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Identification of some native entomopathogenic fungal species and their pathogenicity against Apple Blossom Beetle, *Tropinota (Epicometis) hirta* (Poda, 1761) (Coleoptera: Cetoniidae) adults

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Abstract

Background: Entomopathogenic fungi (EPFs) have more important role in biological control of the important insect pests. In the present study 15 EPF isolates, selected from the EPF culture collection, were identified using molecular methods and their effectiveness against adults of Apple Blossom Beetle (ABB) [*Tropinota* (=*Epicometis*) *hirta* (Poda, 1761) (Coleoptera: Cetoniidae)] was investigated. To determine virulence of isolates against ABB adults, primarily a single-concentration trial was conducted at 1×10^7 conidia ml^{-1}. Furthermore, concentration-mortality trials were conducted with some isolates that were determined to have a high effect, employing concentrations of 1×10^6, 1×10^8, and 1×10^9 conidia ml^{-1}. For isolates tested in concentration-mortality trials, LT_{30}, LT_{50} and LT_{90} values at a concentration of 1×10^8 conidia ml^{-1} were calculated using probit analysis.

Results: Molecular diagnostic tests revealed that the 13 isolates were *Beauveria bassiana*, 1 isolate was *Clonostachys rosea*, and 1 isolate was *Purpureocillium lilacinum*. According to the single- concentration trial data, some isolates with high efficacy were selected (GN16, GN22-1, GN29-1, HP3-1 and HP33-1) and concentration-mortality trials were established with these isolates using 1×10^6, 1×10^8 and 1×10^9 conidia ml^{-1} concentrations. According to the results of the concentration-mortality trials, all isolates used in 1×10^8 and 1×10^9 conidia ml^{-1} concentrations started to show a significant effect from the 11th day, and at the end of the 17th day, the effect in all concentrations was over 90% (except 1×10^6 conidia ml^{-1} concentration of GN22-1).

Conclusions: According to the data obtained, all isolates used in concentration-mortality trials were effective against ABB adults. With these isolates, more detailed studies should be conducted under orchard conditions.

Keywords: *Tropinota hirta*, Adult, Entomopathogenic fungi, Effect

Background

There are many factors that limit fruit production. Among them, diseases, pests and weeds have an important place. When these factors are not managed properly, significant economic losses occur. Apple Blossom Beetle (ABB) *Tropinota* (=*Epicometis*) *hirta* (Poda, 1761) (Coleoptera: Cetoniidae), is one of the fruit pests (Yaşar and Dahham 2019). It is an important species that spreads to across the Palaearctic region and to almost all regions of Turkey (Aydın 2011). In the spring, ABB adults cause significant damage by feeding on different parts of the flowers of the plants, especially fruit trees,

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strawberries and roses (Aslan and Aslan 2015). They live on leaves during non-flowering periods (Atanasova et al. 2017). Kutinkova and Andreev (2004) reported that the ABB is one of the most important pests of cherry trees in Bulgaria that causes up to 70% damage by feeding on the flowers of young cherry trees.

Chemical control against ABB adults is not recommended due to the presence of pollinators and beneficial insects in the orchards during the flowering period of fruit trees. As a result, eco-friendly control strategies are needed to limit the damage caused by ABB while also protecting the non-target species (Yaşar and Dahham 2019). Biological control with entomopathogenic microorganisms is one of the most effective non-chemical pest control approaches (Mantzoukas and Eliopoulos 2020). EPFs are common in terrestrial environments and serve a crucial role in insect population control (Khan et al. 2012). Approximately 750 EPF species have been found to infect a wide variety of insects and mites (Inglis et al. 2001). Strains of EPFs are concentrated in Hypocreales (various genera), Onygenales (*Ascosphaera* genus), Entomophthorales, and Neozygitales (Entomophthoromycota) (Mora et al. 2017). These biocontrol agents are the most important of all microbial biocontrol agents because they can be mass-produced easily, quickly, and cheaply, and they can be utilized with the same technological methods as traditional contact insecticides. Many commercial mycoinsecticides are being developed from EPFs however most of these products are based in only four genera of entomopathogenic fungi: *Beauveria, Metarhizium, Lecanicillium* and *Isaria* (Vega et al. 2009).

In this study, EPF isolates selected from the culture collection in Mycology Laboratory (Tokat Gaziosmanpaşa University, Faculty of Agriculture, Plant Protection Department) were identified using morphological and molecular methods and their virulence against adults of ABB was investigated under laboratory conditions.

Methods

Insect sources

ABB adults were collected from apple orchards in the research area of Tokat Gaziosmanpaşa University, Faculty of Agriculture (N40°20′01″ E36°28′27″, 622 m), in Tokat-Central-Büyükyıldız (N40°20′11″ E36°23′27″, 606 m) and in Tokat-Central-Akyamaç (N40°20′47″ E36°29′53″, 615 m). The beetles were kept in plastic containers, covered with fine mesh for aeration until used in the bioassay.

Fungal isolates

Fifteen EPF isolates were chosen for the current investigation from the fungal culture collection of the Tokat Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection in Tokat, Turkey. These isolates were initially isolated from *Hypera postica* (Gyllenhal 1813) (Coleoptera, Curculionidae) and *Gonioctena fornicata* (Brüggemann 1873) (Coleoptera, Chrysomelidae) adults gathered from alfalfa fields in Tokat Province, Turkey (Baysal 2017).

Morphological characterization

Fifteen isolates were preliminarily identified using morphological characteristics such as appearance of the fungal infection, colony shape, spore size and spores shape (Humber 1997).

Molecular characterization

DNA isolation and PCR studies

DNA isolation of the EPF isolates was performed according to the "Genomic DNA Purification Kit Thermo Fisher Scientific" method. ITS5 (5′-GGAAGTAAAAGTCGT AACAAGG-3′) as the forward primer and ITS4 (5′-TCC TCCGCTTATTGATATGC-3′) as reverse primers were used for ITS PCR-amplification (White et al. 1990). For PCR, 1 µl template DNA, 2.5 µl 10X Taq Buffer, 0.2 µl dNTP (25 mM), 0.5 µl ITS5 primer (100 pmol), 0.5 µl ITS4 primer (100 pmol), 1.5 µl MgCl2 (25 mM), 0.25 µl Taq polymerase enzyme (Thermo) and 1 µl of Dimethylsulfoxside (DMSO) were mixed in PCR tubes and made up to 25 µl with distilled water and placed in the termocycler (Techne Prime). Thirty-five cycles were performed in thermocycler following the denaturation at 95 °C for 5 min, 95 °C for 1 min, 55 °C for 55 s, 72 °C for 2 min, with a final extension at 72 °C for 10 min (Sevim et al. 2014). The PCR products obtained as a result of PCR were subjected to electrophoresis at 100 V for 1 h in an agarose gel containing 10 mg/ml ethidium bromide, prepared at a rate of 1.2% and visualized under the imaging device (Sevim et al. 2014).

Phylogenetic analysis

For phylogenetic studies, the products obtained at the end of PCR were sent to Atlas Biotechnology (Ankara-Turkey) for the sequencing. The data obtained at the end of the sequencing were analysed with the MEGAX (Kumar et al. 2018) computer program. The data were then compared with reference isolates registered in the National Center for Biotechnology Information (NCBI) gene bank, and molecular identification of the isolates was made.

Inoculum preparation from entomopathogenic fungal isolates

Fungal isolates were cultured on Potato Dextrose Agar (PDA) in Petri dishes. A small part of mycelia was taken from each fungal isolates and inoculated in of PDA and

incubated at 25 ± 2 °C with a 16/8 (L/D) photoperiod. At the end of the 21-day incubation period, spores were harvested with 10 ml of sterilized water containing 0.02% Tween 80. The conidial suspensions were filtered through 3 layers of sterile muslin to remove particles and then conidial concentration adjusted to 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia ml^{-1} (Saruhan et al. 2017).

Bioassays

To determine virulence of isolates against ABB adults, primarily a single- concentration trial was conducted at 1×10^7 conidia ml^{-1}. To test the effect of each of the isolates, ABB adults were immersed into 1×10^7 conidia ml^{-1} suspension of each isolate for 10 s. and transferred into a Petri dish (5 adults per plate) containing fresh apple leaf and flower. The control group was treated with sterile water including 0.02% Tween 80. Mortality of the adults was assessed on the 3rd, 5th, 7th, 9th, 13th, 15th and 17th days of incubation periods. Furthermore, concentration-mortality trials were conducted similar to single-concentration trials. These tests were performed with some isolates that were determined to have a high effect, employing concentrations of 1×10^6, 1×10^8, and 1×10^9 conidia ml^{-1}. A completely randomized block design with 5 replications was used for the experiments and replicated 2 times.

Statistical analysis

Data was analysed with analysis of variance (ANOVA) and the means were compared to Tukey's multiple comparison test by using the MINITAB Release 16 packet program. To determine the statistical interactions between treatments, MINITAB Release 16 was used with a general linear model. For isolates tested in concentration-mortality trials, LT_{30}, LT_{50} and LT_{90} values at a concentration of 1×10^8 conidia ml^{-1} were calculated using probit analysis.

Results

Molecular identification of the fungi

The 15 isolates used in the study were identified by molecular methods, and 13 isolates were *Beauveria bassiana*, 1 isolate was *Clonostachys rosae* and 1 isolate was *Purpureocillium lilacinum* (Table 1; Fig. 1).

Virulence of the fungi

According to the results of the single- concentration trial, all isolates, except HP36-3(1), HP5-2, HP22, HP14 and HP19, had an effect of more than 60% against ABB at the end of the 17th day. Concentration-mortality trials were carried out using concentrations of 1×10^6, 1×10^8 and 1×10^9 conidia ml^{-1} with some of the effective isolates (GN29-1, HP3-1, GN16, HP33-1, and GN22-1 isolates of *B. bassiana*) (Table 2).

Table 1 Species, hosts and GenBank accession numbers of isolates of entomopathogenic fungi

Isolate	Host	Species	Genbank accession numbers
HP5-2	*Hypera postica*	*Beauveria bassiana*	MW599847
GN8-1(1)	*G. fornicata*	*B. bassiana*	MW599848
GN2	*G. fornicata*	*B. bassiana*	MW599849
GN29-1	*G. fornicata*	*B. bassiana*	MW599850
GN22-1	*G. fornicata*	*B. bassiana*	MW599851
HP15	*H. postica*	*B. bassiana*	MW599852
HP22	*H. postica*	*B. bassiana*	MW599853
GN16	*G. fornicata*	*B. bassiana*	MW599854
HP33-1	*H. postica*	*B. bassiana*	MW599855
HP14	*H. postica*	*B. bassiana*	MW599856
GN14	*G. fornicata*	*B. bassiana*	MW599857
GN22-3(2)	*G. fornicata*	*B. bassiana*	MW599858
HP3-1	*H. postica*	*B. bassiana*	MW599859
HP19	*H. postica*	*Clonostachys rosae*	MW599956
HP36-3(1)	*H. postica*	*Purpureocillium lilacinum*	MW600488

Based on the results of concentration-death tests, the mortality rates caused by EPF isolates varied depending to period, isolates, and concentration. EPFs concentration, exposure time, and mortality all had a positive relationship. The GN16 isolate started to show a remarkable effect (68.5%) at a concentration of 1×10^9 conidia ml^{-1} after 7 days post-inoculation. This effect reached 99% after 11 days (F: 133.94; DF: 8.81; $P < 0.05$). At the end of the incubation period, the effectiveness was found above 90% at all concentrations (F: 265.18; DF: 3.36; $P < 0.05$) (Table 3). GN22-1 isolate reached 50% effect only at the end of the 9th day at a concentration of 1×10^9 conidia ml^{-1}. It was able to show 100% efficiency after 17 days exposure at same concentration (F: 103.74; DF: 8.81; $P < 0.05$) (Table 4). Similar to the GN16 isolate, GN29-1 caused a mortality rate of over 60% at a concentration of 1×10^9 conidia ml^{-1} after seven days post-inoculation and this effect was 100% at the end of the 11th day (F: 157.38; DF: 8.81; $P < 0.05$) (Table 5). HP3-1 isolate caused more than 50% mortality from the end of the 9th day at 1×10^8 and 10^9 conidia ml^{-1} concentrations (F: 209.61; DF: 3.36; $P < 0.05$). At the end of the 17th day exposure, all concentrations, including the lowest concentration, produced an effect of almost 100% (F: 622.47; DF: 3.36; $P < 0.05$) (Table 6). In HP33-1 isolate, 58% mortality was determined at a concentration of 1×10^9 conidia ml^{-1} at the end of 7th day exposure time, this rate reached 100% at the end of the 13th day (F: 208.19; DF: 8.81; $P < 0.05$).

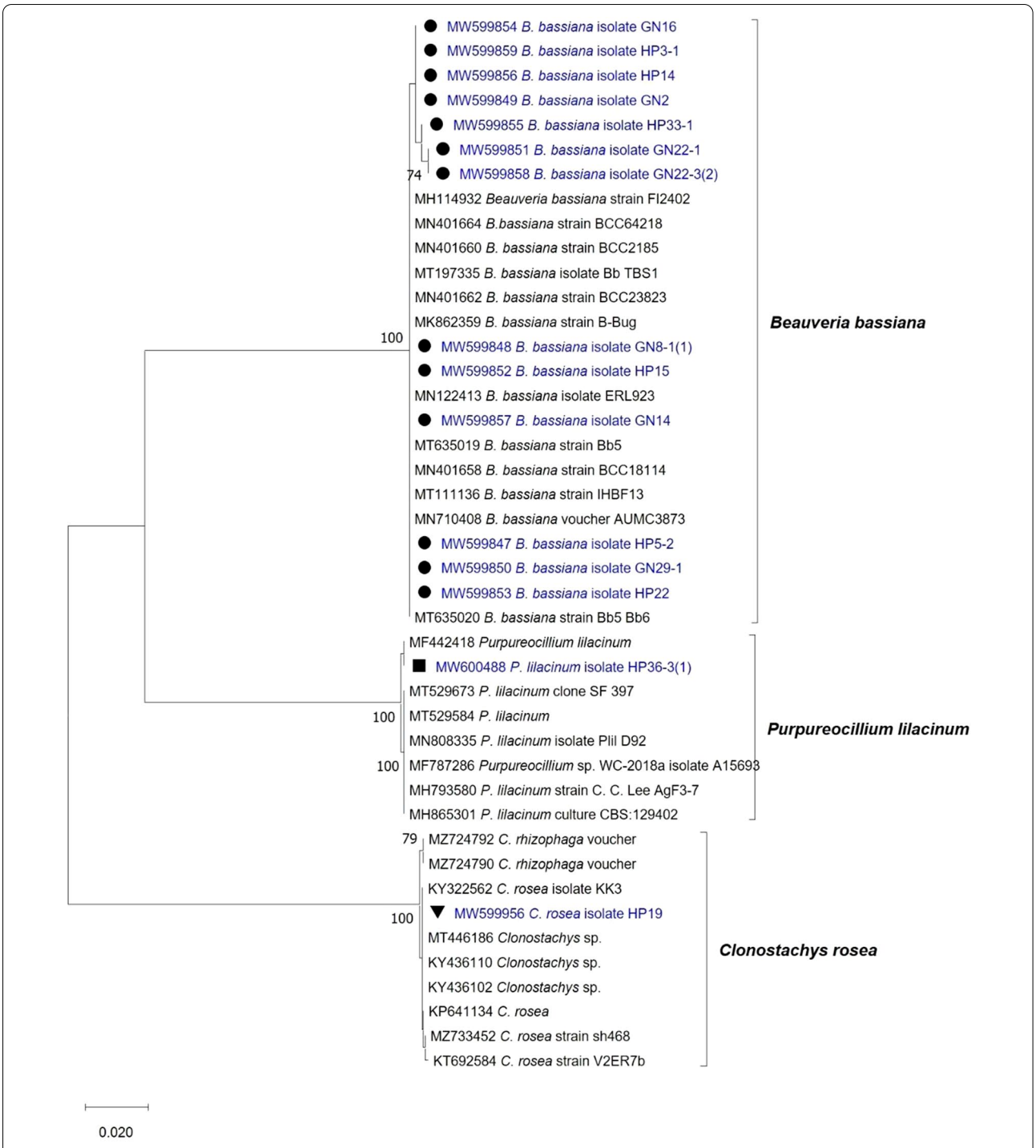

Fig. 1 Phylogenetic tree constructed based on ITS region of the obtained isolates and references using the Neighbor-Joining method. The isolates obtained in this study are shown in blue. Obtained *Beauveria bassiana*, *Purpureocillium lilacinum* and *Clonostachys rosea* isolates are indicated using a black-filled circle, black-filled square, black-filled triangle respectively

Similar to the HP3-1 isolate, after seventeen days post-inoculation, all concentrations, caused an effect of almost 100% (F: 622.47; DF: 3.36; $P < 0.05$) (Table 7).

A complete ANOVA analysis was performed to determine the effects of isolate, concentration, and time for ABB. Isolate*concentration, isolate*time,

Table 2 Mortality of *Tropinota hirta* exposed to the fifteen isolates at 1×10^7 conidia ml^{-1}

Mortality (%)							
ISOLATES	**5 DAT[a]**	**7 DAT**	**9 DAT**	**11 DAT**	**13 DAT**	**15 DAT**	**17 DAT**
GN29-1	5.3a[b]D[c]	31.2aC	80.7aA	92.57aAB	99.8aA	100.0aA	100.0 aA
HP36-3(1)	0.2aBC	0.2bcBC	0.2gBC	0.86 ± 1.2	8.8fghAB	14.5efA	19.7fA
GN14	0.9aD	1.9bcD	8.8defgCD	29.33cdeBC	43.8cdeAB	56.8cdAB	67.4cdA
HP33-1	2.6aD	7.2abcD	43.8abcC	56.63bcBC	73.4bcBC	82.2bcAB	98.7abA
HP5-2	0.0aB	0.9bcB	1.9fgB	1.92fghB	1.9hB	7.4fgAB	27.0efA
GN22-3–2	0.0aD	1.9bcCD	10.2cdefgBC	27.14cdefB	54.0cdA	60.4cdA	76.9bcA
GN2	3.4aCD	5.3abcCD	11.4cdefgC	22.96cdefgBC	39.0cdefAB	47.6cdeAB	62.5cdeA
GN8-1(1)	8.8aDEF	17.9abCDEF	28.8bcdeBCDE	34.73cdBCD	45.2cdeBC	67.0cdAB	85.6abcA
HP3-1	4.2aD	11.4abcD	39.4bcdC	80.34abB	96.6abAB	100.0aA	100.0aA
HP22	3.4aABC	7.5abcABC	10.2cdefgABC	12.99defghAB	13.0efghAB	16.1efA	21.3fA
GN22-1	9.8aDE	15.7abCDE	26.2bcdefBCDE	45.59bcdABCD	54.2cdABC	67.0cdAB	77.2bcA
HP14	0.9aAB	1.9bcAB	3.4efgAB	4.24efghAB	6.2ghAB	7.2fgAB	14.1fgA
GN16	4.2aD	16.1abD	49.8abC	77.39abBC	90.0abAB	98.7abA	99.5aA
HP15	0.0aD	0.2bcCD	5.2efgC	35.33cdB	52.0cdAB	60.4cdA	72.5cdA
HP19	3.4aC	7.4abcBC	10.0cdefgABC	25.36cdefAB	27.3defgAB	31.2defAB	35.1defA
Control	0.0a	0.0bc	0.0 g	0.0 h	0.0 h	0.0 g	0.0 g

[a] DAT: Days after treatment

[b] Means in a column followed by the same lowercase letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

[c] Means in a line followed by the same capital letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

Table 3 Mortality of *Tropinota hirta* exposed to GN16 isolate

Mortality (%)								
Concentration (conidia ml^{-1})	**3 DAT[a]**	**5 DAT**	**7 DAT**	**9 DAT**	**11 DAT**	**13 DAT**	**15 DAT**	**17 DAT**
1×10^6	0.0b[b]D[c]	0.2bcD	1.9cD	16.3cC	39.6cB	46.0bB	60.4bB	90.0bA
1×10^8	0.0bE	3.4bE	27.5bD	56.4bC	83.7bB	92.5aB	99.8aA	100.0aA
1×10^9	5.3aE	29.5aD	68.5aC	90.0aB	99.1aAB	99.1aAB	100.0aA	100.0aA
Control	0.0b	0.0c	0.0c	0.0d	0.0d	0.0c	0.0c	0.0c

[a] DAT: Days after treatment

[b] Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

[c] Means in a line followed by the same capital letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

Table 4 Mortality of *Tropinota hirta* exposed to GN22-1 isolate

Mortality (%)								
Concentration (conidia ml^{-1})	**3 DAT[a]**	**5 DAT**	**7 DAT**	**9 DAT**	**11 DAT**	**13 DAT**	**15 DAT**	**17 DAT**
1×10^6	0.0D	0.2b[b]D[c]	3.4bCD	14.7bC	35.7cB	47.6bB	56.0bB	83.7bA
1×10^8	0.0E	3.4abE	23.7aD	39.6aCD	62.4bC	83.7aB	92.5aAB	99.1aA
1×10^9	0.0F	7.5aE	31.5aD	52.0aD	80.3aC	88.6aBC	96.6aAB	100.0aA
Control	0.0	0.0b	0.0c	0.0c	0.0d	0.0c	0.0c	0.0b

[a] DAT: Days after treatment

[b] Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

[c] Means in a line followed by the same capital letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

Table 5 Mortality of *Tropinota hirta* exposed to GN29-1 isolate

Mortality (%)								
Concentration (conidia ml^{-1})	**3 DAT[a]**	**5 DAT**	**7 DAT**	**9 DAT**	**11 DAT**	**13 DAT**	**15 DAT**	**17 DAT**
1×10^6	0.0b[b]D[c]	0.0bD	5.3bD	27.5bC	43.6cBC	64.7bB	90.0bA	94.8bA
1×10^8	0.0bE	10.2aD	52.0aC	92.5aB	96.6bAB	100.0aA	100.0aA	100.0aA
1×10^9	1.9aD	23.5aC	65.5aB	99.1aA	100.0aA	100.0aA	100.0aA	100.0aA
Control	0.0b	0.0b	0.0b	0.0c	0.0d	0.0c	0.0c	0.0c

[a] DAT: Days after treatment

[b] Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

[c] Means in a line followed by the same capital letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

Table 6 Mortality of *Tropinota hirta* exposed to HP3-1 isolate

Mortality (%)								
Concentration (conidia ml^{-1})	**3 DAT[a]**	**5 DAT**	**7 DAT**	**9 DAT**	**11 DAT**	**13 DAT**	**15 DAT**	**17 DAT**
1×10^6	0.0a[b]E[c]	0.0bE	4.3bE	27.5cD	48.0cCD	64.7cBC	83.7bB	99.1aA
1×10^8	0.0aE	0.9bE	23.7aD	52.0bC	82.1bB	96.6bA	100.0aA	100.0aA
1×10^9	0.9aE	13.0aD	41.6aC	72.5aB	96.6aA	100.0aA	100.0aA	100.0aA
Control	0.0a	0.0b	0.0c	0.0d	0.0d	0.0d	0.0c	0.0b

[a] DAT: Days after treatment

[b] Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

[c] Means in a line followed by the same capital letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

Table 7 Mortality of *Tropinota hirta* exposed to HP33-1 isolate

Mortality (%)								
Concentration (conidia ml^{-1})	**3 DAT[a]**	**5 DAT**	**7 DAT**	**9 DAT**	**11 DAT**	**13 DAT**	**15 DAT**	**17 DAT**
1×10^6	0.0a[b]E[c]	0.0bE	5.3cD	25.6cC	41.8cC	68.5cB	83.7bB	99.1aA
1×10^8	0.0 ± 0.0aG	10.2aF	33.4bE	52.0bD	78.2bC	96.6bB	100.0aA	100.0aA
1×10^9	0.2 ± 0.7aE	23.5aD	58.4aC	83.7aB	99.1aA	100.0aA	100.0aA	100.0aA
Control	0.0 ± 0.0a	0.0b	0.0d	0.0d	0.0d	0.0d	0.0c	0.0b

[a] DAT: Days after treatment

[b] Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

[c] Means in a line followed by the same capital letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test)

concentration*time, isolate* concentration*time interactions were statistically significant (Table 8).

When the values were examined, it was seen that GN29-1 isolate caused the fastest effect in all LT rates among the isolates applied against ABB (LT_{30}: 5.897, LT_{50}: 6.817, LT_{90}: 9.715). HP33-1 isolate is the second isolate that causes the fastest death in respect of LT_{30} (6.722) and LT_{50} (8.060) rates. HP3-1 isolate was found to be the second isolate causing the fastest mortality in terms of LT_{90} (12.333) rate. The slowest effect in all LT values was determined in GN22-1 isolate (LT_{30}: 7.651, LT_{50}: 9.416, LT_{90}: 15.638) (Table 9).

Discussion

The results showed that all *B. bassiana* EPF isolates used in concentration-mortality trials were significantly effective at 1×10^8 and 1×10^9 conidia ml^{-1} concentrations from the 11th day. This effect increased above 80% in all isolates by the end of the 13th day. 17 days after post-inoculation, it was observed that the effect exceeded 90%, including the lowest concentration (except 1×10^6 conidia ml^{-1} concentration of GN22-1). Only one investigation had reported the susceptibility of ABB to EPFs. In that study, similar to the present, it was reported that the effectiveness of *B. bassiana* and *Fusarium* sp. against

Table 8 ANOVA parameters for main effects and interactions for mortality of *Tropinota hirta* adults

Source	*DF*	*F*-value	*P* value
Isolate	4	62.91	0.000
Concentration	3	4175.51	0.000
Time	8	1996.21	0.000
Isolate* Concentration	12	15.73	0.000
Isolate*Time	32	5.74	0.000
Concentration*Time	24	255.34	0.000
Isolate* Concentration*Time	96	2.27	0.000
Error	1620		
Total	1799		

ABB adults at 1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1} concentrations was found as 70, 90 and 90% for *B. bassiana* and 50, 70 and 75% for *Fusarium* sp., respectively on the 14th day (Atmaca et al. 2018).

According to the results of the single concentration trial, the effects of HP36-3(1), HP5-2, HP22, HP14 and HP19 isolates against ABB at the end of the 17th day did not exceed 40%. The virulence of isolates obtained from the same fungal species may differ from each other depending on some factors such as toxins produced by pathogens (Wang 2021).

Since native isolates can be ecologically compatible with the pest species, their use against the target pest always has advantages in comparison to exotic isolates (Sutanto et al. 2021). All of the isolates used in concentration-mortality studies were native isolates and showed a significant effect against ABB. In addition, ABB spends its larval and pupal stages in the soil and overwinters in the soil as adults (Kara 1995). Many hypomycetous fungal entomopathogens are considered soil-borne microorganisms and have significant potential in the control of pests in the soil (Keller and Zimmermann 1989).

Conclusions

All of the isolates (GN16, GN22-1, GN29-1, HP3-1 and HP33-1) used in concentration-mortality studies showed significant activity against ABB adults. The present study showed that these EPFs can be used as a bio-control agent against adults of ABB. Further studies should be conducted under orchard conditions.

Abbreviations

ABB: Apple Blossom Beetle; EPFs: Entomopathogenetic fungi; PDA: Potato Dextrose Agar; LT: Lethal time.

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Author contributions

SU, TA and YY conceived and designed the research. SU conducted the experiments. TA analyzed the data and wrote the manuscript. YY corrected and revised the manuscript, corrected language mistakes and translation, and corrected references. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset(s) supporting the conclusions of this article is (are) included within the article (and its additional file(s)).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Table 9 Lethal time (LT_{30}, LT_{50} and LT_{90}) values of adults of *Tropinota hirta* treated the isolates of entomopathogenic fungi at 1×10^8 conidia ml^{-1} (day)

Isolates	Slope ± SE	LT_{30} (95% fiducial limit)	LT_{50} (95% fiducial limit)	LT_{90} (95% fiducial limit)	Heterogeneity	chi-square
GN16	7.065 ± 0.617	7.058 (6.501- 7.541)	8.374 (7.864–8.865)	12.715 (11.842–13.920)	0.44	34.404
GN22-1	5.817 ± 0.518	7.651 (7.002–8.217)	9.416 (8.815–10.016)	15.638 (14.334–17.539)	0.43	33.563
GN29-1	8.330 ± 0.814	5.897 (5.421–6.300)	6.817 (6.389–7.222)	9.715 (9.074–10.609)	0.31	24.408
HP3-1	8.248 ± 0.739	7.449 (6.925–7.901)	8.624 (8.150–9.081)	12.333 (11.566–13.390)	0.42	33.033
HP33-1	6.649 ± 0.579	6.722 (6.166–7.205)	8.060 (7.548–8.556)	12.562 (11.655–13.823)	0.33	26.213

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