Roberts D (2009) Genetic diversity among Brazilian isolates of *Beauveria bassiana*: comparisons with non-Brazilian isolates and other *Beauveria* species. J Appl Microbiol 107:760–774

Fernandes EK, Keyser CA, Chong JP, Rangel DE, Miller MP, Roberts DW (2010) Characterization of *Metarhizium* species and varieties based on molecular analysis, heat tolerance and cold activity. J Appl Microbiol 108(1):115–128

Furlong MJ, Groden E (2001) Evaluation of synergistic interactions between the Colorado potato beetle (Coleoptera: Chrysomelidae) pathogen *Beauveria bassiana* and the insecticides, imidacloprid, and cyromazine. J Econ Entomol 94(2):344–356

Grafius E (1997) Economic impact of insecticide resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae) on the Michigan potato industry. J Econ Entomol 90:1144–1151

Grafius EJ, Douches DS (2008) The present and future role of insect-resistant genetically modified potato cultivars in IPM. In: Romeis J, Shelton AM, Kennedy GG (eds) Integration of insect-resistant genetically modified crops within IPM programs: Progress in biological control. Springer, Dordrecht, pp 195–221

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nuc Acids Symp 41:95–98

Humber RA (1997) Entomopathogenic fungal identification. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, London, pp 153–185

Jacques RL (1988) The Potato beetles: The genus *Leptinotarsa* in North America. E.J. Brill, New York

Jaronski ST (2010) Ecological factors in the inundative use of fungal entomopathogens. BioControl 55(159):185

Klingen I, Westrum K, Meyling NV (2015) Effect of Norwegian entomopathogenic fungal isolates against *Otiorhynchus sulcatus* larvae at low temperatures and persistence in strawberry rhizospheres. Biol Cont 81:1–7

Lacey LA (2008) Microbial control of insects. In: Capinera JL (ed) Encyclopedia of entomology. Springer, Dordrecht

Lacey LA, Kroschel J, Wraight SP, Goettel MS (2009) An introduction to microbial control of insect pests of potato. Fruit Veg Cereal Sci Biotechnol 3(1):20–24

Mascarin GM, Jaronski ST (2016) The production and uses of *Beauveria bassiana* as a microbial insecticide. World Microbiol Biotechnol 32(11):177

Maurer P, Couteaudier Y, Girard PA, Bridge PD, Riba G (1997) Genetic diversity of *Beauveria bassiana* and relatedness to host insect range. Mycol Res 101(2):159–164

Meyling NV, Eilenberg J (2006) Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agr Ecosyst Environ 113:336–341

Meyling NV, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol Control 43:145–155

Öztürk HE, Güven Ö, Karaca I (2015) Effects of some bioinsecticides and entomopathogenic fungi on Colorado potato beetle (*Leptinotarsa decemlineata* L.). Commun Agric Appl Biol Sci 80(2):205–211

Petek M, Coll A, Ferenc R, Razinger J, Gruden K (2020) Validating the potential of double-stranded RNA targeting Colorado potato beetle *Mesh* gene in laboratory and field trials. Front Plant Sci 19(11):1250

Poprawski TJ, Carruthers RI, Speese J III, Vacek DC, Wendel LE (1997) Early-season applications of the fungus *Beauveria bassiana* and introduction of the hemipteran predator *Perillus bioculatus* for control of Colorado potato beetle. Biol Cont 10(1):48–57

Půža V, Nermuť J, Konopická J, Skoková Habuštová O (2021) Efficacy of the applied natural enemies on the survival of Colorado potato beetle adults. Insects 12(11):1030

Rajula J, Karthi S, Mumba S, Pittarate S, Thungrabeab M, Krutmuang P (2021) Current status and future prospects of entomopathogenic fungi: A potential source of biopesticides. In: De Mandal S, Passari AK (eds) Recent advancement in microbial biotechnology. Academic Press, Oxford, pp 71–98

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98

Rehner SA, Posada F, Buckley EP, Infante F, Castillo A, Vega FE (2006) Phylogenetic origins of African and Neotropical *Beauveria bassiana s.l.* pathogens of the coffee berry borer *Hypothenemus hampei*. J Invertebr Pathol 93:11–21

Rehner SA, Minnis AM, Sung GH, Luangsa-ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103(5):1055–1073

Scott IM, Tolman JH, MacArthur DC (2015) Insecticide resistance and cross-resistance development in Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) populations in Canada 2008–2011. Pest Manag Sci 71:712–721

Sevim A, Demir I, Demirbağ Z (2010a) Molecular characterization and virulence of *Beauveria* spp. from the Pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). Mycopathologia 170:269–277

Sevim A, Demir I, Höfte M, Humber RA, Demirbağ Z (2010b) Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. BioControl 55:279–297

Sevim A, Demir I, Tanyeli E, Demirbag Z (2010c) Screening of entomopathogenic fungi against the European spruce bark beetle, *Dendroctonus micans* (Coleoptera: Scolytidae). Biocontrol Sci Technol 20:3–11

Sorokan AV, Burkhanova GF, Benkovskaya GV, Maksimov IV (2019) Colorado potato beetle microsymbiont *Enterobacter* BC-8 inhibits defense mechanisms of potato plants using crosstalk between jasmonate- and salicylate-mediated signaling pathways. Arthr Plant Interact 14:161–168

Sorokan A, Cherepanova E, Burkhanova G, Veselova S, Rumyantsev S, Alekseev V, Mardanshin I, Sarvarova E, Khairullin R, Benkovskaya G, Maksimov I (2020) Endophytic *Bacillus* spp. as a prospective biological tool for control of viral diseases and non-vector *Leptinotarsa decemlineata* Say. in *Solanum tuberosum* L. Front Microbiol 15(11):569457

Stiller JWB, Hall D (1997) The origin of red algae: Implications for plastide volution. Proc Natl Acad Sci 94:4520–4525

Storch RH, Dill JF (1987) *Bauveria bassiana* for control of Colorado potato beetle (Coleoptera: Chrysomelida) in Maine. Tech Bull-Me Agric Exp Stn 128:1–8

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 38(7):3022–3027

Wang S, Miao X, Zhao W, Huang B, Fan M, Li Z, Huang Y (2005) Genetic diversity and population structure among strains of the entomopathogenic fungus, *Beauveria bassiana*, as revealed by inter-simple sequence repeats (ISSR). Mycol Res 109(12):1364–1372

Wang C, Hawthorne D, Qin Y, Pan X, Li Z, Zhu S (2017) Impact of climate and host availability on future distribution of Colorado potato beetle. Sci Rep 7(1):4489

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: A guide to methods and applications. Academic Press, New York, pp 315–322

Wraight SP, Ramos ME (2002) Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata*. Biol Cont 23(2):164–178

Wraight SP, Ramos ME (2005) Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. J Invertebr Pathol 90(3):139–150

Wraight SP, Ramos ME (2015) Delayed efficacy of *Beauveria bassiana* foliar spray applications against Colorado potato beetle: impacts of number and timing of applications on larval and next-generation adult populations. Biol Cont 83:51–67

Zimmermann G (1986) The ''*Galleria* bait method'' for detection of entomopathogenic fungi in soil. Z Angew Entomol 102:213–215

Wan H (2003) Molecular biology of the entomopathogenic fungus *Beauveria bassiana*: Insect-cuticle degrading enzymes and development of a new selection marker for fungal transformation, Dissertation, University of Heidelberg

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