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ORIGINAL RESEARCH ARTICLE

Phylogeny of indigenous *Beauveria bassiana* isolates from *Leptinotarsa decemlineata* in Türkiye and their biocontrol potential

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Abstract

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), is a serious pest of potatoes and other solanaceous plants such as eggplant, tobacco and tomato. Chemical insecticides are the main management tool for CPB, but it has been reported that resistance to the insecticides can develop rapidly. Therefore, it is important to seek indigenous biocontrol agents and develop environmentally friendly control methods against this pest. In this study, 13 entomopathogenic fungi (11 from adults and 2 from larvae) were isolated from the field collected CPB in Kırşehir, Türkiye and they were identified as *Beauveria bassiana* based on *bloc* gene sequence data. These *B. bassiana* isolates were also tested against CPB larvae and adults under laboratory conditions. The highest mortality and mycoses values against both larvae and adults were obtained from *B. bassiana* LD-3 and LD-9 with 90, 93.33% and 56.66, 86.66% for larvae and 96.66, 93.33% and 93.33, 90% for adults, respectively. Some of these *B. bassiana* isolates are promising candidates for inundative biological control of CPB in Türkiye, but field testing is required to further elucidate their biocontrol potential.

Keywords CPB · Potato · Biocontrol · *Beauveria bassiana* · Phylogeny

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is one of the most important pests of potato and other solanaceous crops worldwide. Adults and larvae are voracious feeders and, when left unmanaged, populations can completely defoliate potatoes and other crops, resulting in yield loss (Kadoić Balaško et al. 2020; Kekillioğlu and Yılmaz 2018; Cingel et al. 2016). Management of CPB is particularly complicated because this pest can feed on locally abundant solanaceous plants. Chemical insecticides can successfully manage CPB populations in potato fields, but this pest has developed resistance to these insecticides from various chemical groups over time (Chen et al. 2023). Today, more than 300 cases of insect resistance have been documented, according to data from Asia, Europe, and North America (Rondon et al. 2021; Chen et al. 2023). Additionally, the abundant use of chemical pesticides brings potential risks to the environment, wildlife, and human health (Aktar et al. 2009). Therefore, there is an urgent need to develop safe, alternative control strategies for pests like CPB in potato.

Apart from the use of chemical insecticides, management strategies such as biological control, biotechnological control such as the use of transgenic crops, cultural control, mechanical control, and crop rotation have been developed for management of CPB (Göldel et al. 2020; Timani et al. 2023; Malekmohammadi 2014). There is evidence that natural enemies, including predators, parasitoids and entomopathogens, can help to manage CPB populations in integrated pest management (IPM) programs (Göldel et al. 2020). Many potato growers still rely on chemical insecticides. Adoption of biocontrol practices is lagging, and one explanation is that the speed of CPB suppression is not sufficient. Incorporating bioinsecticides into IPM programs for CPB is viewed as one solution to help slow the development of insecticide resistance while also improving environmental safety (Malekmohammadi 2014). In this respect, it is still desirable to identify safer and more effective biocontrol

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agents that are compatible with the current management practices for CPB in potato.

Among the microbial insecticides used for CPB, products including entomopathogenic fungi (EPFs) (especially *Beauveria bassiana* (Bals. - Criv.) Vuill. et al. (1912) and bacteria (especially *Bacillus thuringiensis* (*Bt*) are the most common and widespread (Göldel et al. 2020). In contrast to *Bt*, *B. bassiana* can provide effective control for both adults and larvae of CPB because this fungus can infect all developmental stages of the pest, survive, and propagate in the soil for a long time after application in the field, providing an effective control, especially during the entire potato production season (Göldel et al. 2020; Wraight and Ramos et al., 2005). In addition, EPFs have many advantages in terms of biological control of insect pests. For instance, they are relatively easy to produce, do not need to be ingested by their hosts because the infection starts on the host cuticle, there is no identified resistance development in any target pest, they have no or very few side effects on non-target organisms, they can be listed as providing long-term control and being suitable for biotechnological development (Zemek et al. 2021; Sevim et al. 2015). In addition, synergistic combinations of entomopathogenic fungi with other microbial control agents or chemical insecticides are proven in some studies and well expected to happen in the future (Lacey et al. 2001; Rice and Furlong 2023). Today, more than 150 commercial mycoinsecticides based on entomopathogenic fungi have been developed, of which more than 75% are based on the hypocrealean fungi such as *Metarhizium anisopliae* (Metch) Sorok, *Beauveria bassiana*, *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (formerly known as *Isaria fumosorosea* Wize, and *B. brongniartii* (Saccardo) Petch (Faria and Wraight 2007).

When any biological control agent (especially EPFs) is to be used against a target pest, it may be necessary to consider not only the degree of pathogenicity but also the environmental and ecological requirements and limitations (Jackson et al. 2010). Especially in field applications, EPFs are affected by a variety of factors such as solar radiation (UV), rain, temperature, humidity, surface chemistry, and phylloplane microbiota (Wu et al. 2020). In addition, habitat, agricultural practices, and many unexpected factors in various layers and compartments of the existing ecosystem have led to evolutionary genotype differentiation in entomopathogenic fungi (Meyling and Eilenberg 2007). In this sense, considering that entomopathogenic fungi can adapt to various environmental factors in the existing ecosystem and especially to the target pest, it is important to use native isolates in microbial control (Bilgo et al. 2018). It is thought that this approach might increase the chances of survival and longer-term control of the mycoinsecticide after field application against the target pest.

Therefore, the first objective of the study was to isolate various local entomopathogenic fungi from the field collected CPB adults and larvae in Kırşehir, Türkiye and to molecularly characterize them using *Bloc* gene sequence and phylogenetic analysis. The second objective was to evaluate the virulence of native *B. bassiana* isolates against wild-caught adults and larvae of CPB under the controlled laboratory conditions. Some of the isolates seem to have a good potential for controlling CPB.

Materials and methods

Collection of insect samples

The adults and larvae of CPB were collected from Kırşehir province, Türkiye in the spring and summer of 2022. A total of 678 insect samples (348 adults and 330 larvae belongings to different instar) were randomly collected from different locations (12), 122 adults and 94 larvae from the second generations in the summer (Table 1). The collected insects were inspected in the field with respect to any fungal infection and the suspected ones were separately brought to the laboratory. For the others, adults and larvae were separately put into plastic boxes (40 × 50 × 20 cm) with freshly collected potato leaf and brought to the laboratory and fed for up to two weeks. Finally, during this time, the samples were examined, and the samples suspected of fungal infection were separated and used for fungal isolation.

Fungal isolation

The fungi were isolated by an inoculation needle from the insect samples showing external fungal growth in the field. The other suspected samples, which were found dead in the laboratory, were first surface sterilized with 10% sodium hypochlorite for 5 min and then rinsed in 96% ethanol for 1 min. After that, they were twice washed with sterile distilled water and put into moisture chamber to promote fungal growth. After that, the fungi growing on the insects were isolated by an inoculation needle. All fungi were first inoculated into three different places on PDAY (Potato Dextrose Agar + 1% Yeast extract) containing 50 µg/ml tetracycline and 75 µg/ml ampicillin to prevent bacterial growth and incubated for two weeks in the dark. After adequate sporulation, all were purified and grown from single conidium by an inoculation loop on antibiotic free PDAY and stocked in 15% glycerol at -20ºC for further studies (Sevim et al. 2010a).

Table 1 *Beauveria bassiana* isolates from CPB larvae and adults collected from Kırşehir, Türkiye and their sources, infection status, locality, and GenBank accession numbers

No	Isolate	Species	Source	Infection status	Locality		GenBank accession numbers
					City	Village	Bloc
1	LD-1	*Beauveria bassiana*	Adults	Mycosed insect in the field	Kırşehir	Kesikköprü	PP790920
2	LD-2	*B. bassiana*	Adults	Mycosed insect in the field	Kırşehir	Mucur/Yörücek	PP790921
3	LD-3	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Karaboğaz	PP790922
4	LD-4	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Ulupınar	PP790923
5	LD-5	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Dedeli	PP790924
6	LD-6	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Karaboğaz	PP790925
7	LD-7	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Değirmenkaşı	PP790926
8	LD-8	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Akçaağıl	PP790927
9	LD-9	*B. bassiana*	Larvae	Mycosed insect in the laboratory	Kırşehir	Mucur/Rişvan	PP790928
10	LD-10	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Mucur/Karakuyu	PP790929
11	LD-11	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Kaman/Demirli	PP790930
12	LD-12	*B. bassiana*	Larvae	Mycosed insect in the laboratory	Kırşehir	Akpınar/Köşker	PP790931
13	LD-13	*B. bassiana*	Adults	Mycosed insect in the laboratory	Kırşehir	Akpınar/Çiftlik Mehmet Ağa	PP790932

Gene sequencing and species identification

All fungal isolates were first morphologically characterized according to the identification key of Humber (1997). After making sure that they are within *Beauveria* genus, they were molecularly characterized by gene sequencing and phylogenetic analysis. For this, the fungi were grown in 100 ml PDBY (Potato Dextrose Broth + 1% Yeast extract) for two weeks under 12:12 (L: D) photoperiod in orbital-shaker incubator at 150 rpm (SI-600R, Lab. Companion, Des Plaines, USA). After that, they were filtered through sterile two-layers of cheese cloth and the mycelium mass on the cloth was dried at 37ºC for 6 h. Then, they were crushed in liquid nitrogen by a mortar and pestle and 50 mg for each isolate was used for DNA extraction. Genomic DNAs were extracted using "Powersoil DNA isolation kit (MO BIO Laboratories, Carlsbad, CA, USA) in accordance with the recommendations of the manufacturer. All extracted DNAs were roughly quantified by agarose gel electrophoresis and were stored at -20°C until use.

Approximately 1.000 pb region of *Bloc* gene was amplified by PCR and sequenced. For this, the primer pairs of B5.1F (5'-CGACCCGGCCAACTACTTTGA-3') as forward and B3.1R (5'-GTCTTCCAGTACCACTACGCC-3') as reverse were used in PCR amplification (Rehner et al. 2006). The PCR conditions were as follows: initial activation of the *Taq* DNA polymerase for 10 min at 95 °C, followed by 35 cycles of 1 min denaturation at 95 °C, annealing for 1 min at 57ºC and extension for 1 min at 72 °C. The program ended with a final extension at 72ºC for 10 min. The PCR composition of a 50 µl mixture: 25 µl *Taq* 2× master mix (Ampliqon, Odense, Denmark), 25 mM $MgCl_2$, forward and reverse primer (10 µM for each), 19 µl PCR-grade H_2O and 1 µl template DNA. After PCR amplification, all PCR products were electrophoresed on 1.5% agarose gel at 90 V for 45 min with a 100 bp marker (New England Biolabs, MA, USA) and the correct bands were purified, quantified, and sent to Macrogen (The Netherlands) for sequencing. The sequenced DNAs were used for BLAST search to compare each isolate with the known species in GenBank (Benson et al. 2013). Finally, all *Beauveria* isolates were phylogenetically compared with the reference species/isolates indicated in the study of Rehner et al. (2011). The partial *Bloc* gene sequences of the fungal isolates were deposited in GenBank under accession numbers of PP790920-PP790932.

Virulence tests

All fungal isolates were tested against both larvae and adults of CPB under the controlled laboratory conditions. For this, all isolates, which were propagated from a single conidium, were spread on PDAY, and incubated at 28 °C in the dark for two weeks. After the incubation period, the harvesting of conidia for each isolate was performed by adding 10 ml of sterile 0.01% tween 80 onto the medium. After that adding tween 80, conidia were scraped with a glass cell spreader and the final solution was filtered through sterile two-layers of cheese cloth into 50 ml falcon conical tube to remove unwanted mycelium/agar pieces. The conidial concentration was adjusted to the 1×10^8 conidia/ml by a neubauer hemocytometer for each isolate and used for virulence tests. The viability of conidia was tested by spreading 100 µl of conidia suspension on PDAY and determining germination after a 24 h incubation. The germ tube which was greater

than spore diameter was considered as germinated. As a result, spores that germinate 90% or over was used in virulence tests (Sevim et al. 2010a).

Larvae and adults of CPB were collected from Kırşehir province, Türkiye for virulence tests. All collected insects were put into plastic boxes (40 × 50 × 20 cm) with fresh potato leaves and brought to the laboratory at the same day. They were fed in the laboratory for two days and healthy insects showing no disease symptoms were used in bioassays. Larvae and adult bioassays were separately performed. For both larvae and adults, 10 insects were put into plastic boxes (20 × 20 × 10 cm) with freshly collected potato leaves and treated with the conidial concentration (1×10^8 conidia/ml) by aerosol type sprayer for 3 s (approximately 3 ml) for each isolate. For each isolate, all experiments were repeated three times with 10 insects (larvae and adults separately). The control group was treated with only sterile 0.01% tween 80. Freshly collected potato leaves and branches were used as food and changed daily if necessary. All boxes were put into the climate cabinet (28 °C, 70% humidity and 12:12 (Light: Dark) light period) and incubated for 15 days. At the end of the incubation, dead larvae and adults were counted and the percentage mortalities were calculated. Separately, the percent mycoses values were calculated by performing surface sterilization, and then putting into the moisture chamber as described above. The insect samples showing external white fungal growth were evaluated as mycosed (Keçili et al. 2022; Sevim et al. 2010b).

Data analysis

The DNA sequences were edited and aligned with Bioedit (Hall 1999) and used for BLAST search in GenBank (Benson et al. 2013). The reference sequences from the study of Rehner et al. (2011) were downloaded from GenBank and the cluster analysis of all DNA sequences for the multiple sequence alignment was performed with Clustal W packed in Bioedit (Thompson et al. 1994). The data from this was used to construct phylogenetic tree using neighbor-joining (NJ) analysis with p-distance correction in MEGA 11.0.13 (Tamura et al. 2021). Alignment gaps were considered as missing data. The reliability of the dendrogram was tested with 1.000 pseudoreplicates by bootstrap analysis using MEGA 11.0.13.

Mortality data was corrected by Abbott's formula (Abbott 1925) and percent mycosis values were calculated. All fungal isolates were compared with each other by One-way Analysis of Variance (ANOVA) and Fisher's approach was used as post hoc test. Before performing ANOVA, all data was tested with respect to variance homogeneity using Levene statistics (Levene 1960), and all percentage (%) data (in case some of them come out 0 (zero) was subjected to arcsin transformation. In addition, the difference between larval and adult mortality with respect to the susceptibility to fungal infection was assessed by chi-square ($\chi 2$) test. All data was analyzed using Minitab 17 statistical software.

Results

Among the insect samples examined, only two adults (0.57% for adults and 0.29% for total infection rate) were found to be infected (mycosed) with white fungus (later identified as *B. bassiana*) in the field. Also, 11 insect samples (from adults and 2 from larvae) were detected as mycosed in the examinations after they were brought to the laboratory. When all infected samples detected in the field and laboratory are considered, a total of 13 isolates were obtained (11 from adults with 3.16% infection rate and 2 from larvae with 0.60% infection rate). The total infection rate was determined to be 1.91%. Based on the phylogenetic analysis using *Bloc* gene region, all experimental sequences were grouped with *B. bassiana* isolates retrieved from GenBank, with an 82% bootstrap support (Fig. 1).

According to the bioassay results for adults, all isolates caused different mortality values, and all were significantly different from the control ($F = 11.26$, $df = 13$, $p < 0.0001$). The highest mortalities were obtained from *B. bassiana* LD-3 and LD-9 with 96.66 and 93.33% ($F = 11.26$, $df = 13$, $p < 0.0001$). For larvae, all isolates caused different mortality values, and all were significantly different from the control ($F = 22.88$, $df = 13$, $p < 0.0001$). The highest mortalities were obtained from *B. bassiana* LD-3 and LD-9 with 90 and 93.33% ($F = 22.88$, $df = 13$, $p < 0.0001$) (Fig. 2).

For adults, all isolates also caused different mycoses values in comparison to each other and all of them were different from the control ($F = 13.44$, $df = 13$, $p < 0.0001$). The highest mycoses value was obtained from *B. bassiana* LD-3 and LD-9 with 93.33 and 90% ($F = 13.44$, $df = 13$, $p < 0.0001$). For larvae, all isolates also caused different mycoses values in comparison to each other and all of them were different from the control ($F = 18.21$, $df = 13$, $p < 0.0001$). The highest mycoses value was obtained from *B. bassiana* LD-6 and LD-9 with 70 and 86.66% ($F = 18.21$, $df = 13$, $p < 0.0001$) (Fig. 3).

Based on Pearson $\chi 2$ test, there was no significant difference between larval and adult mortality in terms of the susceptibility to fungal infection ($df = 72$, $p > 0.05$).

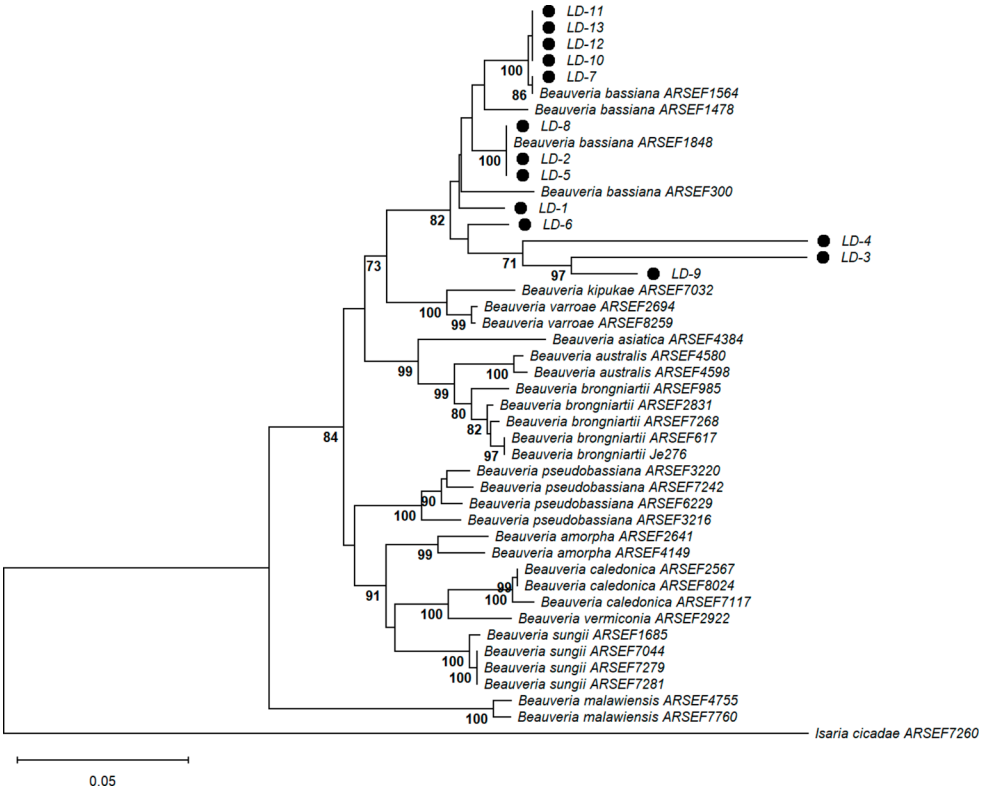

Fig. 1 The phylogenetic tree of *Beauveria* species including 13 white fungi obtained from this study. Reference species/isolates were taken from the study of Rehner et al. (2011). The phylogenetic analysis was based on the neighbor-joining (N-J) method with p-distance correction using the partial sequence of *Bloc* gene region. Numerical values (≥ 70) on branches are the bootstrap values with 1.000 replicates. *B. bassiana* isolates from this study were marked by a solid black circle. *Isaria cicadae* ARSEF7260 was used as an outgroup

Discussion

The popularity of mycoinsecticides has greatly increased, especially in the last two decades, in the control of insect and mite species in agriculture, forestry and veterinary entomology (Jaronski and Mascarin 2017). Twelve polyphyletic fungal species are available within the genus *Beauveria*, of which *B. bassiana* (Bals.) Vuill. and *B. pseudobassiana* S.A. Rehner & Humber are well-known species in terms of microbial control (Rehner et al. 2011; Romon et al., 2017). Among mycoinsecticides, however, *B. bassiana* is the most popular and commercially successful species (Hu et al. 2016). This fungus is known to show a broad spectrum of virulence at different levels against a wide range of insect and mite pests, however, its bio-efficacy greatly depends on many factors such as the degree of pathogenicity, the isolation source and location, life stages of the target pests and resistance to various environmental factors (Islam et al. 2023; Bugti et al. 2020). Besides, the use of native entomopathogenic fungal isolates against the target pest can provide a higher survival and persistence abilities under local ecological and environmental conditions (Islam et al. 2023). Moreover, within the framework of conservative biological control, the use of local isolates is of great importance to minimize contamination risks from imported biopesticides (Islam et al. 2023; Idrees et al. 2022). Therefore, in this study, it was aimed to isolate and characterize (both molecularly and pathogenically) potential native fungal isolates which can be used against CPB.

Different isolates of entomopathogenic fungi, even if they are placed in the same species, differ in virulence against the target pest (Valero-Jiménez et al. 2014). It has been shown that these differences in pathogenicity are related to the morphological, physiological, and genetic characteristics of isolates (hyphal growth rate, conidial viability, expression level of some virulence genes etc.) (Anderson et al. 2011; Varela and Morales 1996; Valero-Jiménez et al. 2014; Peng et al. 2024; Romon et al., 2017). Therefore, it is important to investigate the virulence of different isolates against the target pests because this will increase the potential for the development of EPFs as a biocontrol agent against insect pests, also allowing the selection of the most virulent isolate. In this study, we screened a total of 13 *B. bassiana* isolates against both larvae and adults of CPB with respect to their pathogenicity and found that two isolates (LD-3 and LD-9) had promising results and had the best mycoinsecticidal potential against CPB.

So far, many studies have been carried out on the use of various entomopathogenic fungi against CPB within the scope of microbial control, even some of them have been tested in the field and good results have been obtained (Keçili et al. 2022; Zemek et al. 2021; Wraight and Ramos 2002). In fact, today, some commercial preparations, and products such as Botanigard® 22WP (Lam International Co., Butte, MT, USA), specifically based on *B. bassiana*,

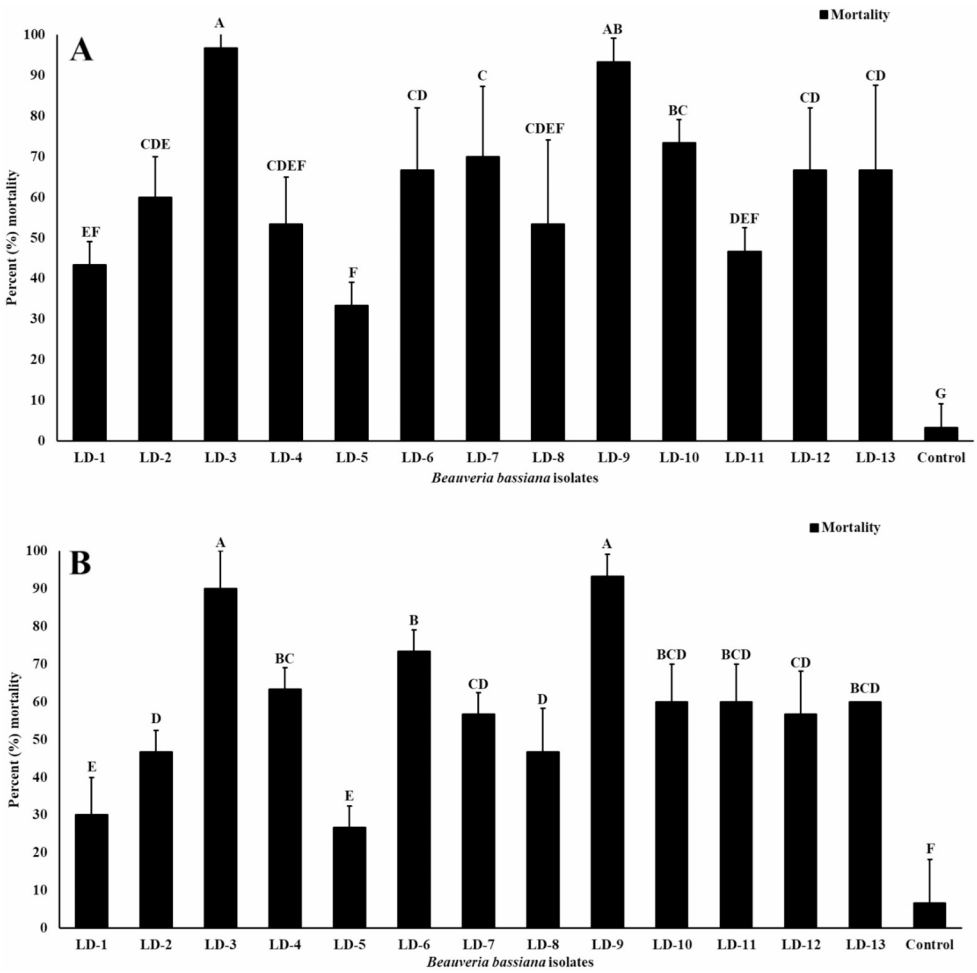

Fig. 2 Percent (%) mortalities (+SE) of *Beauveria bassiana* isolates against adults (**A**) and larvae (**B**) of CPB within 15 days. The adults and larvae were treated with the conidial concentration of 1×10^8 conidia/ml by aerosol type sprayer for each isolate. Mortality rates were corrected by Abbott's formula (Abbott 1925). Different capital letters indicate a significant difference among mortality levels according to Fisher test ($p < 0.05$). 0.01% Tween 80 was used as negative control

are available (Zimmermann 2007). Based on the literature, it has been observed that different isolates of the same species, which are isolated from different geographical regions and even from different insects, have different levels of virulence due to genetic diversity (Goettel et al. 2005; Gasmi et al. 2021; Quesada-Moraga et al. 2006). In this direction, we think that the use of native isolates isolated directly from the target pest is important in terms of biological control. In this study, we can say that entomopathogenic *B. bassiana* isolates that can be used against CPB were obtained, especially in the central Anatolian region of Türkiye.

Although EPFs attack all developmental stages of their hosts, they may vary in virulence (Butt et al. 2016). Ansari and Butt (2012) showed that *Hylobius abietis* L. (Coleoptera: Curculionidae) larvae and pupae were more susceptible to entomopathogenic fungi and died faster than adults after application of fungi. In another study, Sedighi et al. (2013) showed that *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae) nymphs was more susceptible than adults. In the same way, it was determined that the different developmental stages of CPB exhibited different levels of susceptibility to entomopathogenic fungi, and the larval stage was more susceptible than the adults (Fargues 1972; 1991; Baki et al. 2021). In this study, it was determined that there was no significant difference between larvae and adults of CPB in terms of the susceptibility to fungal infection but, it is generally estimated that the larvae are more susceptible than adults, because the cuticula is thicker, harder, and more sclerotized in adults. In larvae, this layer has a softer and more flexible structure (Ansari and Butt 2012). The differences among these studies might be due to the use of different fungal isolates or bioassay methods used against CPB.

In most entomopathogenic fungi (especially the group of Ascomycota), spores or conidia are produced after host's death and the fungus has fully colonized the cadaver in the end of infection and this is also called as mycosis (Goettel et al. 2005). Mycosis is of a great importance since the most insect pathogenic fungi rely on spores for reproduction and the production of spores on cadavers is essential for other insects to become infected after the death of the host (horizontal transmission), providing a better long-term control and causing a decrease in inoculum amount in field applications (Goettel et al. 2005; Chavez et al. 2023). Also, the success of infection in insects depends on the number of

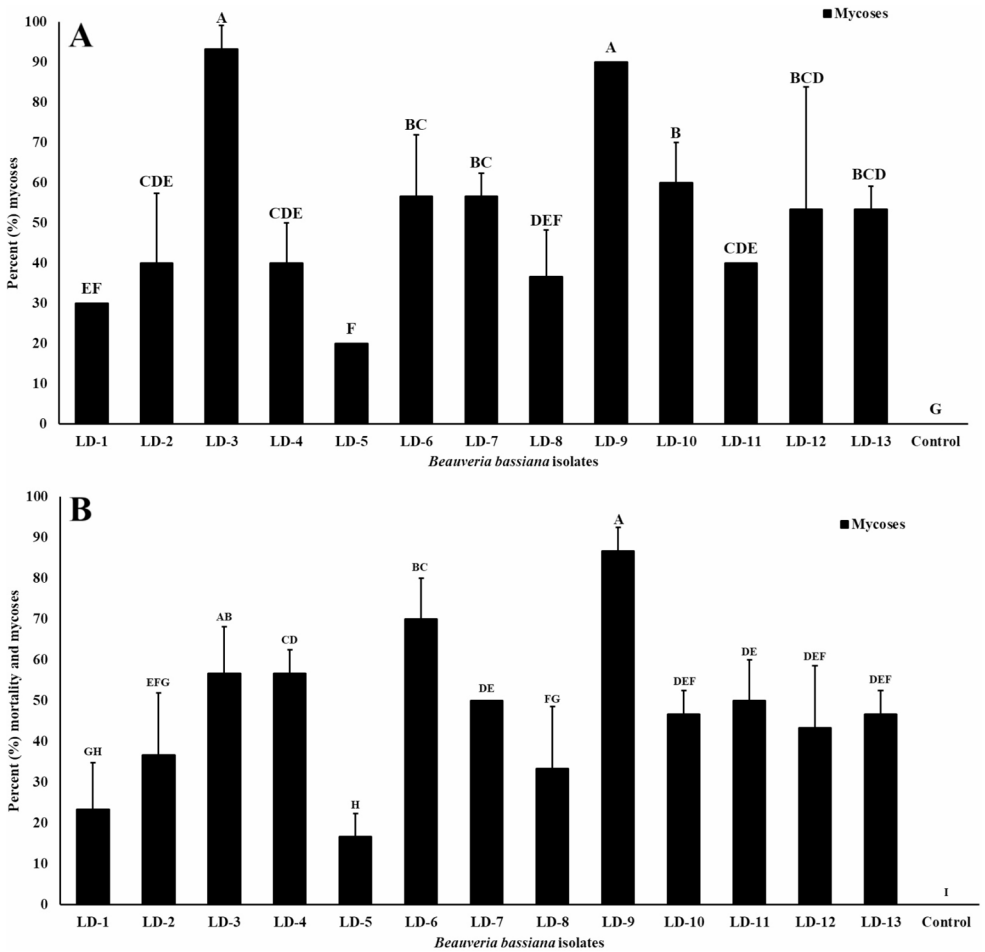

Fig. 3 Percent (%) mycoses (+SE) of *Beauveria bassiana* isolates against adults (**A**) and larvae (**B**) of CPB. The adults and larvae were treated with the conidial concentration of 1×10^8 conidia/ml by aerosol type sprayer for each isolate and mortality values were recorded within 15 days. After that, the dead insects were kept in moisture chamber to encourage mycosis for 10 days and mycosed insects were counted. Different capital letters indicate a significant difference among mortality levels according to Fisher test ($p<0.05$). 0.01% Tween 80 was used as negative control

conidia produced or applied (inoculum amount), and for a successful infection, the number of conidia must exceed the critical threshold value (Meyling and Eilenberg 2007). For all these reasons, it is important to investigate the degree of mycosis after insect death. In this study, we also determined the percent mycosis after insect death and found that two isolates showing the highest virulence (LD-3 and LD-9) also produced a good level of mycosis, except for LD-3 against larvae.

This study aimed to isolate and characterize EPF from *L. decemlineata* in Kırşehir province, Türkiye to find a possible biocontrol agent. The isolated fungi were identified by gene sequencing (*Bloc*) and phylogenetic analysis. They were also tested against *L. decemlineata* adults and larvae under laboratory conditions to find safer, more effective, and ecofriendly native fungal isolates that could be used in IPM strategies. Further studies are needed to determine the efficacy of these isolates in the field.

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Author contributions Ali Sevim (PhD) planned, designed the study, helped to perform all experiments, analyzed data, and wrote the manuscript. Nidanur Değirmenci (BS student) and Sema Gül (BS student) performed fungal isolation and species identification experiments. All the authors have read and approved the final version of the manuscript.

Data availability Data and all fungal isolates will be provided on request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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