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Original article (Orijinal araştırma)

Efficacy of local entomopathogenic fungi isolated from forestlands in Tokat Province (Türkiye) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)[1]

Tokat İli (Türkiye) orman alanlarından izole edilen yerel entomopatojen fungusların patates böceği *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'ya etkisi

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Abstract

Effects of the entomopathogenic fungi, isolated from forest soil samples gathered from Tokat Province (Türkiye) and its 11 districts between 2014-2017, were evaluated on the third instars and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) under laboratory conditions. Initially, single-dose efficacy experiments were conducted to determine the effect of the 33 isolates on *L. decemlineata* larvae and adults at 1×10^8 conidia/ml. The four isolates giving the highest mortality in single-dose efficacy experiments, GOPT-498-4, GOPT-529-2, GOPT-552, GOPT-562 that included *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae), were used in dose-mortality experiments at 1×10^3, 1×10^5, 1×10^7, 1×10^8, 1×10^9 conidia/ml. The lowest LC_{50} and LT_{50} values were recorded for GOPT-552 with 1.4×10^6 conidia/ml and 10.6 days, respectively, followed by GOPT-562 and GOPT-529-2. *Beauveria bassiana* (GOPT-552 and GOPT-562) isolates were more effective against *L. decemlineata* larvae and adults. Accordingly, GOPT-552 and GOPT-562 isolates are considered to have potential for biological control of Colorado potato beetle.

Keywords: *Beauveria bassiana*, microbial control, mycopesticide, pests, virulence

Öz

Tokat İli (Türkiye) ve 11 ilçesinden 2014-2017 yılları arasında toplanan orman toprak örneklerinden izole edilen entomopatojenik fungusların, Kolorado patates böceği, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'nın üçüncü dönem larva ve erginleri üzerindeki etkileri laboratuvar koşullarında değerlendirilmiştir. İlk olarak 33 izolatın *L. decemlineata* larvaları ve erginleri üzerindeki etkinliğini belirlemek için 1×10^8 konidi/ml'de tek doz etkinlik denemeleri yapılmıştır. Tek doz etkinlik denemelerinde en yüksek ölüm oranlarını veren dört izolat olan *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae)'yı içeren GOPT-498-4, GOPT-529-2, GOPT-552, GOPT-562 izolatları 1×10^3, 1×10^5, 1×10^7, 1×10^8, 1×10^9 konidi/ml'de doz-ölüm denemelerinde kullanılmıştır. En düşük LC_{50} ve LT_{50} değerleri sırasıyla 1.4×10^6 konidi/ml ve 10.6 gün ile GOPT-552 izolatı için kaydedilmiş olup, bunu GOPT-562 ve GOPT-529-2 izolatları takip etmiştir. *Beauveria bassiana* (GOPT-552, GOPT-562) izolatları *L. decemlineata* larva ve erginlerine karşı daha etkili olmuştur. Dolayısıyla GOPT-552 ve GOPT-562 izolatlarının Kolorado patates böceğinin biyolojik kontrolü için potansiyele sahip olduğu görülmüştür.

Anahtar sözcükler: *Beauveria bassiana*, mikrobiyal mücadele, mikopestisit, zararlılar, virulans

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Introduction

Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), is one of the most devastating pests of potato that cause significant damage by eating the leaves. Both larvae and adults can cause complete defoliation of potato plants, with considerable yield losses of up to 60% (Jacques & Fasulo, 2015). This pest is a native of North America and has become a devastating pest worldwide. The beetle survives by feeding on cultivated and wild solanaceous plants, like potato, eggplant and tomato (Kıvan & Aysal, 2014).

The most widely used biological agents in biological control after predators and parasitoid insects are entomopathogens (Deacon, 1983). Generally, four groups of microbial pathogens are found in insects. These are bacteria, fungi, viruses and protozoa. Among these biological control agents, entomopathogenic fungi (EPF) especially infect the pest through integument, their ease of production and high adaptability make them potentially more useful than the others. EPF have three mechanisms of action including causing mechanical damage to tissues and producing toxic metabolites. In this way, they can cause the direct death of insects or weaken them, and limit their vital activities (Kulkarni, 2015).

Beauveria bassiana (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae), *Beauveria brongniartii* (Sacc.) Petch (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae), *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae) and *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora (Hypocreales: Cordycipitaceae) are the most commonly used EPF for biological control of soilborne pests (Dragonova et al., 2008). The commercially available *B. bassiana* product has been applied to pests such as *Curculio elephas* J.C.Fabricius, 1781 (Coleoptera: Curculionidae) (chestnut borer) and *Curculio nucum* (L., 1758) (Coleoptera: Curculionidae) (hazelnut borer) that have pupae and adults in the soil, with instar mortality of 35% (Paparatti & Speranza 1999; 2005). This showed that the soil phase has an important stage in the life cycle of pests such as potato beetle, which pupae in the soil and overwinter in the soil as an adult. In addition, EPF are used to control other pests such as rose aphid *Macrosiphum rosae* (L., 1758) (Hemiptera: Aphididae) (Soy, 2017); alfalfa weevil *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae) and lucerne beetle *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae) (Baysal, 2017); *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) (Ateş, 2020); apple blossom beetle *Tropinota* (*Epicometis*) *hirta* (Poda, 1761) (Coleoptera: Scarabaeidae) (Uçar, 2021); common grasshopper species *Poecilimon glandifer* (Karabag, 1950) (Orthoptera: Tettigoniidae) (Doğan, 2022); rice weevil *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) (Solmaz, 2022). Boverin, a mycoinsecticide derived from *B. bassiana*, was produced in 1965 for the control of Colorado potato beetle and codling moth in the former USSR (Kendrick, 2000).

Colorado potato beetle populations in cultivated potato fields have developed resistance to many insecticides used for control, and this species is becoming the most destructive pest of potatoes in the northeast of the USA (Zhao et al., 2000). Since then, a range of control methods have been implemented. Unfortunately, despite all efforts to control this pest, potato beetle has developed resistance to DDT, other chlorinated hydrocarbons, organophosphorus, carbamates and some pyrethroids (Grafius, 1997). Therefore, it is necessary to search for alternative methods to chemical control for potato beetle and to put them into practice, and many studies have been conducted for this purpose worldwide (Kepenekci et al., 2015; Zemek et al., 2021). For example, in a study conducted by Güven et al. (2015), four *B. bassiana* isolates from different sources and sites were tested for their pathogenicity against larvae and adult of *L. decemlineata* with spraying, dipping and residue methods under laboratory conditions at 10^8 conidia/ml, and *B. bassiana* was found to be the more effective on larvae than adults. It was reported that mortality of the adult insect was low but larval mortalities obtained with the spraying, dipping, and residual methods for BMAUM-001 were 73, 65 and 68%; for BMAUM-002 were 84, 93 and 91%; and for BMAUM-003 were 84,

60 and 79%, respectively. In another study Öztürk et al. (2015) evaluated the efficacy of commercial mycopesticides containing entomopathogenic fungi; Priority [*Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.], Nibortem [*Verticillium lecanii* (Zimm.) Viégas], Nostalgist (*B. bassiana*), Bio-Magic (*M. anisopliae*), Bio-Nematon (*Paecilomyces* sp., Bainier) and plant extracts; Nimbedicine EC (azadirachtin) on adult and larva of *L. decemlineata* under laboratory conditions. The biological control agents were applied to second-third instars, fourth instars and adults with spray and leaf dipping methods. Single concentration (10^8 conidia/ml) of entomopathogenic fungi and recommended dose of bioinsecticides were prepared for application. Entomopathogenic fungi and bioinsecticides were found to be more effective against early instars than fourth instars and adults. In spray methods, Bio-Magic, Nibortem, and Nostalgist caused 96, 93 and 82% mortality on second instars and 20, 37 and 33% mortality on adults, respectively, while all local *B. bassiana* isolates caused 100% mortality on second and fourth instars of the insect. Adults showed 59-86% mortality. Similarly, in another study four *B. bassiana* isolates (BbDm-1, BbDs-2, BbMg-2 and BbMp-1) at 1×10^7 conidia/ml were more pathogenic than the others against *L. decemlineata*, causing mortality between 97% and 100% on first and second instars, respectively, between 92 and 97% on the third and fourth instars, respectively, and between 93 and 97% on 0-48h adults, respectively, 9 days post treatment. The highest mortality was seen in larvae; but first and second instars were more susceptible to fungal isolates than third and fourth instars (Baki et al., 2021).

In forest areas, unlike cultivated areas, no pesticides are used and no tillage is done. Therefore, the presence of entomopathogenic fungi in these regions is much higher than in cultivated soils (Vänninen et al., 1989; Miętkiewski et al., 1991; Vänninen, 1995; Chandler et al., 1997; Bałazy, 2004). The isolation of entomopathogenic fungi from forest areas makes the present study different from other studies and adds to its originality. The aim of the present study was to evaluate the efficacy of local *B. bassiana* and *Talaromyces* spp. isolates from forest soils in Tokat Province, Türkiye against third instars and adult of Colorado potato beetle under laboratory conditions.

Materials and Methods

The main material of the experiment was third instars (which consume the most potato leaves) and adults of the Colorado potato beetle (*L. decemlineata*) and the entomopathogenic fungi isolates of forest soil (Table 2) applied against the pest.

Soil sampling

Soil samples were collected in forest areas of different elevation in districts of Tokat Province, Türkiye (Table 1). Soil samples were collected after removing the surface soil at depths of ~5-20 cm. Soil sampling was made from five points at each sampling site. The samples were mixed in a plastic bag, ~750 g of soil from the mixture was placed in polyethylene bags and brought to the laboratory and examined for entomopathogenic fungi as soon as possible (Ali-Shtayeh et al., 2002). Soils brought to the laboratory from forest areas were dried for 4 days in the climate chamber (25 ± 2ºC) and then passed through 2 mm sieves in order to purify them from foreign materials (e.g., leaves, stones and wood chips). Fifty g of each soil sample was then placed in 90 mm sterile glass Petri dishes and moistened to field capacity by spraying 10 ml of sterile distilled water. Fungi were isolated from soil samples using a *Galleria* bait method (Zimmermann, 1986). In order to prevent the *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae from forming pupa, the larvae were transferred to Petri dishes after they were kept at 50°C for 120 s. Five fourth or fifth instar *Galleria* of the same age and size were added to each soil sample in glass Petri dishes, each of which was prepared in three replicates. The Petri dishes were wrapped with Parafilm and incubated at 25 ± 2ºC in the dark for 7-14 days. The Petri dishes were turned and agitated daily to ensure that the larvae came into maximum contact with the soil (Mietkiewski et al., 1991). The larvae were examined once every 2 days for a period of 2 weeks. Dead larvae were removed and surface-sterilized in 1% sodium hypochlorite for 2-3 min, then washed two times in sterile distilled water (Quesada-Moraga et al., 2007) and they were taken

into sterile Petri dishes with moist filter paper and incubated at 25 ± 2 ºC (Ali-Shtayeh et al., 2002). The fungi obtained from the samples showing external fungal growth during the incubation were purified using a needle in a sterile cabinet (Sevim, 2010). For this, potato dextrose agar with 1% yeast extract was used. To prevent bacterial contamination, 50 µg/ml ampicillin, 20 µg/ml broad spectrum antibiotic tetracycline and 200 µg/ml streptomycin were added to the medium (Ihara et al., 2001).

Table 1. Number of soil samples collected in Tokat Province and its districts and number of EPF isolates obtained

Districts of Tokat Province	Forest area* (ha)	Samples collected	EPF isolates obtained
Tokat (Central)	94033	47	11
Almus	71355	36	11
Artova	14520	7	0
Başciftlik	5259	3	0
Erbaa	42000	21	0
Niksar	46244	23	2
Pazar	3355	4	0
Reşadiye	47129	23	1
Sulusaray	9350	4	0
Turhal	42332	21	4
Yeşilyurt	7598	4	1
Zile	26925	13	3
Total	410100	206	33

*Area values are from 2013 (Anonymous, 2014).

Rearing of third-instars and adults of *L. decemlineata*

Potato plants (cv. Marabel) grown during the production season in a 72 m^2 screen house at the Middle Black Sea Transitional Zone Agricultural Research Institute were used for potato beetle rearing. Third-instars were separated based on their pronotum coloration. Insects used in the experiments were first generation larvae and adults that emerged from the underground overwintered adults that came to the soil surface, mated and deposited eggs in the spring with the potato planting. No pesticide application was applied to the potato plants grown in the screen house during the vegetation period.

Fungal isolates

In total, 33 EPF isolates (Table 1) were isolated from soil samples collected from forestlands in Tokat Province in Türkiye in 2014-17 by using the *G. mellonella* bait method (Zimmermann, 1986). These isolates were identified morphologically and molecularly at the genus and species level by Prof. Dr. İsmail Demir (Karadeniz Technical University Faculty of Arts and Sciences Department of Biology in Trabzon Province, Türkiye) (Samson et al., 1988; White et al., 1990; Humber, 2005; 2012). Based on the sequence analysis, 25 isolates were identified as *B. bassiana*, and eight of isolates were identified as *Talaromyces* spp. (C.R. Benjamin) (Eurotiales: Trichocomaceae). All isolates were cultured on potato dextrose agar (PDA) medium at 25 ± 1°C for 15-30 days.

Table 2. Coordinates and altitude of local fungus isolates obtained from forest vegetation soils by trap insect method

Isolates	Sampling site	GPS Coordinates		Altitude (m)	EPF Scientific Name
		N	E		
GOPT-479	Tokat	40°22'20"	36°32'48"	872	*Talaromyces* spp.
GOPT-483	Tokat	40°19'33"	36°33'53"	661	*B. bassiana*
GOPT-498-1	Tokat	40°21'32"	36°42'35"	754	*Talaromyces* spp.
GOPT-498-2	Tokat	40°21'32"	36°42'35"	754	*Talaromyces* spp.
GOPT-498-4	Tokat	40°21'32"	36°42'35"	754	*B. bassiana*
GOPT-501-1	Tokat	40°19'56"	36°30'07"	628	*Talaromyces* spp.
GOPT-510	Tokat	40°19'46"	36°30'30"	606	*Talaromyces* spp.
GOPT-517	Tokat	40°23'42"	36°40'13"	769	*B. bassiana*
GOPT-528	Tokat	40°11'16"	36°29'00"	1161	*B. bassiana*
GOPT-529-1	Tokat	40°12'38"	36°30'36"	1054	*B. bassiana*
GOPT-529-2	Tokat	40°12'38"	36°30'36"	1054	*B. bassiana*
GOPT-537	Almus	40°22'22"	36°52'00"	1004	*B. bassiana*
GOPT-541-1	Almus	40°24'02"	36°54'18"	870	*B. bassiana*
GOPT-541-2	Almus	40°24'02"	36°54'18"	870	*B. bassiana*
GOPT-547-1	Almus	40°25'09"	36°54'29"	808	*B. bassiana*
GOPT-547-2	Almus	40°25'09"	36°54'29"	808	*Talaromyces* spp.
GOPT-547-4	Almus	40°25'09"	36°54'29"	808	*B. bassiana*
GOPT-547-5	Almus	40°25'09"	36°54'29"	808	*B. bassiana*
GOPT-549	Almus	40°24'54"	36°54'42"	872	*B. bassiana*
GOPT-551	Almus	40°24'27"	36°55'17"	810	*B. bassiana*
GOPT-552	Almus	40°24'21"	36°55'20"	837	*B. bassiana*
GOPT-557	Almus	40°21'31"	37°01'22"	866	*B. bassiana*
GOPT-562	Niksar	40°31'29"	36°53'11"	576	*B. bassiana*
GOPT-563	Niksar	40°34'16"	36°55'06"	270	*Talaromyces* spp.
GOPT-580-2	Turhal	40°14'21"	36°06'52"	708	*B. bassiana*
GOPT-580-3	Turhal	40°14'21"	36°06'52"	708	*Talaromyces* spp.
GOPT-583	Turhal	40°14'03"	36°04'03"	711	*B. bassiana*
GOPT-584	Turhal	40°13'42"	36°04'09"	783	*B. bassiana*
GOPT-597	Zile	40°13'29"	36°00'37"	952	*B. bassiana*
GOPT-615	Zile	40°14'12"	35°57'29"	820	*B. bassiana*
GOPT-617	Zile	40°13'08"	35°58'10"	970	*B. bassiana*
GOPT-624	Reşadiye	40°22'45"	37°21'19"	516	*B. bassiana*
GOPT-665	Yeşilyurt	40°00'32"	36°16'32"	1129	*B. bassiana*

Inoculum production

The fungi isolates were subcultured on PDA medium and incubated for 4 weeks. At the end of the incubation period, 10 ml of sterile distilled water with 0.02% Tween 80 was poured into the Petri dishes and spores were harvested with glass spreader. The spore suspension was filtered through three layers of sterile cheesecloth and the mycelia and agar pieces were removed. Spore density in stock solutions was determined using a hemocytometer. Spore concentration was adjusted to 1×10^8 conidia/ml for each isolate.

Single-dose efficacy experiments

Third instars and adults of *L. decemlineata* were immersed in the spore suspensions (1×10^8 conidia/ml) of each fungal isolate for 8-10 s. The excess water on the larvae and adults was removed with filter paper. Treated larvae and adults were put in Petri dishes (9 cm) containing fresh potato leaves their stem wrapped with wet cotton. Larvae and adults in control treatment were immersed in distilled water containing 0.02% Tween 80. Each treatment had a group of five larvae and adults. Mortality of the larvae and adults were registered daily from days 1 to 7 and days 1 to 11 of incubation under the laboratory conditions, respectively. The experiments had three replicates and were conducted twice.

Dose-mortality experiments

Only adults were evaluated in dose-mortality studies because the mortality was high in the control larvae 7 day after treatments. As mentioned above adults of *L. decemlineata* were immersed with different spore concentrations (1×10^3, 1×10^5, 1×10^7, 1×10^8 and 1×10^9 conidia/ml) and in distilled water containing 0.02% Tween 80 (control). Mortality of the adults were registered daily from day 1 to 14 of incubation. Experiments included three replicates and were conducted twice.

Statistical analysis

The obtained mortality data were then analyzed by one-way ANOVA using the SPSS 17 package program (IBM, Armonk, NY, USA). Prior to ANOVA, the mortality data were tested for normality (Kolmogorov Smirnov test) and then arcsine transformed ($n' = \arcsin \sqrt{n}$) to obtain a normally distributed data set. Post-hoc Tukey's HSD test was performed to separate and compare means ($P = 0.05$) were detected. LC_{50} and LT_{50} values of isolates were calculated by probit analysis of dose-mortality experiments with Polo-PC program (LeOra, 1994).

Results

Single-dose efficacy experiments

The results in Tables 3 and 4 show the mortality of *L. decemlineata* larvae and adults treated with 1×10^8 conidia/ml of each isolate, respectively.

There were no significant differences in the mortality caused by the isolates after 1 day ($F = 1.3$; $df = 33$; $p > 0.05$) and 3 days ($F = 3.8$; $df = 33$; $p > 0.05$). After 3 days, mortality observed in GOPT-597 and GOPT-549 (75%) isolates belonging to *Beauveria* genera were higher than the control and other isolates. Significant differences in mortality were evident on day 5 compared to the control ($F = 6.5$; $df = 33$; $p < 0.05$). Isolates GOPT-624 (85%), GOPT-547-1, GOPT-617, GOPT-549 and GOPT-665 (90%), GOPT-584 and GOPT-552 (95%), GOPT-498-4 (100%) caused a higher mortality than others after 5 days. The differences in mortality were also significant on day 7 compared to the control ($F = 11.6$; $df = 33$; $p < 0.05$). GOPT-597, GOPT-617, GOPT-549, GOPT-624, GOPT-665, GOPT-557, GOPT-583, GOPT-584, GOPT-541-2, GOPT-541-1, GOPT-580-2, GOPT-547-5, GOPT-498-4, GOPT-551, GOPT-552, GOPT-562 (100%) isolates belonging to *Beauveria* genera caused high mortality. GOPT-498-4 caused highest mortality (100%) in third instars on day 5. Among the *Talaromyces* isolates, GOPT-547-2 caused the highest mortality (85%) after 7 days. Although both GOPT-510 and GOPT-498-2 *Talaromyces* isolates caused mortality of 70%, there was no a statistically significant difference ($F = 11.6$; $df = 33$; $p > 0.05$) between them when compared to the control (Table 3).

Table 3. Mortality (%) of *Leptinotarsa decemlineata* larvae treated with suspension of EPF isolates at 1×10^8 conidia/ml

Fungal isolate	Species	Mortality ± SDM*(%)			
		1 DAT	3 DAT	5 DAT	7 DAT
Control		0 ± 0 a	0 ± 0 a	5 ± 10 a	15 ± 10 a***
GOPT-498-1	*Talaromyces* spp.	0 ± 0 a	20 ± 23 ab	30 ± 25 abcd	65 ± 30 abc
GOPT-479	*Talaromyces* spp.	0 ± 0 a	30 ± 11 abc	35 ± 19 abcde	60 ± 16 abcde
GOPT-547-2	*Talaromyces* spp.	0 ± 0 a	40 ± 28 abc	55 ± 19 abcdef	85 ± 19 bcdef
GOPT-580-3	*Talaromyces* spp.	0 ± 0 a	20 ± 23 ab	45 ± 30 abcde	55 ± 19 abc
GOPT-501-1	*Talaromyces* spp.	0 ± 0 a	20 ± 16 ab	30 ± 11 abc	40 ± 0 ab
GOPT-510	*Talaromyces* spp.	5 ± 10 a	45 ± 10 abc	65 ± 10 abcdefgh	70 ± 11 abc
GOPT-615	*B. bassiana*	0 ± 0 a	50 ± 11 abc	75 ± 25 efghi	90 ± 11 def
GOPT-483	*B. bassiana*	0 ± 0 a	45 ± 10 abc	65 ± 25 abcdefghi	80 ± 23 cdef
GOPT-547-1	*B. bassiana*	0 ± 0 a	65 ± 19 bc	90 ± 11 efghi	95 ± 10 def
GOPT-563	*Talaromyces* spp.	0 ± 0 a	0 ± 0 a	10 ± 11 ab	25 ± 10 abc
GOPT-498-2	*Talaromyces* spp.	5 ± 10 a	25 ± 25 abc	45 ± 10 abcdefg	70 ± 11 abcd
GOPT-597	*B. bassiana*	10 ± 11 a	75 ± 37 c	80 ± 28 bcdefghi	100 ± 0 def
GOPT-537	*B. bassiana*	5 ± 10 a	35 ± 19 abc	60 ± 16 bcdefghi	85 ± 19 def
GOPT-617	*B. bassiana*	5 ± 10 a	70 ± 11 bc	90 ± 11 ghi	100 ± 0 f
GOPT-549	*B. bassiana*	10 ± 20 a	75 ± 10 c	90 ± 11 fghi	100 ± 0 f
GOPT-517	*B. bassiana*	0 ± 0 a	30 ± 25 abc	70 ± 20 bcdefghi	95 ± 10 def
GOPT-624	*B. bassiana*	10 ± 11 a	50 ± 11 abc	85 ± 19 efghi	100 ± 0 f
GOPT-665	*B. bassiana*	5 ± 10 a	45 ± 10 abc	90 ± 11 defghi	100 ± 0 ef
GOPT-557	*B. bassiana*	5 ± 10 a	50 ± 11 abc	80 ± 16 efghi	100 ± 0 ef
GOPT-583	*B. bassiana*	10 ± 20 a	50 ± 11 abc	85 ± 19 fghi	100 ± 0 f
GOPT-529-1	*B. bassiana*	15 ± 10 a	35 ± 19 abc	65 ± 19 cdefghi	95 ± 10 ef
GOPT-584	*B. bassiana*	0 ± 0 a	65 ± 19 bc	95 ± 10 i	100 ± 0 f
GOPT-528	*B. bassiana*	15 ± 19 a	50 ± 28 abc	80 ± 16 cdefghi	95 ± 10 def
GOPT-529-2	*B. bassiana*	0 ± 0 a	35 ± 30 abc	70 ± 34 cdefghi	95 ± 10 ef
GOPT-541-2	*B. bassiana*	5 ± 10 a	45 ± 19 abc	80 ± 16 efghi	100 ± 0 f
GOPT-541-1	*B. bassiana*	20 ± 16 a	35 ± 19 abc	60 ± 16 cdefghi	100 ± 0 f
GOPT-547-4	*B. bassiana*	5 ± 10 a	40 ± 0 abc	70 ± 25 efghi	95 ± 10 ef
GOPT-580-2	*B. bassiana*	0 ± 0 a	50 ± 25 abc	75 ± 19 efghi	100 ± 0 f
GOPT-547-5	*B. bassiana*	0 ± 0 a	40 ± 16 abc	70 ± 11 abcdefghi	100 ± 0 f
GOPT-498-4	*B. bassiana*	5 ± 10 a	55 ± 10 bc	100 ± 0 hi	100 ± 0 ef
GOPT-551	*B. bassiana*	10 ± 11 a	50 ± 25 abc	75 ± 10 defghi	100 ± 0 f
GOPT-552	*B. bassiana*	0 ± 0 a	50 ± 11 abc	95 ± 10 fghi	100 ± 0 f
GOPT-562	*B. bassiana*	0 ± 0 a	20 ± 0 ab	70 ± 11 cdefghi	100 ± 0 f

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

There were no significant differences in mortality between isolate or compared to the control after 1 day ($F = 0.7$; $df = 33$; $p > 0.05$), 3 days ($F = 1.0$; $df = 33$; $p > 0.05$) and 5 days ($F = 0.9$; $df = 33$; $p > 0.05$). Significant differences in mortality compared to the control were evident after 7 days ($F = 11.5$; $df = 33$; $p < 0.05$). *Beauveria* isolates GOPT-541-1 (53%), GOPT-547-4 (56%), GOPT-529-2 (63%), GOPT-498-4 (66%), GOPT-547-5 and GOPT-551 (70%), GOPT-562 (93%), GOPT-552 (100%) caused high mortality (>50%) after 7 days. Differences in mortality on day 11 were also significant compared to control ($F = 18.3$; $df = 33$; $p < 0.05$). GOPT-584 (80%), GOPT-528 (83%), GOPT-529-2 and GOPT-541-2 (86%), GOPT-541-1, GOPT-547-4 and GOPT-580-2 (90%), GOPT-547-5 (93%), GOPT-498-4 and GOPT-551 (96%), GOPT-552 and GOPT-562 (100%) caused high mortality after 11 days. Mortality caused by *Talaromyces* isolates after 11 days did not differ from the control or from each other ($F = 18.3$; $df = 33$; $p > 0.05$) (Table 4).

Table 4. Mortality (%) of *Leptinotarsa decemlineata* adults treated with a suspension of EPF isolates at 1×10^8 conidia/ml

Fungal isolate	Species	Mortality ± SDM*(%)				
		1DAT	3DAT	5DAT	7DAT	11DAT
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	6 ± 10 ab	10 ± 10 ab***
GOPT-498-1	*Talaromyces* spp.	3 ± 8 a	6 ± 10 a	6 ± 10 a	6 ± 10 ab	6 ± 10 a
GOPT-479	*Talaromyces* spp.	0 ± 0 a	6 ± 16 a	10 ± 16 a	10 ± 16 ab	10 ± 16 ab
GOPT-547-2	*Talaromyces* spp.	6 ± 16 a	10 ± 16 a	10 ± 16 a	10 ± 16 ab	10 ± 16 ab
GOPT-580-3	*Talaromyces* spp.	3 ± 8 a	6 ± 10 a	6 ± 10 a	6 ± 10 ab	10 ± 16 ab
GOPT-501-1	*Talaromyces* spp.	0 ± 0 a	10 ± 10 a	13 ± 10 a	13 ± 10 abc	16 ± 8 abc
GOPT-510	*Talaromyces* spp.	3 ± 8 a	6 ± 10 a	10 ± 10 a	13 ± 10 abc	16 ± 15 ab
GOPT-615	*B. bassiana*	0 ± 0 a	3 ± 8 a	10 ± 10 a	16 ± 8 abcd	16 ± 8 abc
GOPT-483	*B. bassiana*	0 ± 0 a	0 ± 0 a	3 ± 8 a	6 ± 10 ab	20 ± 12 abc
GOPT-547-1	*B. bassiana*	0 ± 0 a	10 ± 10 a	13 ± 16 a	16 ± 19 abc	20 ± 21 ab
GOPT-563	*Talaromyces* spp.	0 ± 0 a	6 ± 10 a	6 ± 10 a	16 ± 15 abcd	20 ± 17 abc
GOPT-498-2	*Talaromyces* spp.	6 ± 16 a	16 ± 19 a	23 ± 23 a	23 ± 23 abcde	23 ± 23 abc
GOPT-597	*B. bassiana*	3 ± 8 a	13 ± 16 a	13 ± 16 a	16 ± 19 abc	23 ± 23 abc
GOPT-537	*B. bassiana*	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	26 ± 10 abc
GOPT-617	*B. bassiana*	3 ± 8 a	13 ± 20 a	20 ± 25 a	23 ± 29 abcd	26 ± 35 abc
GOPT-549	*B. bassiana*	0 ± 0 a	6 ± 16 a	10 ± 16 a	16 ± 15 abcd	33 ± 24 abcd
GOPT-517	*B. bassiana*	3 ± 8 a	16 ± 15 a	16 ± 15 a	23 ± 15 abcdefg	36 ± 19 abcde
GOPT-624	*B. bassiana*	0 ± 0 a	6 ± 10 a	16 ± 15 a	26 ± 16 abcdefg	40 ± 21 abcde
GOPT-665	*B. bassiana*	0 ± 0 a	0 ± 0 a	10 ± 16 a	30 ± 20 abcdefg	40 ± 25 abcde
GOPT-557	*B. bassiana*	3 ± 8 a	3 ± 8 a	10 ± 10 a	23 ± 19 abcdef	50 ± 20 bcdef
GOPT-583	*B. bassiana*	0 ± 0 a	0 ± 0 a	10 ± 16 a	16 ± 15 abcd	50 ± 16 bcdef
GOPT-529-1	*B. bassiana*	0 ± 0 a	6 ± 10 a	10 ± 10 a	36 ± 15 abcdefg	66 ± 16 cdefg
GOPT-584	*B. bassiana*	6 ± 10 a	6 ± 10 a	6 ± 10 a	30 ± 20 abcdefg	80 ± 21 efg
GOPT-528	*B. bassiana*	0 ± 0 a	6 ± 10 a	10 ± 10 a	43 ± 15 bcdefg	83 ± 8 defg
GOPT-529-2	*B. bassiana*	3 ± 8 a	10 ± 16 a	10 ± 16 a	63 ± 26 defgh	86 ± 16 fg
GOPT-541-2	*B. bassiana*	0 ± 0 a	6 ± 16 a	6 ± 16 a	37 ± 8 abcdefg	86 ± 24 fg
GOPT-541-1	*B. bassiana*	0 ± 0 a	0 ± 0 a	3 ± 8 a	53 ± 10 cdefg	90 ± 10 fg
GOPT-547-4	*B. bassiana*	0 ± 0 a	0 ± 0 a	0 ± 0 a	56 ± 15 cdefgh	90 ± 10 fg
GOPT-580-2	*B. bassiana*	3 ± 8 a	6 ± 10 a	10 ± 10 a	36 ± 15 abcdefg	90 ± 10 fg
GOPT-547-5	*B. bassiana*	0 ± 0 a	6 ± 10 a	6 ± 10 a	70 ± 20 fghi	93 ± 10 g
GOPT-498-4	*B. bassiana*	6 ± 16 a	16 ± 15 a	16 ± 15 a	66 ± 27 ghi	96 ± 8 g
GOPT-551	*B. bassiana*	0 ± 0 a	6 ± 10 a	16 ± 15 a	70 ± 16 efghi	96 ± 8 g
GOPT-552	*B. bassiana*	0 ± 0 a	10 ± 16 a	13 ± 16 a	100 ± 0 i	100 ± 0 g
GOPT-562	*B. bassiana*	0 ± 0 a	13 ± 16 a	20 ± 17 a	93 ± 10 hi	100 ± 0 g

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

Dose-mortality experiments

The results of a single-dose screening of both larvae and adult experiments in Tables 3 & 4 were used to select isolates causing high mortality for further testing. GOPT-498-4 (*B. bassiana*) (100-96%), GOPT-529-2 (*B. bassiana*) (95-86%), GOPT-552 (*B. bassiana*) (100-100%), GOPT-562 (*B. bassiana*) (100-100%) were selected for dose-mortality experiments (1×10^3-1×10^9 conidia/ml) (Table 5).

The highest mortality was observed with GOPT-552 at 1×10^3 conidia/ml compared to other isolates. Mortality increased with increasing dose and 90% mortality was observed at the 1×10^9 conidia/ml, the highest mortality with this isolate. GOPT-498-4 caused the second highest mortality at 1×10^3 conidia/ml. Mortality increased with increasing dose, and a mortality of 81% was observed at the 1×10^9 conidia/ml.

With GOPT-562, mortality was the same as the control at 1×10^3 conidia/ml ($F = 41$; $df = 5$; $p > 0.05$), and a significant increase from 16 to 70% at the 1×10^7 conidia/ml was observed on day 14 ($F = 41$; $df = 5$; $p < 0.05$). Mortality of 86% was observed at 1×10^9 conidia/ml. With GOPT-529-2 at 1×10^9 conidia/ml mortality of 86% was observed, similar to GOPT-562. After 14 days, the mortality observed at 10^7, 10^8 and 10^9 conidia/ml with all isolates were significantly different from the control, but there was no significant differences between isolates (Table 5). The data acquired from the dose-death experiments were examined by probit analysis.

Table 5. Mortality percentage of *Leptinotarsa decemlineata* adults treated with a range of doses of the four selected *Beauveria bassiana* isolates

Fungal isolate	Doses (conidia/ml)	Mortality ± SDM*(%) 1DAT**	3DAT	5DAT	7DAT	9DAT	11DAT	14DAT
Control		0 ± 0 a*	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
GOPT-498-4 *B. bassiana*	1×10^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	6 ± 10 a	6 ± 10 a***
	1×10^5	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	6 ± 10 a	10 ± 10 a	20 ± 25 a
	1×10^7	0 ± 0 a	0 ± 0 a	0 ± 0 a	21 ± 4 b	25 ± 5 b	40 ± 0 b	60 ± 0 b
	1×10^8	3 ± 8 a	3 ± 8 a	6 ± 16 a	38 ± 4 bc	48 ± 9 c	66 ± 8 bc	73 ± 8 b
	1×10^9	0 ± 0 a	0 ± 0 a	3 ± 8 a	50 ± 8 c	60 ± 8 c	73 ± 8 c	81 ± 9 b
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
GOPT-529-2 *B. bassiana*	1×10^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
	1×10^5	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	16 ± 15 a	26 ± 10 b
	1×10^7	3 ± 8 a	3 ± 8 a	3 ± 8 a	26 ± 10 b	41 ± 9 b	53 ± 8 b	63 ± 5 c
	1×10^8	0 ± 0 a	0 ± 0 a	0 ± 0 a	33 ± 10 b	56 ± 8 bc	70 ± 8 bc	76 ± 5 cd
	1×10^9	0 ± 0 a	3 ± 8 a	3 ± 8 a	40 ± 0 b	66 ± 10 c	81 ± 9 c	86 ± 10 d
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 a	3 ± 8 a
GOPT-552 *B. bassiana*	1×10^3	3 ± 8 a	3 ± 8 a	6 ± 10 a	6 ± 10 a	6 ± 10 a	6 ± 10 a	13 ± 16 a
	1×10^5	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	6 ± 10 a	20 ± 25 a	23 ± 32 a
	1×10^7	0 ± 0 a	6 ± 10 a	6 ± 10 a	26 ± 10 b	43 ± 8 b	60 ± 0 b	63 ± 5 b
	1×10^8	0 ± 0 a	6 ± 10 a	6 ± 10 a	40 ± 0 b	60 ± 0 b	68 ± 7 b	78 ± 4 b
	1×10^9	0 ± 0 a	0 ± 0 a	3 ± 8 a	46 ± 10 b	66 ± 10 b	85 ± 12 b	90 ± 10 b
Control		0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a	3 ± 8 a	3 ± 8 ab	3 ± 8 a
GOPT-562 *B. bassiana*	1×10^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	3 ± 8 a
	1×10^5	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a	16 ± 15 b	16 ± 15 a
	1×10^7	0 ± 0 a	0 ± 0 a	0 ± 0 a	26 ± 10 b	50 ± 10 b	60 ± 6 c	70 ± 6 b
	1×10^8	0 ± 0 a	0 ± 0 a	0 ± 0 a	41 ± 4 c	53 ± 10 b	56 ± 8 c	78 ± 4 b
	1×10^9	0 ± 0 a	3 ± 8 a	6 ± 16 a	50 ± 6 c	60 ± 6 b	70 ± 8 c	86 ± 10 b

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

The doses that killed 50% (LC_{50}) of the population on day 14 and the assay parameters are presented in Table 6. The minimum LC_{50} values were with GOPT-552 with 1.4×10^6 conidia/ml. Therefore, the most virulent isolate was GOPT-552, followed by GOPT-562.

Table 6. LC_{50} values (conidia/ml) of the isolates against adults of *Leptinotarsa decemlineata* at 14 days after treatment

Fungal isolate	Slope ± SE	X^2	LC_{50} (conidia/ml)	95% confidence interval
GOPT-498-4	0.319 ± 0.060	31.1	3.8×10^7	$8x10^6$-$2.4x10^8$
GOPT-529-2	0.405 ± 0.064	18.7	1.1×10^7	$3x10^6$-$3.8x10^7$
GOPT-552	0.390 ± 0.060	36.4	1.4×10^6	$2.9x10^5$-$5.4x10^6$
GOPT-562	0.438 ± 0.067	21.7	9.5×10^6	$3.3x10^6$-$2.7x10^7$

Also, in order to determine the effect of time on death, LT_{50} values calculated at 1×10^8 conidia/ml is given in Table 7. GOPT-529-2 caused the fastest death (LT_{50} = 10.2) of the isolates tested, followed by GOPT-552 (LT_{50} = 10.6) and GOPT-562 (LT_{50} = 10.7).

Table 7. LT_{50} values (days) of *Leptinotarsa decemlineata* adults treated with suspension of selected isolates at a dose of $1x10^8$ conidia/ml

Fungal isolate	Slope ± SE	X^2	LT_{50} (days)	95% confidence interval
GOPT-498-4	2.32 ± 0.477	97.4	15.1	10.9-36.4
GOPT-529-2	5.74 ± 0.927	15.7	10.2	9.47-11.2
GOPT-552	3.58 ± 0.608	28.2	10.6	9.43-12.5
GOPT-562	4.34 ± 0.753	13.2	10.7	9.64-12.1

Discussion

Mortality of adult insects caused by 33 different fungal isolates tested in this study on days 7 and 11 (Table 4) were lower than mortality of the third instars on days 5 and 7 (Table 3). The probable reason for this lower efficacy against adult beetles is the hardening of the insect cuticle as it develops from a larva to an adult. The insect cuticle is an important barrier against the invasion of fungal pathogens and the initial condition for the initiation of infection is the adhesion of the spore to the integument of the pest. Cuticular waxes accumulated during insect molting and subsequent intermolt period of insect life contain chemical components that inhibit the growth and penetration of microorganisms (David, 1967). In the instar cuticle, by comparison, the chemical components differentiate with maturation and cause the hardening of the cuticle, as well as an increase in the internal defense mechanisms against microbial infections. (Boman, 1981). According to the study of Wraight & Ramos (2002), *B. bassiana* was found to be more virulent against the early instars of the potato beetle. In another study, the mortality of insects due to *B. bassiana* infection in the late stage instars was low due to the hardening of the insect cuticle (Charnley, 2003). In our study of third instars, mortality ranging from 20 to 100% were observed among all isolates. The diversity in the virulence of isolates, and the amount of enzyme produced by them should be considered as the reason for this difference. The results obtained in this study show similarities and differences with previous studies. Çam et al. (2002), in their preliminary study to determine the effect of *B. bassiana* isolate at 1×10^8 conidia/ml on the third instars of potato beetle, reported that 89% mortality from fungal infection occurred after 6 days, and this mortality was different from the control but not imidacloprid application. McCoy et al. (1988) reported that the application of *B. bassiana* at 2×10^9 spore/g resulted in high mortality in potato beetle larvae. Akbarian et al. (2012) immersed the second and fourth instars of potato beetle in suspension of *B. bassiana* at 1×10^8 conidia/ml. Mortality after 15 days were 39.3% for the second instars and 25.6% for the fourth instars, which was lower mortality than found in our study. Shafighi et al. (2012) applied five concentrations of two local isolates of *B. bassiana*, DEBI007 and IR1217C, on second instars using the immersion method. Mortality after 15 days were 60 and 58% for the high concentration (1×10^9 conidia/ml) DEBI007 and IR1217C isolates, respectively. These results generally had low mortality compared to the mortality after 3, 5 and 7 days in our study.

Çam et al. (2002) in their preliminary study to determine the effect of a *B. bassiana* isolate at 1×10^8 conidia/ml on potato beetle adults, while the mortality observed in adult potato beetles was 11% after 3 days, it increased slightly with the extension of the incubation period and was 20% to 6 days. In our study, mortality in adults was generally the same as the mortality on day 3 of Çam et al. (2002), but were generally higher than the mortality on day 7. Karaman (2019) determined that *Simplicillium lamellicola* ((F.E.V.Sm.) Zare & W.Gams (Hypocreales: Cordycipitaceae), *Lecanicillium muscarium* (Petch) Zare & Gams (Hypocreales: Cordycipitaceae), *B. bassiana*, *M. anisopliae*, *C. fumosorosea* entomopathogenic fungi caused 80, 90, 90, 100 and 95% mortality in potato beetle adults after 7 days, respectively, under laboratory conditions.

Metarhizium anisopliae showed the highest effect with 100%. When these values are compared to our study, GOPT-552 gave the same mortality (100%) as *M. anisopliae*. Kılınç (2020), in his study, found the highest mortality (72%) after 7 days with isolate GOPT-258, followed by GOPT-321 with a mortality of 66%. In our study, the mortality after 7 days was the highest. The highest mortality (100%) was obtained with GOPT-552, followed by GOPT-562 with a mortality of 93%. However, there was no significantly difference between these isolates ($F = 11.5$; $df = 5$; $p > 0.05$) (Table 4). Watt & LeBrun (1984) reported similar results to our study, and found that soil treatment with *B. bassiana* was effective in controlling the first and second progeny pupae of *L. decemlineata* with a reduction of 74 and 77%, respectively. The treatment caused a decrease in the adults emerging from the pupa and an increase in the formation of mycosis. Todorova et al. (2000) determined that different *B. bassiana* isolates were highly effective at 100, 93, 90 and 87% against potato beetle adults 8 days after the application, and showed similarity with the high mortality of 86, 93 and 100% obtained after 7 and 11 days in our study (Table 4). However, it was low at 1×10^9 conidia/ml in the dose-mortality experiment (Table 5). The reason for this is thought to be the continuous subculture of EPF from PDA to PDA after their initial isolation from the insect. Güven et al. (2015) determined the effects of four *B. bassiana* isolates at 1×10^8 conidia/ml on third instars and adults of potato beetle using three application methods (spraying, dipping and residue method). *Beauveria bassiana* isolates were found to be more effective on larvae than adults in the three application methods, similar to our study. Mortality of third instars in the immersion method were 93% for BMAUM-002, 65% for BMAUM-001 and 60% for BMAUM-003, and mortality on day 5 for GOPT-498-4, GOPT-552 and GOPT-562 isolates in our study were 100, 95 and 70%, respectively (Table 3). Öztürk (2016), *B. bassiana* BMAUM-LDE-001, BMAUM-LDL-002 and BMAUM-LDE-002 isolates were sprayed on the second and third instars of potato beetle. Similar to our study, after 7 days, the same high (100%) mortality was found with GOPT-597, GOPT-617, GOPT-549, GOPT-624, GOPT-665, GOPT-557, GOPT-583, GOPT-584, GOPT-541-2, GOPT-541-1, GOPT-580-2, GOPT-547-5, GOPT-498-4, GOPT-551, GOPT-552, GOPT-562. The same isolates caused mortality of 59, 62, 86% in adults after 7 days with the three spray methods, respectively, and they were generally higher than after 7 days in our study. The reason for this difference is thought to be due to the different application methods (dipping-spraying) of the fungi solutions to adults.

In dose-mortality experiments, there was no significant differences ($p > 0.05$) between doses in terms of mortality caused by fungal isolates after 1, 3 and 5 days. From day 7 onwards, there was an increase in the mortality due to the increasing dose. Noronha & Goettel (2009) reported that inoculation concentration in adult potato beetles significantly affects the mortality and that most adult insects are infected when exposed to a surface concentration of 10^7 conidia/cm^2 compared to a surface concentration of 10^6 conidia/cm^2. Kılınç (2020) investigated the effects of two different isolates (GOPT-228 and GOPT-375) of *B. bassiana* against the adults of potato beetle at five concentrations (1×10^3, 1×10^5, 1×10^7, 1×10^8 and 1×10^9 conidia/ml) and observed 64-48, 72-52, 80-52, 80-68, and 88-72% mortality, respectively, after 11 days. This was higher mortality than at our day 11. Taking into account the mortality, mycosis and sporulation density in the culture media in single-dose efficacy experiments, Kılınç (2020) conducted a dose-mortality experiment on potato beetle adults using isolates GOPT-228 and GOPT-375. In that study, isolates GOPT-228 and GOPT-375 of *B. bassiana* gave low mortality of 48 and 32%, respectively, after 7 days. Likewise, in our dose-mortality experiment with four isolates, mortality at 1×10^8 conidia/ml was lower than in the single-dose efficacy experiments. In addition, Çerçi (2010) indicated that the results of the similar dose acquired from the single-dose efficacy experiments and dose-death experiments varied. These variations may be due to the difference in application date or the continuous subculture of fungus to purify and reproduce them. Butt & Goettel (2000) argued that when an isolate is used in biological activity experiments, its virulence should be enhanced by subculture through an insect host before being cultured in an artificial medium. Yıldırım (2021) determined the adult LC_{50} value of *B. bassiana* isolate LdA 1 as 0.17×10^8 conidia/ml. The LC_{50} of 0.17×10^8 conidia/ml obtained was less virulent than the LC_{50} of 1.4×10^6 conidia/ml for GOPT-552, which was the most virulent isolate in our study (Table 6). This high virulence in our isolate is attributable to its

isolation from forest soil rather than potato beetle cadavers. Despite the fact that both *B. bassiana* and *M. anisopliae* are common, *B. bassiana* is known to be very sensitive to the harmful effects of tillage and thus its natural habitat is limited. The persistence of *M. anisopliae* in cultivated soil has been determined as more common in the forest soil than in arable land (Rath et al., 1992; Vänninen, 1995; Quesada-Moraga et al., 2007; Sánchez-Peña et al., 2011). Most reports indicate that the incidence of entomopathogenic fungi in heavily cultivated soils is lower than in forest soils (Vänninen et al., 1989; Miętkiewski et al., 1991; Vänninen, 1995; Chandler et al., 1997; Bałazy, 2004).

In conclusion, *B. bassiana* isolates, GOPT-552, GOPT-562 and GOPT-529-2, were been found to be promising for biocontrol of *L. decemlineata* larvae and adults. However, further studies under field conditions is needed to confirm their usefulness.

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