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Efficacy of Entomopathogenic Fungi, *Beauveria bassiana, Isaria fumosorosea* and *Metarhizium anisopliae* Against Green Shield bug, Palomena prasina L. (Heteroptera: Pentatomidae)

Şeyma YİĞİT[1], İslam SARUHAN[2]
[1,2]Ondokuz Mayıs Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Samsun-Türkiye
[1]https://orcid.org/ 0000-0003-2268-5103, [2]https://orcid.org/0000-0003-0229-9627
✉:seyma.yigit @omu.edu.tr

ABSTRACT
In this study, the entomopathogenic fungi; *Beauveria bassiana*, *Isaria fumosorosea* and *Metarhizium anisopliae* were evaluated against fourth nymphs and adults of the green shield bug, *P. prasina*. The experiment was conducted both under field and laboratory conditions. Isolates included six *Beauveria bassiana,* one *Metarhizium anisopliae* and one *Isaria fumosorosea*. Isolates were bioassayed against nymphs and adults in both under field and laboratory conditions. Four replications of the bioassays were analyzed and evaluated daily for 14 days. LT_{50} and LT_{95} values for the experimented isolates ranged from 4.19 to 7.11 days and from 6.68 to 11.30 days, respectively in laboratory for nymphs, ranged from 4.98 to 7.18 days and from 8.03 to 12.22 days, respectively in laboratory for adults. LT_{50} and LT_{95} values for the experimented isolates ranged from 6.69 to 10.70 days and from 10.29 to 17.91 days, respectively in field for nymphs, ranged from 7.29 to 10.70 days and from 11.23 to 17.91 days, respectively in field for adults. It was obvious that BB1/21b *(Beauveria bassiana)* was the most virulent on adults of *P. prasina*. As a result, while the effect of the isolates used in the study was high due to controlled conditions in the laboratory, it was low in field conditions.

Plant Protection

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Entomopatojenik funguslar*; Beauveria bassiana, Isaria fumosorosea* ve *Metarhizium anisopliae*'nın *Palomena prasina* L. (Heteroptera: Pentatomidae)'ya karşı etkileri

ÖZET
Bu çalışmada *Beauveria bassiana*'nın altı farklı ırkı), *Isaria* (*Paecilomyces*) *fumosorosea* ve *Metarhizium anisopliae* izolatlarının birer ırkı *Palomena prasina* L. (Heteroptera: Pentatomidae)'nın nimf ve erginlerine karşı laboratuvar (25±1 ^{0}C, % 70 nem ve 16:8 h (ışıklı: karanlık) ve arazi koşullarında biyolojik etkinlik denemeleri yapılmıştır. Ayrıca bazı izolatların laboratuvar şartlarında *P. prasina*'nın yumurtaları üzerine etkinliği belirlenmiştir. Laboratuvar çalışmalarında izolatların $1x10^8$ konidi/mL spor yoğunluğu, 5'er adet ergin ve nimf kullanılmış ve çalışma 4 tekerrürlü olarak yürütülmüştür. Çalışma 14 gün boyunca takip edilerek yüzde ölüm oranları, LT_{50} ve LT_{95} değerleri belirlenmiştir. Çalışma sonucunda *P. prasina*'nın nimflerine karşı laboratuvar şartlarında kullanılan izolatların LT_{50} ve LT_{95} değerlerine bakıldığında sırasıyla; 4.19 - 7.11 gün ile 6.68 - 11.30 gün arasında bulunmuştur. Erginlerde ise LT_{50} ve LT_{95} değerlerine bakıldığında sırasıyla; 4.98 - 7.18 gün ve 8.03- 12.22 gün arasında belirlenmiştir. Arazi şartlarında ise nimflere karşı yapılan çalışmada izolatların LT_{50} ve LT_{95} değerlerine bakıldığında sırasıyla; 6.69 - 10.70 gün ve 10.29 -17.91 gün olarak belirlenmiştir. Erginlerde ise LT_{50} ve LT_{95} değerleri 7.29 - 10.70 gün ve 11.23 - 17.91 gün arasında değişmektedir. Yapılan çalışmada kullanılan tüm izolatların *P. prasina* ergin ve nimflerine karşı arazi şartlarındaki etkinliği

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labaoratuvar şartlarına nazaran daha düşük olmuştur. Sonuç olarak çalışmada kullanılan tüm izolatların *P. prasina*'ın ergin ve nimflerine karşı biyolojik mücadele de kullanılma potansiyeli sahip olduğu ortaya konulmuştur.

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INTRODUCTION

Hazelnut is one of the most important Turkish export products with 2, 3 billion US dollars yearlyApproximately 70% of the world's hazelnuts are grown in Turkey especially in the Black Sea region and some areas of Marmara which are equal to nearly 700.000 ha. Almost 400.000 families earn their living from hazelnut cultivation (Erper et al., 2016; Anonymous, 2019a, Anonymous, 2019b). There are many insect pests attacking hazelnut orchards that affect hazelnut production as well as quality in Turkey. The one of the most crucial is the Pentatomidae family, which was not well understood to reduce the quality of hazelnut seed by the farmers (Tavella *et al.*, 2001, Tuncer *et al.*, 2004). The most vital member of the Pentatomidae family is *Palomena prasina* (Hemiptera: Heteroptera: Pentatomidae) which is widely spread in Turkey's hazelnut orchards (Tuncer *et al.*, 2005). On average, spotted kernel damage caused by *P. prasina* in Italy was 1.3-4.0 % and in Turkey 9.58% (Tavella *et al.,* 2001; Saruhan and Tuncer, 2010).

This pest insect stays for almost five months in the hazelnut orchard causing a high economic loss nowadays, some chemicals have been used to control *P. prasina*. However, there is a need to develop alternative methods to cope with *P. prasina* due to the known side effects of pesticides used in hazelnut orchards. Biological control using entomopathogenic fungi is an alternative method instead of using chemicals pesticides. Entomopathogenic fungi (EPF) are common natural enemies of arthropods. Hence, they are attracting attention worldwide as potential biological control agents. There are more than 700 species of entomopathogens in the fungal kingdom (Roy *et al.,* 2006; Sandhu *et al.*, 2012). Fungal entomopathogens such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikof) Sorokin, *Isaria farinosa* (Holm: Fries), *Lecanicillium* spp. and *Simplicillium* spp. play an important role in regulating insect populations (Shah and Pell 2003, Zimmermann 2008, Gurulingappa *et al.,* 2011).

Beauveria bassiana has wide host range of hosts including hemipteran species (Gouli et al., 2011). Due to its environmentally friendly nature, bio-persistence and ability to kill pests at various developmental stages in their life cycle, the use of *B. bassiana* is a great importance in Integrated Pest Management (IPM) programs is of great importance (Kumar and Sultana, 2017). The spores of *B. bassiana* attach to the insect's cuticle, they germinate, the hyphae penetrate the insect's body, and proliferate. The insects die after about 3–5 days and infected cadavers may serve as a source of spores for secondary spread of the fungus. Entomopathogenic fungi especially insects with stinging sucking mouth structure used in the fight against cuticle and beetles by infecting agents. Entomopathogenic most of the fungi are *Metarhizium*, *Beauveria*, *Trichoderma*, *Verticillium*, *Nomuraea*, It belongs to the genus Entomophtora and Neozygite and is effective on many insect species (Deshpande, 1999). *Beauveria bassiana* and *Metarhizium* many harmful effects of *Anisopalia* entomopathogens used in biological control against insect species reported and their commercial preparations developed and released to the market (Wraight et al., 2001; Copping, 2004).

The aim of this study was to determine the pathogenicity of six isolates of *B. bassiana* (BB 1/23, BB1/216, B kür 1/3a, B kür 1/b, BÇE9 and BB 1/a), one isolate of *I. fumosorosea* (TR78-3), and one isolate *M. anisopliae* (TR-106) against fourth instar nymphs, adults and eggs of *P. prasina* L. under laboratory and field conditions.

MATERIALS and METHODS

Insect culture (adults)

The adults of *P. prasina* were collected from different hazelnuts orchards by beating-sheet method during april and may in Samsun province. Then, they have transferred to the insects were conserved in climate chamber for egg-laying. The eggs were surface disinfected by immersion in ethyl alcohol (70%) for 45 seconds. So fourth instar nymphs and adults were used. The insects were maintained in a climate chamber at 25±1°C, 70±5% Relative Humidity, and 16:8 hours light: dark photoperiods. After the pest was adapted to the laboratory condition, the insect culture was fed with fresh bean pods (Sırık 97) (*Phaseolus vulgaris* L.) and food was renewed daily. They developed into nymphs and adults.

Fungal Cultures

(The EPFs) *Metarhizium anisopliae* (TR-106) and *Isaria* (*Paecilomyces*) *fumosorosea* (TR-78-3), and *Beauveria bassiana* (BB1/23, BB1/216, B kür 1/3a, B kür 1/b, BB 1/a, BÇE9) used in the study were obtained from the stock cultures of Mycology Laboratories of, Ondokuz Mayıs University and Ankara University. The eight isolates of EPF (Table 1) were incubated on potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) at 25 ± 1 °C for 10–14 days. Conidia were harvested by sterile distilled water, containing 0.02% Tween 20. Then, conidia suspensions were filtered through four layers of sterile cheesecloth to remove mycelium, and conidia were counted under an Olympus CX-31 compound microscope (Olympus America Inc., Lake Success, NY), using a Neubauer hemocytometer to calibrate a suspension of 1×10^8 conidia mL^{-1} of each isolate (Erper et al. 2016).

Table 1. Isolates of entomopathogenic fungi used in this study.
Tablo1. Çalışmada kullanılan entomopatojen fungusların detayları

Isolates	Isolate denomination	Host	Location of collection
Metarhizium anisopliae	TR-106	*Xylosandrus germanus*	Samsun province, Turkey
Isaria fumosorosea	TR-78-3	*Hyphantria cunea*	Samsun province, Turkey
Beauveria bassiana	BB 1/23	*Eurygaster integriceps*	Ankara province, Turkey
Beauveria bassiana	BB 1/216	*Eurygaster integriceps*	Ankara province, Turkey
Beauveria bassiana	B Kür 1/3a	*Eurygaster integriceps*	Ankara province, Turkey
Beauveria bassiana	B Kür 1/b	*Eurygaster integriceps*	Ankara province, Turkey
Beauveria bassiana	BÇE -9	*Eurygaster integriceps*	Ankara province, Turkey
Beauveria bassiana	BB 1/a	*Eurygaster integriceps*	Ankara province, Turkey

Conidial germination assessment

The viability of conidia of the eight isolates belonging to *B. bassiana, I. fumosorosea, M. anisopliae* was determined. A conidial suspension (200 µl) of each isolate at (1×10^4 conidia ml^{-1}) obtained by dilution was sprayed onto Petri plates (9-cm dia.), containing PDA (Merck Ltd., Darmstadt, Germany). These plates were incubated at 25 ± 1 °C. After 24 h of incubation, the percentage of germinated conidia was counted, using an Olympus CX-31 compound microscope at × 400 magnification. Conidia were regarded as germinated, when they produced a germ tube, at least half of the conidial length. The germination ratios for each isolate were calculated after examining a minimum of 200 conidia from each of the three replicate plates (Saruhan et al. 2015).

Experimental design

This study used the nymphs and adults of *P. prasina* that were cultured in the laboratory in advance. During the experiments, 1 L plastic embedded cups were used. Bottoms of the cups were embedded with filter papers moisturized by sterile-distilled water. Holes were pierced into the cover of cups to allow aeration. Five nymphs and 5 adults of *P. prasina* were placed on plastic cups. onidial suspensions (1×10^8 conidia mL^{-1}) of the entomopathogenic fungi were applied to the nymphs and adults of *P. prasina* (5 mL per cup) using a Potter spray tower (Burkard, Rickmansworth, Hertz UK). Only sterile-distilled water was sprayed to control the plastic cups. The nymphs and adults were fed with fresh (Sırık 97) been and renewed daily. After supplying the feed to the nymphs and the adults, the plastic cups were closed and incubated at 25 ± 1°C, 75 ± 5% RH, and 16:8 hours light: dark photoperiod for 14 days in a Binder incubator (Model KBWF 240; Germany). The above culture was observed daily. The trials were observed for 14 days and during this period after the dead individual numbers were recorded and the cadavers were removed. The laboratory bioassay for eggs was conducted to examine effects of isolates on egg mortality. The conidial suspensions ($1x10^8$ conidia mL^{-1}) (2 mL per Petri dish) were applied to *P. prasina*'s eggs. For each isolates two egg mass were treated.

The eggs of *P. prasina* taking from the field culture were washed with sterile distilled water and two eggs of 28 pieces (28x2=56) eggs masses were placed on bottoms of Petri dishes cup cover with filter paper.

Field experiments

The hazelnut orchards were selected and cheesecloth cages (30 x 75 cm) were attached to each branch (Cages are attached to branches with fruit for feeding insects). Ten nymphs and the ten adults/replicate were put into separate cages. Then conidial suspensions ($1x10^8$ conidia mL^{-1}) were sprayed into those bags. Then conidial suspensions ($1x10^8$ conidia mL^{-1}) were sprayed into those cages. However, only sterile distilled water was sprayed to the control cages. It was carried out in four repetitions according to the coincidence blocks trial design.

The above cultures were observed for 14 days, and

during this period we recorded the dead ones on days of 1, 3, 5, 7 and 14.

Dead individuals on which the fungal sporulation was observed, were counted under a Leica EZ4 educational stereomicroscope at 40-70X magnification. Evidence of *B. bassiana, I. fumosorosea* and *M. anisopliae* on nymph and adult cadavers was verified by microscopic inspection (Meng,2017).

The biological efficiency values were obtained by using Abbott's formula.

Statistical analysis

The death rate was calculated by dividing the dead insects by the initial number of insects. The mortality data were corrected with Abbott's formula (Abbott, 1925). Serial-time mortality data from bioassays were examined by probit analysis, also, SPSS software (SPSS Inc., Chicago, Illinois, USA, Version 21) was used to calculate 50% lethal time (LT_{50}) and 95% lethal time (LT_{95}).

RESULT and DISSCUSSION

All fungi evaluated in this study were effective against the nymphs and the adults of *P. prasina* in the laboratory. More than 85% of the *P. prasina* nymph died at the end of 14th days. *Beauveria bassiana* B kür 1/b, BB1/a, BB1/21b and Bkür1/3a isolates caused complete (100%) mortality on the nymphs at the end of the 14th days. On the other hand, *M. anisopliae* TR-106, *B. bassiana* BB1/23, and *I. fumosorosea* TR-78-3 showed 95, 90 and 85% mortality at the same application period, respectively (Figure 1). Statistical analysis of the efficacy rate was found to be significant. Under laboratory conditions, over 60% mortality rate for all the adults of *P. prasina* was achieved at the end of the 14th day (Table 2).

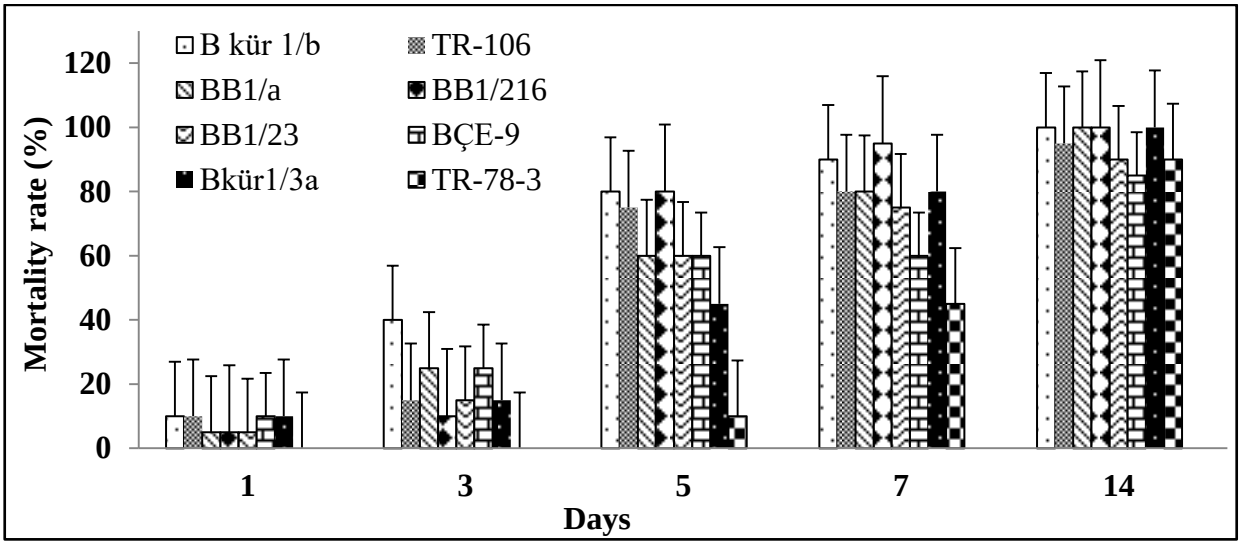

Figure 1. Effect of entomopathogenic fungi on 4th instar nymphs of *Palomena prasina* (% mortality rate) on laboratory conditions

Şekil 1. Laboratuvar koşullarında Palomena prasina 4. dönem nimflerine entomopatojen fungusların etkisi

Table 2. LT_{50} and LT_{95} values of Entomopathogenic fungi against 4th instar nymphs of *Palomena prasina* for laboratory condition

Tablo 2. Laboratuar koşullarında Palomena prasina 4. dönem nimfleri için entomopatojen fungusların LT_{50} and LT_{95} değerleri

Isolates	LT_{50} (95% fiducial limits for days)	LT_{95} (95% fiducial limits for days)
BÇE -9	5.26 (3.72-6.89)	11.30 (8.91-18.30)
BB 1/23	5.14 (4.04-6.24)	9.47 (7.94-12.82)
BB1/a	4.59 (3.82-5.33)	8.25 (7.17-10.19)
BB1/216	4.19 (2.83-5.54)	6.68 (5.38-11.03)
Bkür 1/3a	4.93 (3.63-6.30)	8.75 (7.12-13.06)
TR – 106	4.50 (2.44-6.30)	8.63 (6.68-15.72)
TR-78-3	7.11 (6.85-7.37)	9.61 (9.16-10.21)
Bkür 1/b	3.58 (2.76-4.31)	7.15 (6.11-9.05)

Figure 2 show that the isolates of B kür 1/b was 100% efficient, while the isolates of Bkür1/3a, TR-106, BÇE-9, TR-78-3, BB 1/a, BB1/216, and BB 1/23; 80%,75%, 70%, 70% 65%, 65%, and 60% mortality, respectively at the end of the 14th day.

It was obvious that BB1/216 was the fastest killer of *P. prasina*. Under laboratory conditions, over 60% death rate for all the adults of *P. prasina* was achieved at the end of the 14th day (Table 3).

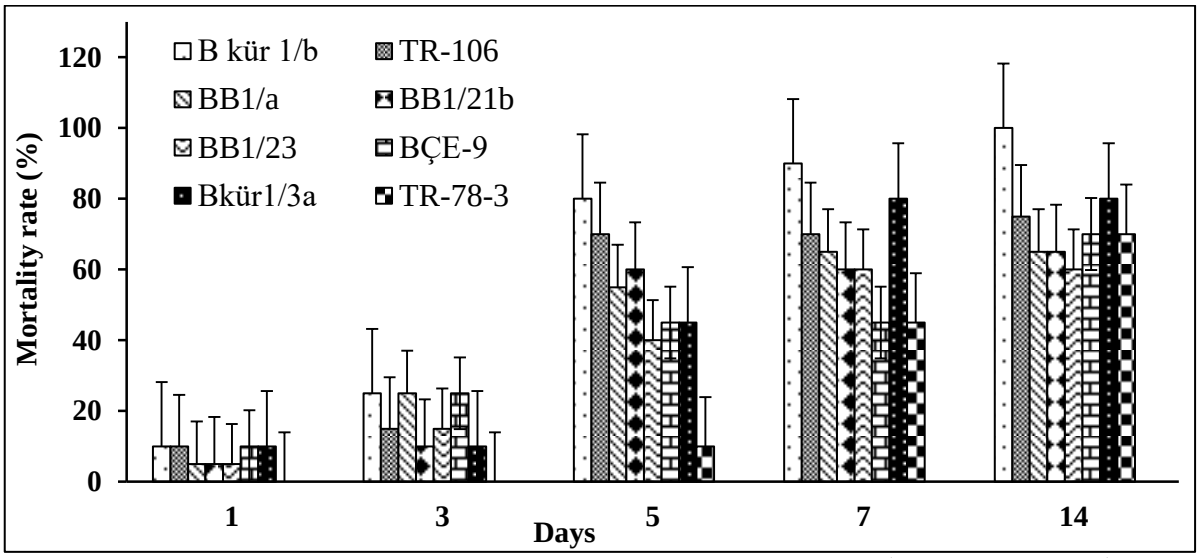

Figure 2. Effect of entomopathogenic fungi on adults of Palomena prasina (% mortality rate) on laboratory conditions ($P \geq 0.05$)

Şekil 2. Laboratuvar koşullarında Palomena prasina erginlerine entomopatojen fungusların etkisi

Table 3. Entomopathogenic fungal isolates against adults of Palomena prasina LT_{50} and LT_{95} values 14 days post- treatment on laboratory conditions.

Tablo 3. Laboratuvar koşullarında Palomena prasina erginlerine entomopatojen fungusların uygulanmasıyla 14 gün sonundaki LT_{50} and LT_{95} değerleri

Isolates	LT_{50} (95% fiducial limits for days)	LT_{95} (95% fiducial limits for days)
BÇE -9	6.02(3.52-10.33)	12.22(8.88-36.99)
BB 1/23	6.39(5.26-7.82)	11.44(9.44-16.38)
BB1/a	6.15(4.79-7.94)	11.37(9.11-18.04)
BB1/216	4.98(2.32-7.30)	9.48(7.21-20.82)
Bkür 1/3a	5.35(4.41-6.29)	9.26(7.93-11.92)
TR – 106	5.07(3.65-6.41)	8.03(7.39-13.44)
TR-78-3	7.18(6.53-7.95)	10.58(9.44-12.78)
Bkür 1/b	5.30(3.66-7.03)	8.91(7.14-15.33)

In a similar study with *P. prasina,* Erper et al. (2016) found that *Lecanicilium musacarium* and *B. bassiana* gave 98% and 95% mortality, respectively. Many studies claimed that *M. anisopliae* and *B. bassiana* were effective against Coleoptera species at different life stages (Prazak, 1991; Gindin *et al.*, 2006; Castrillo *et al.*, 2011; Ansari and Butt, 2012; Hirsch and Reineke, 2014; Carrillo *et al.,* 2015; Tuncer *et al.,* 2016; Kushiyev *et al.,* 2017; Liu *et al.,* 2017). Gindin *et al.* (2006) studied under laboratory conditions and discovered 85% death rate for adults of the *Rhynchophorus ferrugineus* Olivier that were sprayed with 1×10^8 spore mL^{-1} of *M. anisopliae* Ru isolates.

Another study found that *M. anisopliae* and *B. bassiana* were effective on adults of *Hylobius abietis* L. which is known as the most dangerous pests in the forest, especially *M. anisopliae* ARSEF4556 isolate was entirely efficient (%100 mortality) on the death rate of pest at the 12th day (Ansari and Butt, 2012). Concentrations of $1x10^6$ and $1x10^8$ from *Isaria farinosa* (Holm.) and *B. bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) were tested against *Aelia rostrata* Boh. (Hemiptera: Pentatomidae) and $1x10^8$ of *I. farinosa* was observed as efficient giving mortality rate of 70% on the 12th day whereas $1x10^8$ of *B. bassiana* (Balsamo) Vuillemin (Sordariomycetes:

Hypocreales) was found to be 100% efficient on the 9th day (Muştu et al. 2011).

Gouli et al. (2012) declared that three isolates of *B. bassiana* and two isolates of *M. anispoliae* were tested against *Halyomorpha halys* and were found to be 85% and 100% effective on the 9th and 12th day respectively. Moreover, Gouli *et al.*, (2012) claimed that *B. bassiana* had more efficacy than *M. anisopliae*. Goettel et al. (2005) postulated that entomopathogenic fungi could survive in different environmental conditions. Even though the isolates of B kür 1/6b, BÇE-9, BB1/216, Bkür1/3a, and TR-78-3 were efficient in the laboratory, under the field conditions they had a lower efficiency (Figure 3).

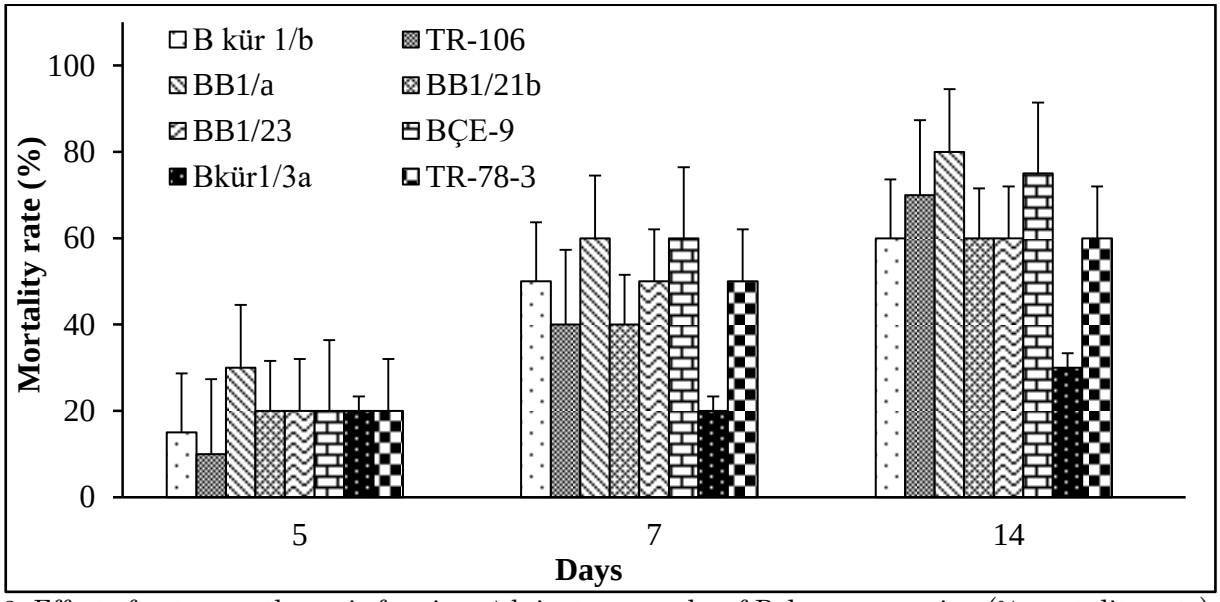

Figure 3. Effect of entomopathogenic fungi on 4th instar nymphs of Palomena prasina (% mortality rate) on field conditions. (P≥0.05)

Şekil 3. Tarla koşullarında Palomena prasinanın 4. dönem nimflerine entomopatojen fungusların etkisi

Under field conditions, at the lethal time values, for the isolates of the fungi sprayed against the nymphs of *P. prasina* were as follows: LT_{95} values were 10.29 days for BB1/a, 10.57 days for Bkür1/3a, 11.14 days for BB 1/23, 11.92 days for BÇE-9, 12.39 days BB 1/216, 13.06 days for B kür 1/b, 17.91 days for BB 1/216, and for TR-78-3 (Table 4).

According to Figure 4, at the end of the 14th day in the field conditions, the efficiency of the fungi Bkür1/3a, BB 1/a, B kür 1/b, BB1/216, BB 1/23, BÇE-9, TR-78-3 and TR-106 against the adults of *P. prasina* was 75, 70, 60, 60, 60, 50, 45, and 30%, respectively. Even though the isolates B kür 1/b was (100%) more efficient in the laboratory, than under the field conditions which decreased to reach only 50%.

Table 4. Entomopathogenic fungal isolates against 4th instar nymphs of *Palomena prasina* LT_{50} and LT_{95} values 14 days post- treatment on field conditions.

Tablo 4. Tarla koşullarında Palomena prasinanın 4. Dönem nimflerine entomopatojen fungusların uygulanmasıyla 14 gün sonundaki LT_{50} and LT_{95} değerleri

Isolates	LT_{50} (95% fiducial limits for days)	LT_{95} (95% fiducial limits for days)
BÇE -9	7.77(6.51-10.29)	11.92(9.71-20.76)
BB 1/23	7.74(7.41-8.11)	11.14(10.44-12.11)
BB1/a	6.69(5.58-8.04)	10.29(9.34-16.53)
BB1/216	7.95(6.85-10.01)	12.39(10.24-19.06)
Bkür 1/3a	7.02(5.94-8.38)	10.57(8.97-15.11)
TR – 106	10.70(Unable)	17.91(Unable)
TR-78-3	10.70(Unable)	17.91(Unable)
Bkür 1/b	8.09(6.19-18.54)	13.06(9.77-53.85)

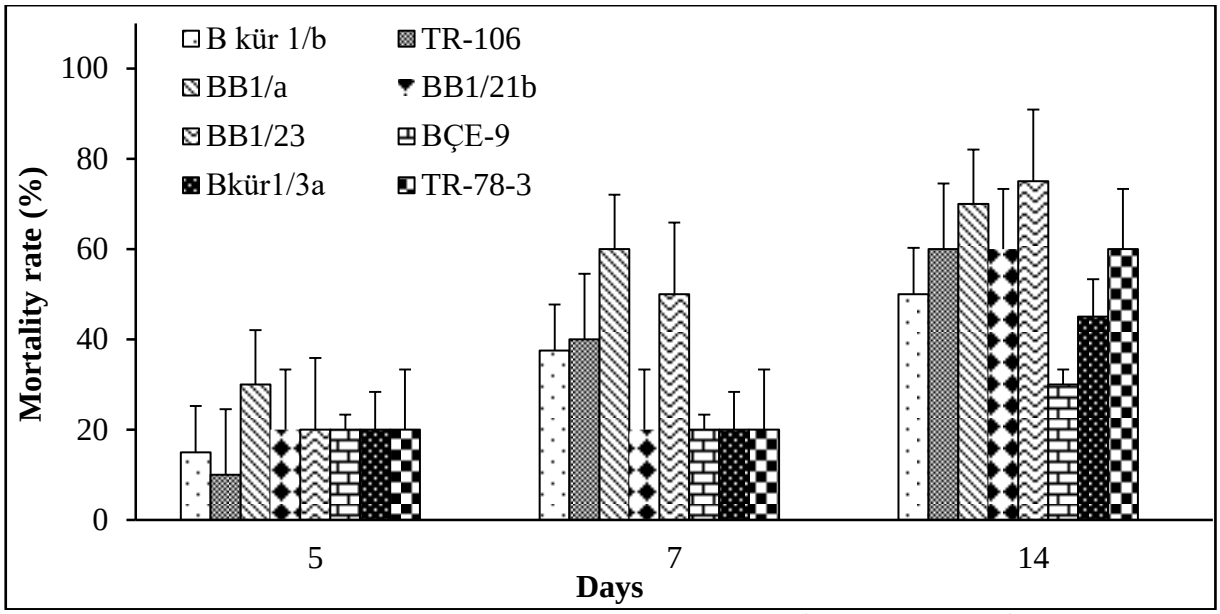

Figure 4. Effect of entomopathogenic fungi on adults *Palomena prasina* (% mortality rate) on field conditions
Şekil 4. Arazi koşullarında Palomena prasina'nın erginlerine entomopatojen fungusların etkisi

Under field conditions, at the lethal time values, in table 5, for isolates of fungi sprayed against the adult of *P. prasina* were as mentioned below LT_{95} values were 11.23 days for Bkür1/3a, 11.94 days for BB1/a, 11.95 days for BB1/23, 14.23 days for B kür 1/b, 14.89 days for BÇE-9, 17.91 days for BB1/216, TR-78-3 and TR-106.

When the entomopathogenic fungi were sprayed on the eggs of *P. prasina* the egg hatchability was 6, 40 and 75% for *Beaveria bassiana* Bkür 1/b, BB1/216 and BB 1/a, respectively (Table 6). Samuels *et al.* (2002) reported that egg hatching fluctuated between7.8%-43.3% when six different *B. bassiana* isolates applied against *Blissus antillus* (Hemiptera: Lygaeidae) at $5x10^6$ conidia/mL. As a result of the application of different entomopathogenic fungi isolates against *Nezara viridula* eggs, the egg hatching ranged from 0 to 63.33% (Permadi et al, 2020).

Table 5. Entomopathogenic fungal isolates against adults of *Palomena prasina* LT_{50} and LT_{95} values 14 days post- treatment on field conditions.
Tablo 5. Tarla koşullarında Palomena prasinanın erginlerine entomopatojen fungusların uygulanmasıyla 14 gün sonundaki LT_{50} and LT_{95} değerleri

Isolates	LT_{50} (95% fiducial limits for days)	LT_{95} (95% fiducial limits for days)
BÇE -9	9.21(7.22-31.42)	14.89(10.78-85.77)
BB 1/23	8.07(7.27-9.33)	11.95(10.34-15.78)
BB1/a	7.29(4.94-16.19)	11.94(8.95-55.81)
BB1/21	10.70(Unable)	17.91(Unable)
Bkür 1/3a	7.37(6.43-8.67)	11.23(9.59-15.39)
TR - 106	10.70(Unable)	17.91(Unable)
TR-78-3	10.70(Unable)	17.91(Unable)
Bkür 1/b	9.01(7.23-20.71)	14.23(10.62-51.34)

Table 6. Effects of entomopatogenic fungi isolates on *Palomena prasina* eggs hatchability.
Tablo 6. Entomopatojen fungusların Palomena prasina yumurta açılımına etkisi

Isolates	Days (% hatchability rate)			
	1	3	7	14
BB 1/a	0	0	60	75 **b***
B kür 1/b	0	0	6	6 **d**
BB1/21	0	0	35	40 **c**
Control	0	0	75	100 **a**

*Within columns, means followed by the same small letter do not differ significantly

In a study, *B. bassiana, M. anisopliae* isolates against *Orosanga japonica* (Melichar, 1898) (Hemiptera: Ricaniidae) were used both in the laboratory and in field conditions. As a result of the study, *B. bassiana* isolate was effective against the nymphs and adults of the pest at a rate of 74% and 18.5% in the laboratory, respectively, while it was 50.5% and 11% in field conditions. In the same study, *M. anisopliae* isolate was effective against the nymphs and adults of the pest 80% and 59.5% in laboratory. But this ratios were, 36% and 20% in field conditions, respectively (Göktürk, 2020). The effects of the isolates used in the study on adults were found to be lower than nymphs both in laboratory and field conditions. *M. brunneum* ısolate was used against *Dichelops furcatus* (Hemiptera: Pentatomidae) and it was stated that nymphs are more sensitive than adults (Romero et al.2020). In another study, it was determined that nymphs of *Halyomorpha halys* were more susceptible than their adults to some entomopathogenic fungi (Pike, 2014).

CONCLUSIONS

When considering the strategies with in which entomopathogenic fungi can be used in biological control, it is sometimes difficult to apply. But biological control (entomopatogenic fungi used to insects) is very important. It is possible to use the tested entomopathogenic fungi as potential biocontrol agents against *P. prasina* in quite large hazelnut plantations of Turkey, rather than using detrimental chemical pesticides. It is virulence that biocontrol fungi (especially BB1/a, BCE-9 and TR-106 for nymphs, BB1/23 and BB1/a for adults) to field conditions for *P. prasina*. These results show that this isolates could constitute a viable biological control agent for *P. prasina.*

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The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Abbott WS 1925. A method of computing the effectiveness of an insecticide. J. econ. Entomol, 18(2): 265-267.

Anonymous 2019a. Fındık raporu, Şubat 2015, Ankara. T.C. Gümrük ve Ticaret Bakanlığı, Kooperatifçilik Genel Müdürlüğü, 29s. http://koop.gtb.gov.tr/data/53319cec487c8eb1e43d7299/2014%20F%C4%B1nd%C4%B1k%20Raporu.pdf. Accessed 25 April 2020

Anonymous 2019b. 2014 yılı Fındık sektör raporu. Toprak Mahsulleri Ofisi Genel Müdürlüğü, http://www.tmo.gov.tr/Upload/Document/raporlar/2014findiksektorraporu.pdf. Accessed 25 April 2020.

Ansari MA, Butt TM 2012. Susceptibility of different developmental stages of large pine weevil *Hylobius abietis* (Coleoptera: Curculionidae) to entomopathogenic fungi and effect of fungal infection to adult weevils by formulation and application methods. Journal of Invertebrate Pathology. 111(1):33-40.

Carrillo D, Dunlap CA, Avery PB, Navarrete J, Duncan RE, Jackson MA, Behle RW, Cave RD, Crane J, Rooney AP, Peña JE 2015. Entomopathogenic fungi as biological control agents for the vector of the laurel wilt disease, the redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae). Biological Control. 81:44-50.

Castrillo LA, Griggs MH, Ranger CM, Reding ME, Vandenberg JD 2011. Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) against adult *Xylosandrus germanus* (Coleoptera: Curculionidae) and impact on brood. Biological Control, 58: 121-126.

Copping, L.G., 2004. The Manual of Biocontrol Agents. 3rd Ed., The BioPesticide, British Crop Protection Council, Alton, UK, pp. 702

Deshpande, M.V., 1999. Mycopesticide production by fermentation: Potential and challenges. Journal Critical Reviews in Microbiology, 25(3): 229-243.

Erper I, Saruhan I, Akca I, Aksoy HM, Tuncer C 2016. Evaluation of some entomopathogenic fungi for controlling the Green Shield Bug, *Palomena prasina* L. (Heteroptera: Pentatomidae). Egyptian Journal of Biological Pest Control. 26(3):573-578.

Gindin G, Levinski S, Glazer I, Soroker V 2006. Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil. Phytoparasitica. 34 (4):370-379.

Goettel MS, Eilenberg J, Glare T 2005. Entomopathogenic fungi and their role in regulation of insect populations. In: Gilbert, L.I.,

Iatrou, K., Gill, S.S. (eds). Comprehensive Molecular Insect Science. pp. 361-405.

Gouli V, Gouli S, Skinner M, Hamilton G, Kim JS, Parker BL 2012. Virulence of select entomopathogenic fungi to the brown marmorated stink bug, *Halyomorpha halys*(Stål) (Heteroptera: Pentatomidae). Pest management science. 68(2): 155-157.

Göktürk T 2020. Determination of the Lethal Effects of Some Entomopathogens on Orosanga japonica (Melichar, 1898) (Hemiptera: Ricaniidae). Turk J Agric Res. 7(3): 305-314

Gurulingappa P, Mcgee P, Sword GA 2011. In vitro and in plants compatibility of insecticides and the endophytic entomopathogen, *Lecanicillium lecanii*. Mycopathologia. 172: 161–168.

Hirsch J, Reineke A 2014. Efficiency of commercial entomopathogenic fungal species against different members of the genus Otiorhynchus (Coleoptera: Curculionidae) under laboratory and semi-field conditions. Journal of Plant Diseases and Protection, 121(5): 211-218.

Kumar S, Sultana R, Yanar, D 2017. Application of entomopathogenic fungi for insect pests control. Journal of Entomology and Zoology Studies, 5(6): 07-13.

Kushiyev R, Tuncer C, Erper I, Saruhan I 2017. Effectiveness of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against Anisandrus dispar (Coleoptera: Curculionidae: Scolytinae). ıx. International congress on Hazelnut. 152 p, 15-19.

Liu J, Zhang Y, Liu T, Tuncer C, Cheng Y 2017. Screening of a highly pathogenic strain against Hazelnut weevil and microscopic observation on its infection process. Journal of Beijing Forestry University. 39(3): 32-37.

Meng, X., Hu, J., Ouyang, G. (2017) The isolation and identification of pathogenic fungi from *Tessaratoma papillosa* Drury (Hemiptera: Tessaratomidae). Peerj, 5: 1-14 https://doi.org/ 10.7717/peerj.3888

Mustu M, Demirci F, Kocak E 2011. Mortality effects of *Isaria farinosa* (Holm.) and *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) on *Aelia rostrata* Boh. (Hemiptera:Pentatomidae). Turkish Journal of Entomology. 35(4): 559-568.

Pike TJ 2014. Interactıons Between The Invasıve Brown Marmorated Stınk Bug, *Halyomorpha halys* (Hemıptera: Pentatomıdae), And Entomopathogenıc Fungı. Thesis submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Master of Science. P 58.

Prazak RA 1991. Studies on indirect infectionof *Trypodendron lineatum* Oliv with *Beauveria bassiana* (Bals.) Vuill. Zeitschrift for Angewandte Entomologie. 111: 431–441.

Romero GR, Antúnez C, Sarubbi-Orue H, Garrido-Jurado B, Valverde-García P, Schade M, Popo TM (2020). Virulence of *Metarhizium brunneum* (Ascomycota: Hypocreales) Strains Against Stinkbugs *Euschistus heros* and *Dichelops furcatus* (Hemiptera: Pentatomidae). Journal of Economic Entomology, 113 (5): 2540–2545

Roy HE, Majerus MEN 2006. *Harmonia axyridis*: A successful biocontrol agent or an invasive threat? In: Eilenberg J, Hokkanen HMT (Eds) An ecological and societal approach to biological control. Dordrecht, The Netherlands: Kluwer Academic Publishers. 295–309

Samuels RI, Coracini DLA, Dos Santos C M, Gava CAT 2002. Infection of *Blissus antillus* (Hemiptera: Lygaeidae) eggs by the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Biological control, 23(3): 269-273.

Sandhu SS, Sharma AK, Beniwal V, Goel G, Batra P, Kumar A, Jaglan S, Sharma AK and Malhotra S 2012. Myco-Biocontrol of Insect Pests: Factors Involved, Mechanism, and Regulation. Journal of Pathogens. Article ID:126819. http://dx.doi.org/ 10.1155/ 2012/126819.

Saruhan I, Tuncer C 2010. Research of damage rate and type of green shield bug (*Palomena prasina* L. Heteroptera: Pentatomidae) of hazelnut. Anadolu Journal of Agricultural Sciences. 25(2): 75-83.

Saruhan I, Erper I, Tuncer C, Akca I 2015. Efficiency of some entomopathogenic fungi as biocontrol agents against Aphis fabae Scopoli (Hemiptera: Aphididae). Pak. J. Agri. Sci, 52(2): 273-278.

Shah PA, Pell JK 2003. Entomopathogenic fungi as biological control agents. Applied microbiology and biotechnology. 61(5-6): 413-423.

Tavella L, Arzone A, Miaja ML, Sonnati C 2001. Influence of Bug (Heteroptera, Coreidae and Pentatomidae) feeding activity on hazelnut in Northwest Italy. Acta Hort.556: 461-468.

Tuncer C, Saruhan I, Akça I 2004. The Insect Pest Problem Affecting Hazelnut Kernel Quality in Turkey. 6. International Hazelnut Congress. Tarragona, Spain ActaHort., p. 88.

Tuncer C, Saruhan I, Akça I 2005. The Insect Pest Problem Affecting Hazelnut Kernel Quality in Turkey. Acta Hort. 668: 367-376.

Tuncer C, Kushiyev R, Saruhan I, Erper I 2016. Determination of the effectiveness of entomopathogenic fungi *Metarhiziumanisopliae* and *Beauveria bassiana* against *Xylosandrus germanus* (Coleoptera: Curculionidae: Scolytinae). Turkey 6th Plant Protection Congress with International Participation. p 127.

Wraight, S.P., Jackson, M.A., Kock, S.L., 2001. Production, stabilization and formulation of fungal biocontrol agents. In: T.M. Butt, C. Jackson and N. Magan (Eds.), Fungi as biocontrol agents: Progress problems and potential, CABI Publishing, pp. 253-287.

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.