Artvin Çoruh Üniversitesi
Orman Fakültesi Dergisi
ISSN:2146-1880, e-ISSN: 2146-698X
Yıl: 2022, Cilt: 23, Sayı:2, Sayfa: 209-216

ofd.artvin.edu.tr

Artvin Coruh University
Journal of Forestry Faculty
ISSN:2146-1880, e-ISSN: 2146-698X
Year: 2022, Vol: 23, Issue:2, Pages: 209-216

Characterization and biological activity of two new entomopathogenic Beauveria bassiana strains isolated from Hyphantria cunea larvae in Turkey

Türkiye'de Hyphantria cunea larvalarından izole edilen iki yeni entomopatojenik Beauveria bassiana suşunun karakterizasyonu ve biyolojik aktivitesi

Zeynep BAYRAMOĞLU[1]

[1]Recep Tayyip Erdoğan Üniversitesi, Pazar Meslek Yüksekokulu, Pazar, Rize

Eser Bilgisi / *Article Info*
Araştırma makalesi / *Research article*
DOI: 10.17474/artvinofd.1181151

Sorumlu yazar / *Corresponding author*
Zeynep BAYRAMOĞLU
e-mail:
zeynep.bayramoglu@erdogan.edu.tr

Geliş tarihi / *Received*
28.09.2022
Düzeltme tarihi / *Received in revised form*
21.10.2022
Kabul Tarihi / Accepted
25.10.2022
Elektronik erişim / *Online available*
28.10.2022

Keywords:
Hyphantria cunea
Beauveria bassiana
Biological control

Anahtar kelimeler:
Hyphantria cunea
Beauveria bassiana
Biyolojik mücadele

Abstract

In this study, two fungal strains (HC-Z1 and HC-Z2) from *Hyphantria cunea* (fall webworm) larvae were evaluated for their potential as biocontrol agents against the *H. cunea* larvae. Based on morphological and molecular (ITS1-5.8S-ITS2 region) characterization, the strains were identified as *Beauveria bassiana* (HC-Z1: OP415530 and HC-Z2: OP415531). To determine the biological activities of the two fungal strains, a concentration-response assay ($1 \times 10^{4-8}$ conidia/ml) was performed against third stage *H. cunea* larvae. In addition, two *B. bassiana* strains were tested on five (1-5) larval stages at 1×10^7 conidia/ml concentration. Both isolates produced mortalities over 96% within 7 days for the first larval stage of *H. cunea*. The LC_{50} and LT_{50} of HC-Z1 and HC-Z2 strains against third instar *H. cunea* larvae were calculated as 0.6×10^4 and 1.2×10^4 conidia/ml, respectively. LT_{50} values were obtained in 2.82 and 3.44 days for HC-Z1 and HC-Z2 isolates, respectively. As a result, it can be concluded that HC-Z1 and HC-Z2 strains can potentially be used as biological control agents against *H. cunea*.

Özet

Bu çalışmada, *Hyphantria cunea* (Amerikan beyaz kelebeği) larvalarından izole edilen iki fungus suşunun (HC-Z1 ve HC-Z2), *H. cunea* larvalarına karşı biyokontrol etmeni olarak potansiyelleri araştırıldı. Morfolojik ve moleküler (ITS1-5.8S-ITS2 bölgesinin dizileri) karakterizasyonlarına göre suşlar *Beauveria bassiana* (HC-Z1: OP415530 ve HC-Z2: OP415531) olarak tanımlandı. İki fungus suşunun biyolojik aktivitelerini belirlemek için *H. cunea* larvalarının 3. evresinde konsantrasyon yanıt testi ($1 \times 10^{4-8}$ konidia/ml) yapıldı. Ayrıca, iki *B. bassiana* suşu, 1×10^7 konidia/ml konsantrasyonu *H. cunea* larvalarının beş evresi (1-5.) üzerinde test edildi. Her iki suş da *H. cunea*'nın birinci evre larvalarına karşı 7 gün içinde %96'nın üzerinde ölüm oranı oluşturdu. Üçüncü evre *H. cunea* larvalarına karşı HC-Z1 ve HC-Z2 suşlarının LC_{50} değerleri sırasıyla 0.6×10^4 ve 1.2×10^4 konidia/ml olarak hesaplandı. LT_{50} değerleri ise HC-Z1 ve HC-Z2 suşları için sırasıyla 2.82 ve 3.44 gün olarak belirlenmiştir. Sonuç olarak, HC-Z1 ve HC-Z2 suşlarının potansiyel olarak *H. cunea*'ya karşı biyolojik kontrol etmeni olarak kullanılabileceği sonucuna varılabilir.

INTRODUCTION

The fall webworm, *Hyphantria cunea* Drury (Lepidoptera: Erebidae) is an important polyphagous pest that attacks about 600 plant hosts. It is native to the United States, southern Canada, and northern Mexico (Schwalter and Ring 2017). This pest has spread to Europe, Russia, Georgia, China, New Zealand, Iran, Korea, Japan, and Turkey (Ito and Miyashita 1968, Yang et al. 2006, 2008). Fall webworm is difficult to control because of its polyphagous, high reproductive potential, and rapid spread (Tuncer and Kansu 1994, Saruhan et al. 2014). likely indicates broad detoxification ability by this moth (Yamamoto et al. 2007).

For a long time, chemical insecticides such as permethrin, cyfluthrin and bifenthrin are mainly used to control *H. cunea.* Chemical control has a faster and significant effect, but the use of chemicals has caused many problems such as environmental pollution and resistance (Jialin et al. 2013). Biological control of entomopathogens against insect pests can be considered as a suitable alternative to the use of chemicals (Bayramoglu et al. 2018, Edosa et al. 2019). Entomopathogenic fungi (EPF) have remarkable potential in controlling insect pests in the world. Fungal entomopathogens such as *Beauveria bassiana* and *Metarhizium anisopliae* play an important role in controlling pests (Shah and Pell 2003, Zimmermann 2008, Gurulingappa et al. 2011).

Beauveria bassiana (Balsamo) (Ascomycota: Hypocreales: Clavicipitaceae) is a widely used fungal species that is effective on a wide range of insects and is used as biopesticide (Dembilio et al. 2010, Xiong et al. 2013). There have been some studies on the effectiveness of EPF on *H. cunea* (Aker and Tuncer 2016a, 2016b, Aker and Kushiyev 2016, İskender et al. 2012, Saruhan et al. 2017, Wang et al. 2019). Among these, *B. bassiana* isolates tested against H. cunea larvae had over 90% mortality (Iskender et al. 2012, Qin et al. 2012). The efficacy of *B. bassiana* was investigated against H. cunea, and high larval mortality was observed (Zibaee et al. 2013). Entomopathogenic fungi (PaF04, PaF09, PaF76) were also tested, and a larval mortality of 95% was obtained at a concentration of 1×10^6 conidia (Iskender et al. 2012).

The aim of this study was to characterize two EPF isolated from cadavers of *H. cunea* larvae from the vicinity of Trabzon and Rize in Turkey and then to investigate their pathogenicity.

MATERIAL AND METHODS

Fungal Isolation

H. cunea larvae were collected from the leaves of apple (*Malus domestica* Borkh, Rosales: Rosaceae) and pear (*Pyrus communis* L., Rosales: Rosaceae) plants in Trabzon (HC-Z1) and Rize (HC-Z2) provinces, Turkey, in June and July of 2020. Fungal pieces from dead larvae of *H. cunea* were transferred to potato dextrose agar medium containing 1% yeast extract (PDAY medium, Merck, Darmstadt, Germany) and chloramphenicol (40 µg/ml) to. After the fungal cultures were obtained in pure form in the medium, they were stored at -80 °C.

Microscopic Identification

The fungal isolates were examined by light microscope for morphological identification. Agar blocks containing fungal isolates incubated for 4-5 days were taken and spores were examined using phase contrast microscopy (400X, Nikon Eclipse E600). The identification process was carried out according to the Humber (2012).

Molecular Identification

Fungal spores were inoculated onto Sabouraud CAF agar (Liofilchem®, Roseto degli Abruzzi (Te) Italy) medium and incubated for 1-2 weeks at 28 °C. Mycelium was harvested from the fungi growing in petri dishes and subjected to DNA extraction. Fungal DNA' were isolated using ZR Fungal/Bacterial DNA MiniPrep kit (50 prep, ZYMO RESEARCH).

PCR reaction was performed using the ITS1-5. 8S-ITS2 region for molecular identification (White et al. 1990). The reaction mixture (50 µl) consisted of 50 ng of DNA template, 5X reaction buffer, 10 mM dNTPs, 10 µM of each primer, and 2-unit Phusion-DNA polymerase (New England Biolabs, Ipswich, MA, USA). The reaction conditions were after a denaturation step at 98 °C for 30 s, 35 cycles of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. PCR products were electrophoresed on a 1% agarose gel, visualized under UV light and sent to MACROGEN (Netherlands) for sequencing. The sequences were compared with similar sequences in the GenBank database (Benson et al. 2012) and aligned with the BIOEDIT v.7.2.5 software (Hall 1999). For phylogenetic analysis, comparison with *Beauveria* species was performed using neighbor-joining (NJ) in MEGA v.11.0 software (Tamura et al. 2013, Kumar et al. 2018). Finally, sequences were comparative with representative sequences described by Rehner and Buckley (2005) to state the position of new strain between *Beauveria* species.

Biological Activities

Larvae of *H. cunea* were collected from mulberry trees in Rize, Turkey in July of 2020. They were reared on mulberry leaves to get adults and larvae were hatched from eggs in laboratory conditions. Neonate *H. cunea* larvae were fed and reared until the 5^{th} stage and 1, 2, 3, 4 and 5 stage larvae were selected for bioassays.

The insecticidal activity of *B. bassiana* HC-Z1 and HC-Z2 strains was evaluated using a concentration-response bioassay. Mulberry leaves were used as food in the bioassays. Fungal suspensions used in biotests were

prepared by harvesting with sterile 0.01% Tween 80 (AppliChem, Darmstadt, Germany) from cultures that were incubated for 4 weeks. After harvest, the suspension was filtered through cheesecloth to remove fungal residues and transferred to a sterile 50 ml falcon. The number of conidia in the suspensions was calculated using a Neubauer hemocytometer and the concentration was set to 1×10^8 conidia/ml. The spores of the fungal strains were diluted to $1 \times 10^{4-8}$ conidia/ml. Thirty 3rd stage larvae of *H. cunea* were used for each experiment, and the experiments were repeated three times at different periods. Then, the selected larvae were transferred into plastic containers (15 cm wide × 8 cm deep) with mulberry leaves. The fungal suspensions were applied by a mini hand-sprayer for each dilute, separately. The control groups were exposed to only 0.01% Tween 80 during the tests. All containers were stored at 25 ± 1 °C, 65 ± 5% RH under 12/12 photoperiod for 7 days. Each day, the containers were inspected, dead larvae were counted, and the presence of mycosis in the dead larvae was noted. Susceptibility of larval age, two *B. bassiana* strains at 1×10^7 conidia/ml concentration were tested on first, second, third, fourth, and fifth instar larvae. The method described in the concentration-response test was used to determine the effect of the fungal strains on the different larval stages. For statistical analyses, mortality rates at 7th day were calculated using the Schneider-Orelli formula (Puentener 1981), and LC_{50} and LT_{50} values were determined by Probit analysis using MS Excell (Finney 1971).

RESULTS

Two fungal strains, HC-Z1 and HC-Z2, infected the *H. cunea* larvae and produced mycelium outside the cadavers (Figure 1). These strains were originally detected in *H. cunea* larvae that were naturally infected with fungi. Morphological examination of two fungal strains revealed morphological and cultural characteristics like those of *Beauveria* species. The strains were identified according to the shape and size of their conidia as they grew on PDAY according to Humber (2012). Colonies were detected as round, slightly raised with white powdery surface and slightly downy with circular rings. Phase contrast micrographs of the fungal isolates are shown in Figure 2. The spores of the strains were characteristically spherical and generally had a zigzag structure.

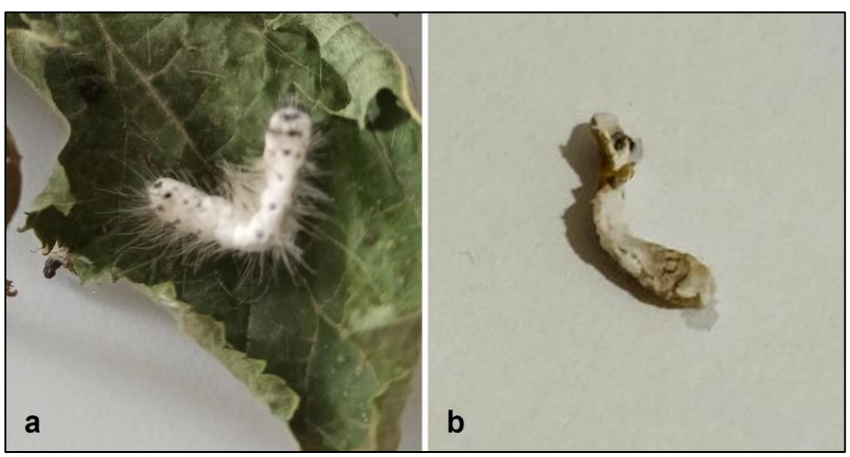

Figure 1. Mycosed *Hyphantria cunea* larvae by HC-Z1 (a) and HC-Z2 (b) strains

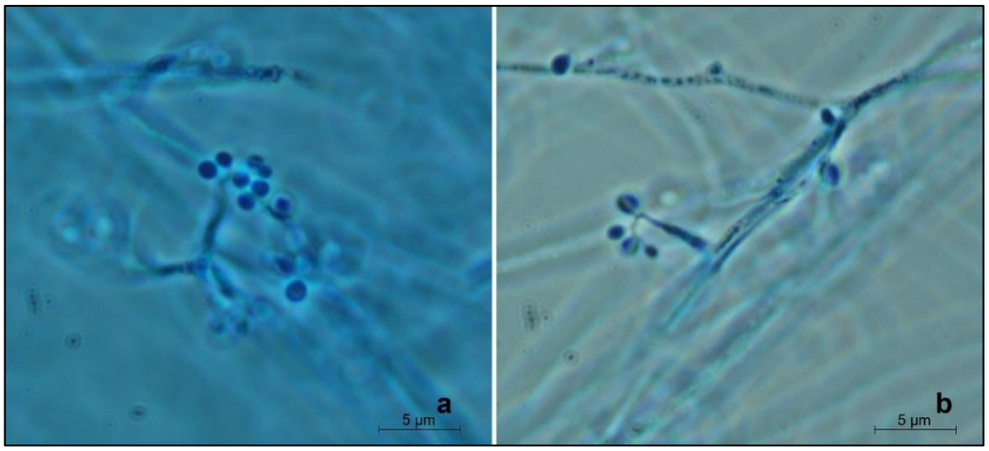

Figure 2. Phase-contrast microscopy of Beauveria bassiana HC-Z1 (a) and HC-Z2 (b) strains.

Amplified PCR products of about 538 and 567 bp in size for HC-Z1 and HC-Z2, respectively were sequenced. The partial sequences of ITS1-5.8S-ITS2 were used to produce phylogenetic trees. Based on phylogenetic analysis, two strains (HC-Z1 and HC-Z2) were found to be identical to *B. bassiana* (Figure 3). The accession numbers in the GenBank database for the newly identified *B. bassiana* HC-Z1 and HC-Z2 strains are OP415530 and OP415531, respectively.

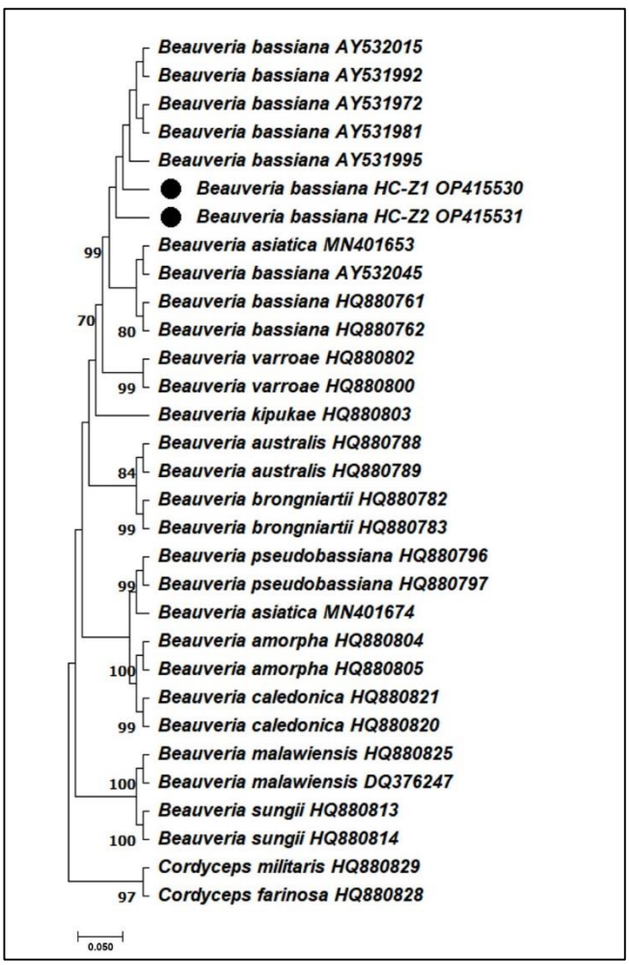

Figure 3. Neighbor-joining tree of Beauveria bassiana HC-Z1 and HC-Z2 strains and closely related fungal species based on the sequences of the ITS1-5.8S-ITS2 gene region. Numbers at nodes are bootstrap percentages based on 1000 repeat.

In the concentration-response experiments, *B. bassiana* strains HC-Z1 and HC-Z2 caused complete mortality on third-stage *H. cunea* larvae within 7 days of treatment with a conidial suspension of 1×10^8 conidia/ml (Figure 4). Mortality of HC-Z1 and HC-Z2 strains differed from the control groups within 7 days of application. The HC-Z1 strain showed 100% mortality on 3rd stage *H. cunea* larvae at the highest concentration (1×10^8 conidia/ml). The HC-Z2 strain produced a mortality rate of 98.48% at the highest concentration against 3rd stage *H. cunea* larvae. The HC-Z1 strain had higher activity than HC-Z2 at all used concentrations. At the other concentrations ($1 \times 10^{4-7}$ conidia/ml) of the HC-Z1 strain, mortalities of about 61.67, 66.75, 75.84, and 97.24% were observed, respectively. The HC-Z2 strain also exhibited 58.97, 60.54, 70.41, and 89.74% mortality at concentrations of $1 \times 10^{4-7}$ conidia/ml, respectively (Figure 4). The LC_{50} of HC-Z1 and HC-Z2 strains was calculated to be 0.6×10^4 (0.07 - 5.8) and 1.2×10^4 (0.1 - 10.6) conidia/ml, respectively (Table 1). Using the LT_{50} value at 1×10^8 conidia/ml concentration, it was found that HC-Z1 and HC-Z2 strains killed half of the *H. cunea* larvae in 2.82 and 3.44 days, respectively (Table 1).

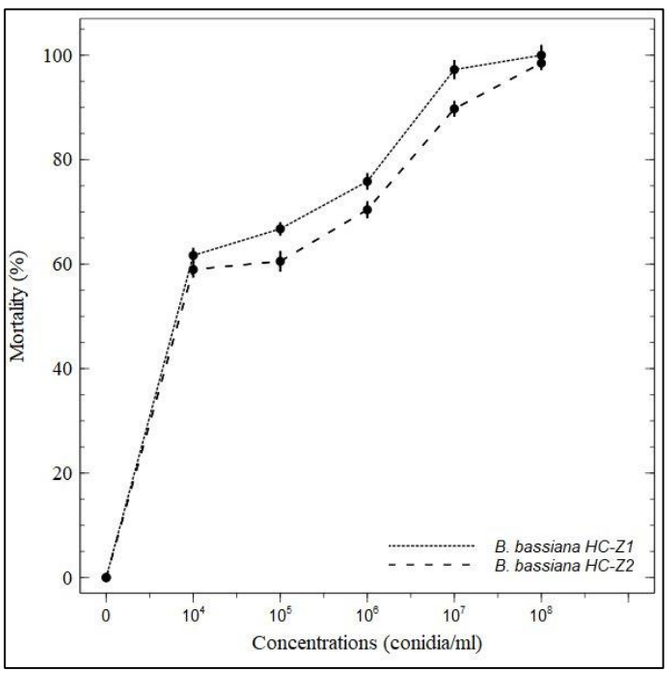

Figure 4. Insecticidal activities of HC-Z1 and HC-Z2 strains on *Hyphantria cunea* third stage larvae within 7 days.

Table 1. Median lethal concentrations (LC_{50}) of two *Beauvera bassiana* HC-Z1 and HC-Z2 strains against *Hyphantria cunea* third stage larvae

Strain	LC_{50} (conidia/ml)	Slope ± SE	*df*	χ^2	LT_{50} (day)*
HC-Z1	0.6×10^4 (0.07 –5.8)	0.448 ± 0.487	3	0.864	2.82 (1.41 – 3.38)
HC-Z2	1.2×10^4 (0.1 –10.6)	0.442 ± 0.476	3	0.671	3.44 (1.94 – 5.18)

*Using 1×10^8 conidia/ml; SE: Standard Error; *df*: Degrees of freedom; χ^2: Chi-Square

Results showed that the susceptibility to fungal infections was higher in the younger stages of *H. cunea* larvae. Mortalities were lower for both HC-Z1 and HC-Z2 strains in the 4th and 5th stages than in the 2nd and 3rd stages of larvae (Figure 5). The mortality of the HC-Z1 strain caused by the 1×10^7 conidia/ml concentration was noted as 96.24, 94.15, 89.46, 77.35, and 67.48% against to the first, second, third, fourth, and fifth stage larvae, respectively. The mortality rates of HC-Z2 strain against five larval stages were 97.5, 93.64, 87.5, 72.18, and 65%, respectively.

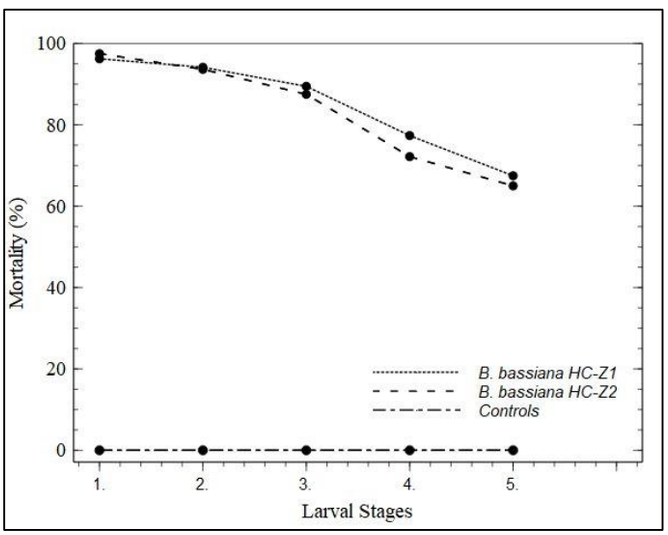

Figure 5. Insecticidal activities of HC-Z1 and HC-Z2 strains at 1 × 108 conidia/ml on five different larval stages of *Hyphantria cunea* within 7 days.

DISCUSSION

Biological control of agricultural and forestry insect pests can be considered an alternative control method to the use of pesticides. The use of biological control agents known as pathogens in the integrated pest management strategy could significantly reduce the use of chemicals. Fungi, bacteria, nematodes, and viruses are the potential pathogens that have potential to be used against *H. cunea* (Edosa et al. 2019).

In the present study, two indigenous *B. bassiana* strains were isolated from *H. cunea* larvae, identified, and their biological characteristics determined. Mycelial structures outside the cadavers were used for isolation and characterization of the fungi (Greenfield et al. 2016). Both strains are morphologically defined in terms of conidia structure and shape (Humber 1997). The conidiogenous cells of *B. bassiana* are short and ovoid and terminate in a narrow apical process called a rachis. The rachis elongates into a long zig-zag extension after conidia production (Humber 2012). Conidiogenous cells with a swollen base and denticulate -shaped rachis extending apically, forming a conidium on each denticle, are *B. bassiana*-specific morphological images under the microscope.

Genetic diversity of *Beauveria* fungal species is determined by sequences of ITS gene regions (Chen et al. 2018). In this study, the molecular sequences of ITS gene regions were used for phylogenetic analysis (Zare and Gams 2001). The activity of *B. bassiana* strains identified in different regions varies in different host insects (Takatsuka 2007). *B. bassiana* is the most common fungal species in many areas (Meyling and Eilenberg 2007). Phylogenetic analysis in the context of molecular identification has revealed that *Beauveria* strains HC-Z1 and HC-Z2 are closely related to other *B. bassiana* isolates.

Currently, many EPF species have been detected in various insect species and found to be highly effective (Ansari et al. 2008, Xiong et al. 2013). In this study, *B. bassiana* fungal species were detected in larvae of *H. cunea* in Turkey. In a previous study, five fungi were recovered from *H. cunea* pupae, namely *Aspergillus westerdijkiae*, *B. bassiana*, *Paecilomyces fumosoroseus*, *P. lilacinus*, and *Trichoderma coningiopsis* (Sullivan 2011). *B. bassiana* was previously detected in *H. cunea* pupae (Sullivan 2011), but it was isolated from larvae for the first time in this study. In addition, *B. bassiana* was previously isolated from *H. cunea* in China (Zhang et al. 2016). In another study, *Isaria fumosorosea* (TR -78.07) was detected from infected pupae of *H. cunea* in Turkey (Aker and Tuncer 2016a). Wang et al. (2020) reported that *Isaria javanica* was detected from *H. cunea* larvae in China.

Nowadays, many studies have shown that EPFs are effective in controlling *H. cunea* (Sullivan et al. 2011, Qin et al. 2012, İskender et al. 2012, Ajamhassani 2013, Zhang et al. 2016, Zibaee et al. 2013, Aker and Tuncer 2016a, 2016b, Saruhan et al. 2017, Wang et al. 2020, Karabörklü et al. 2020, 2022). The present study showed that two indigenous *B. bassiana* strains have significant pathogenic activity against *H. cunea*. In general, these results confirm the susceptibility of *H. cunea to B. bassiana* as reported by some researchers (İskender et al. 2012, Zhang et al. 2016, Saruhan et al. 2017, Karabörklü et al. 2020, 2022). In a study by Iskender et al. (2012), *B. bassiana* isolates were reported to kill *H. cunea* larvae at a dose of 1×10^6 conidia/ml at the end of fifth day with mortality ranging from 90% to 96.66%. In the current study, strains *B. bassiana* HC-Z1 and HC-Z2 achieved mortality of 100% and 97.42%, respectively, at a concentration of 1×10^8 conidia/ml at day 7. In the study conducted by Zibaee et al. (2013), the highest mortality rate of 76% was observed when the isolates of *B. bassiana* EUT 105 and EUT 106 at a dose of 1×10^7 conidia/ml were applied to the *H. cunea* larvae at the fourth instar stage. However, the present study showed that strains HC-Z1 and HC-Z2 of *B. bassiana* produced mortality of 77.35 and 72.18%, respectively, on fourth instar *H. cunea* larvae at the same concentration. Consistent with the results of the current study, it was also reported that increasing concentrations significantly increased mortality (Wang et al. 2020).

In another study, *B. bassiana* and *Paecilomyces farinosus* strains were evaluated for their pathogenicity against *H. cunea* (Qin et al. 2012). The results of their study showed that *B. bassiana* FD and *P. farinosus* SH9-4 strains had

mortality of 92.4% and 87.06%, respectively, and LT_{50} values of 87.06 h (~ 3.62 days) and 92.34 h (~ 3.84 days), respectively. In our study, *B. bassiana* HC-Z1 and HC-Z2 strains were found to have similar mortality rates on *H. cunea* larvae of 2.82 and 3.44 days, respectively. Saruhan et al. (2017) showed that LT_{50} values of *L. muscaricum* isolates were determined between 5.64 and 9.38 days. In addition, İskender et al. (2012) determined LT_{50} values between 2.36 and 2.53 days for three *B. bassiana* strains. All these studies and present study show that *B. bassiana* strains had higher mortality and low mortality both LC_{50} and LT_{50} values against *H. cunea* larvae.

The results demonstrated that at 1×10^8 conidia/ml concentration of both HC-Z1 and HC-Z2 strains, mortality exceeded 65% for each of the five different stages of *H. cunea* larvae after 7 days. Karabörklü et al. (2020) recorded the efficacy of ten isolates of *B. bassiana* and *M. anisopliae* against third, fourth, and fifth instar *H. cunea* larvae at 1×10^5 conidia/ml. Isolate *B. bassiana* YK23 showed the highest insecticidal activity at 89.72% in third instar larvae. As the larval stage increased, the activity of the isolates also decreased (Karabörklü et al. 2020). Similarly, in this study, the highest efficacy (96.24 and 97.5%) was observed in second and third larval stages larvae at 1×10^7 conidia/ml concentration. The efficacy of fungal applications on larval stages was found to be higher in the initial stages (Saruhan et al. 2017, Aker and Tuncer 2016a, Wang et al. 2020). In the study conducted by Saruhan et al. (2017), isolates of *L. muscarium* and *S. lamellicola* were used at a dose of 1×10^8 conidia/ml against third instar *H. cunea* larvae. They found that *L. muscarium* isolates showed lethal efficacy between 72.7% and 93.9% and *S. lamellicola* isolates between 57.6% and 78.8% (Saruhan et al. 2017). Similarly, in a study by Aker and Tuncer (2016a), isolates of *M. anisopliae*, *L. muscarium*, *S. lamellicola*, and *Isaria fumosorosea* were applied to 2nd and 3rd instar *H. cunea* larvae at a dose of 1×10^8 conidia/ml. As a result of the application, it was found that the second instar larvae were more sensitive, and the most effective isolate was *M. anisopliae* with a mortality rate of 85% (Aker and Tuncer 2016a). Another study reported that when *B. bassiana* was applied to *H. cunea* larvae at the 4th instar at 1×10^8 conidia/ml concentration, the larval mortality rate reached 100% by the end of day 9 (Aker and Tuncer 2016b). In our study, the effect of *B. bassiana* strains on 4th instar *H. cunea* larvae at 1×10^7 conidia/ml concentration was calculated to be 77.35% and 72.18% at day 7. When isolate *I. javanica* BE01 was applied to 3rd, 4th, and 5th instar *H. cunea* larvae at a dose of 1×10^8 conidia/ml, mortality of 86.67%, 86.67%, and 73.33%, respectively, was observed at 15 days (Wang et al. 2020). In summary, this study shows that *B. bassiana* strains HC-Z1 and HC-Z2 have good potential to infect and kill first-, second-, and third-stage *H. cunea* larvae. These strains are of immense importance for sustainable control of *H. cunea* in forests. Further field studies are needed to investigate their efficacy under natural conditions.

REFERENCES

Ajamhassani M, Sendi JJ, Zibaee A, Askary H, Farsi MJ (2013) Immunoliogical responses of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) to entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criy) and *Isaria farinosae* (Holmsk.) Fr. J Plant Prot Res 53:2

Aker O, Kushiyev R (2016) Pathogenicity of *Metarhizium anisopliae* on larvae of fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) at different temperatures. Int J Zool Stud 1(6):29-32

Aker O, Tuncer C (2016a) Efficacy of *Metarhizium anisopliae* and some entomopathogenic fungi on larvae of fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae). J Entomol Zool Stud 4(5):171-176

Aker O, Tuncer C (2016b) Pathogenicity of *Beauveria bassiana* on larvae of fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) at different temperatures. Int J Entomol Res 1(6):16-20

Ansari MA, Brownbridge M, Shah FA, Butt TM (2008) Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis*, in plant-growing media. Entomol Exp Appl 127:80-87

Bayramoğlu Z, Nalçacıoğlu R, Demirbağ Z, Demir İ (2018) Characterization of a Betabaculovirus from the fall webworm, *Hyphantria cunea* Drury. (Lepidoptera: Erebidae), in Turkey. Biocontrol Sci Technol 28(12):1178-1190

Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW (2012) GenBank. Nucleic Acids Res 40:48-53

Chen WH, Man L, Huang ZX, Yang GM, Han YF, Liang JD, Liang ZQ (2018) *Beauveria majiangensis*, a new entomopathogenic fungus from Guizhou, China. Phytotaxa 333:243-250

Dembilio Ó, Quesada-Moraga E, Santiago-Álvarez C, Jacas JA (2010) Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the Red Palm Weevil *Rhynchophorus ferrugineus*. J Invertebr Pathol 104(3):214-221

Edosa TT, Jo YH, Keshavarz M, Anh YS, Noh MY, Han YS (2019) Current status of the management of fall webworm, *Hyphantria cunea*: Towards the integrated pest management development. J Appl Entomol 143:1-10

Finney DJ (1971) Probit Analysis. Cambridge University Press, Cambridge, UK

Greenfield M, Gomez-Jimenez MI, Ortiz V, Vega FE, Kramer M, Parsa S (2016) *Beauveria bassiana* and *Metarhizium anisopliae* endophytically colonize cassava roots following soil drench inoculation. Biol Control 95:40-48

Gurulingappa P, Gee PM, Sword GA (2011) In Vitro and In Planta Compatibility of Insecticides and the Endophytic Entomopathogen, *Lecanicillium lecanii*. Mycopathologia 172:161-168

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

Humber RA (1997) Identification of entomopathogenic fungi. In: Lacey LA (ed) Manual of Techniques in Insect Pathology, Academic Press, London, UK, pp 151-187

Humber RA (2012) Identification of entomopathogenic fungi. In: Lacey LA (ed) In Manual of Techniques in Invertebrate Pathology. Elsevier, 32 Jamestown Road, London, UK, pp 151-187

İskender NA, Örtücü S, Aksu Y (2012) Pathogenicity of three isolates of the entomopathogenic Fungi *Beauveria bassiana* to control *Hyphantria cunea* Drury Lepidoptera: Arctiidae Larvae. Manas Fen Bilimleri Dergisi 2(13):15-21

Ito Y, Miyashita K (1968) Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan. V. Preliminary life tables and mortality data in urban areas. Res Popul Ecol 10:177-209

Jialin L, Jingyun C, Ping C (2013) Research Progress of Occurrence and Comprehensive Control of Fall Webworm [*Hyphantria cunea* (Drury)]. Plant Dis Pests 4(4):32-35

Karabörklü S, Altın N, Bahadır A (2022) Efficiency of Native *Beauveria bassiana* and *Metarhizium anisopliae* Isolates against the Early Instar Larvae of fall webworm, *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebiidae). Ziraat Mühendisliği 373:4-9

Karabörklü S, Altın N, Yıldırım İ, Öztemiz S, Sadıç E, Aydın Ö (2020) Insecticidal activity of some Turkish strains of entomopathogenic fungi against the fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) under the laboratory conditions. Türkiye Biyol Mücadele Derg 11(1):119-128

Kumar S, Stecher A, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis Across Computing Platforms. Mol Biol Evol 35(6):1547-1549

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

Qin F-q, Yan G-z, He W, Wang J-l, Tao W-q (2012) Elementary Study on Control of *Hyphantria cunea* by *Beauveria bassiana* and *Paecilomyces farinosus*. J Shanxi Agric Sci 17(02):140-145

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

Saruhan İ, Toksöz Ş, Erper İ (2017) Evaluation of some entomopathogenic fungi against the fall webworm (*Hyphantria cunea* Durry, Lepidoptera: Arctidae). Selcuk J Agr Food Sci 31(2):76-81

Saruhan, I., Akca, I., and Kushiyev, R. (2014). Toxicity of some biopesticides to the fall webworm, *Hyphantria cunea* Durry (Lepidoptera: Arctidae). Egypt. J. Biol. Pest. Control 24, 255–257.

Schowalter TD, Ring DR (2017) Biology and management of the fall webworm, *Hyphantria cunea* (Lepidoptera: Erebidae). J Integr Pest Manag 8(1):7

Shah PA, Pell JK (2003) Entomopathogenic fungi as biological control agents. Appl Microbiol Biotechnol 61(5-6):413-423

Sullivan GT (2011) Determination of Biological Control Agents of Overwintering *Hyphantria cunea* Pupae and Efficacy of Entomopathogenic Fungi. Master thesis Süleyman Demirel University Graduate School of Applied and Natural Sciences, Isparta

Sullivan GT, Özman-Sullivan S, Karaca I, Karaca G (2011) *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae)'nın kışlayan pupalarından elde edilen fungusların entomopatojen etkilerinin belirlenmesi. In: Türkiye IV. Bitki Koruma Kongresi Bildirileri. Kahramanmaraş, Turkey, pp 137

Takatsuka J (2007) Characterization of *Beauveria bassiana* isolates from Japan using inter-simple-sequence-repeat-anchored-polymerase chain reaction (ISSR-PCR) amplification. Appl Entomol Zool 42:563-571

Tamura K, Stecher G, Peterson D, Filipski A (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725-2729

Tuncer C, Kansu IA (1994) Konukçu Bitkilerin *Hyphantria cunea* (Drury) (Lepidoptera, Arctiidae)'ya etkileri üzerinde araştırmalar. Turk Entomol Derg 18(4):209-222

Wang W, Zhou L, Dong G, Chen F (2020) Isolation and identification of entomopathogenic fungi and an evaluation of their actions against the larvae of the fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae). BioControl 1-11

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, San Diego, pp 315-322

Xiong Q, Xiea Y, Zhua Y, Xuea J, Li J, Fan R (2013) Morphological and ultrastructural characterization of *Carposina sasakii* larvae (Lepidoptera: Carposinidae) infected by *Beauveria bassiana* (Ascomycota: Hypocreales: Clavicipitaceae). Micron 44:303-311

Yang ZQ, Wang XY, Wei JR, Qu HR, Qiao XR (2008) Survey of the native insect natural enemies of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) in China. Bull Entomol Res 98:293-302

Yang ZQ, Wei JR, Wang XY (2006) Mass rearing and augmentative releases of the native parasitoid *Chouioia cunea* for biological control of the introduced fall webworm *Hyphantria cunea* in China. Biocontrol 51:401-418

Zare R, Gams W (2001) A revision of Verticillium sect. Prostata IV. The genera *Lecanicillium* and *Simplicillium* gen. nov. Nova Hedwigia 73:1-50

Zhang L, Kang K, Liu Y, Zhang T, Sun L, Zhan C, Huang C, Jiang L, Ye K, Ding D (2016) Evaluation of *Beauveria bass*iana isolates as potential agents for control of *Hyphantria cunea* (Lepidoptera: Arctiidae). Acta Entomol Sin 59(1):111-118

Zibaee I, Bandani AR, Sendi JJ (2013) Pathogenicity of *Beauveria bassiana* to fall webworm (*Hyphantria cunea*) (Lepidoptera: Arctiidae) on different host plants. Plant Prot Sci 49(4):169-176

Zimmermann G (2008) The entomopathogenic fungi *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*): biology, ecology and use in biological control. Biocont Sci Technol 18:865-901