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Determination of Lethal Effects of Some Entomopathogenic Fungi on Different Biological Stages of *Leptinotarsa decemlineata* Say

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ABSTRACT

The lethal effects of *Beauveria bassiana* BIM-001 (1x10^6 spore mL^{-1}, 1x10^7 spore mL^{-1}, 1x10^8 spore mL^{-1}), *Fusarium subglutinans* 12A (obtained from cotton aphid in Adana-Karataş) and the commercial preparation of 1.5% *B. bassiana* strain Bb-1 (250 mL da; 1x10^8 spore mL^{-1}) (Nostalgist SL) on *Leptinotarsa decemlineata* were investigated. The isolates were applied to the eggs, larvae (1st, 2nd, 3rd, and 4th larval stages) and adults of *L. decemlineata* by spraying in laboratory conditions (25 ± 1°C, 60 ± 10% relative humidity and 16:8 [L: D] photoperiod). The observations to determine the lethal effect of isolates were performed up to 9 days for the eggs and larvae, and 21 days for the adults of *L. decemlineata* following the first spore suspension application. In the experiments conducted on the larval stages, it was determined that the mortality rates due to all entomopathogenic fungus isolates and at different concentrations were significantly different from the mortality rate of the control. The effect of all spore concentrations of *B. bassiana* BIM-001 was found significantly higher than other treatments and the mortality rates were 100% for all larval stages (P <0.05). In addition, the mortality rates were between 4 and 16% for adults across all treatment groups except for the control. Moreover, it has been determined that each three spore concentrations of *B. bassiana* BIM-001 also suppressed the egg hatching of *L.decemlineata* by 55 to 60%

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Bazı Entomopatojen Fungusların *Leptinotarsa decemlineata* Say'nin Farklı Biyolojik Dönemleri Üzerindeki Ölümcül Etkilerinin Belirlenmesi

ÖZET

Isparta patates üretim alanlarında bulunan patates böceği erginlerinden elde edilen *Beauvaria bassiana* BIM-001 (1x10^6 spor mL^{-1}, 1x10^7 spor mL^{-1}, 1x10^8 spor mL^{-1}), Adana-Karataş pamuk yaprakbitinde bulunan *Fusarium subglutinans* 12A ve 1.5% *Beauveria bassiana* strain Bb-1 (250 mL da; 1x10^8 spor mL^{-1}) (Nostalgist SL)'in püskürtme yöntemi ile *Leptinotarsa decemlineata*'nın ergin, larva (1., 2., 3., ve 4. larva) ve yumurtalarına ölüm etkileri laboratuvar koşullarında (25±1°C, %60±10 nem ve 16:8 fotoperiyot) araştırılmıştır. Entomopatojen fungusların farklı yaşam dönemleri üzerindeki öldürücü etkilerinin belirlenmesi amacıyla gözlemler ilk spor süspansiyonu uygulamasını takip eden 3., 5., 7. ve 9. günlerde gerçekleştirilmiştir. Larva dönemlerinde yapılan deneylerde, tüm entomopatojen fungusların ve spor konsantrasyonlarının etkisinin kontrolden farklı olduğu, *B. bassiana* BIM-001'in farklı spor konsantrasyonlarının etkisi diğerlerinden önemli ve tüm larva dönemleri için ölüm oranlarının %100 olduğu bulunmuştur (P <0.05). Ek olarak, kontrol dışındaki tüm denemelerde erginlerde ölüm oranları %4 ile %16 arasında bulunmuştur. Ayrıca, *B. bassiana* BIM-001'in her üç spor konsantrasyonunun da *L. decemlineata*'nın yumurtadan çıkmasını %55 ila 60 oranında baskıladığı tespit edilmiştir.

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INTRODUCTION

Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae), Colorado potato beetle, is known to cause significant economic damages in many plants belonging to Solanaceae family in the World. This beetle is a polyphagous pest feeding on many plants including potato, eggplant, tomato, pepper and some weeds (Hsiao, 1978; Hare, 1990). It was reported that the most suitable host for this pest is *Solanum tuberosum* L. (potato) (Atak, 1973; Kekillioğlu and Yılmaz, 2018). Chemical management has been almost the only method for controlling this pest for about last 50 years (Kedici et al., 1998; Erdoğan and Toros, 2010). Negligent use of the pesticides is not only detrimental to human health but it also causes a tremendous negative effect on non-target organisms (Topuz, 2005; Nieder et al., 2018; Kılıç, 2019; Saha et al., 2021). Efforts for the protection of nontarget organisms from the destructive effects of pesticides have led scientists to search organisms in nature to be used for plant protection purposes. The studies on the subject revealed that the entomopathogenic fungi with around 700 known species were the most suitable organisms for these purposes. Fungi species such as *Beauveria bassiana* (Bals) Vull. (Ascomycota: Cordycipitaceae), *Metarhizium anisopliae* (Metsch) (Ascomycota: Clavicipitaceae), *Isaria fumosorosea* Wize (Ascomycota: Clavicipitaceae) and *Lecanicillium* (Verticillium) *lecanii* R. Zare & W. Gams, 2001 (Ascomycota: Cordycipitaceae) have been used commonly as biocontrol agent for pest control (Rath, 2000; Luangsa et al., 2005). *B. bassiana*, in particular, is used for controlling of many agricultural pests including *Cydia pomonella* L. (Lepidoptera: Tortricidae) and *L. decemlineata* (Coleoptera) (Kılıç and Yıldırım, 2008; Alves et al., 2002). In addition, *Fusarium subglutinans* (Ascomycota: Nectriaceae) attracting attention with its effectiveness in aphids and thrip species such as *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) (Erkılıç et al., 1999; Mailhot et al., 2007; Demirözer et al., 2010; Arıcı et al., 2012; Demirözer et al., 2015; 2016).

In current study, the effectiveness of *B. bassiana* BIM-001, *F. subglutinans* 12A and *B. bassiana* strain Bb-1 isolates on egg, 1st, 2nd, 3rd and 4th stage larvae and adults of *L. decemlineata* was investigated under laboratory conditions.

MATERIALS and METHODS

Materials

Colorado potato beetles (*L. decemlineata*) were collected from Isparta potato production areas. BIM-001 isolate of *B. bassiana*, obtained from Isparta potato production areas, 12A isolate of *F. subglutinans* isolated from *Aphis gossypii* Glover (Hemiptera: Aphididae) collected in the cotton production areas of Adana-Karataş, and Nostalgist (*B. bassiana* strain Bb-1, Agrobets Group Inc.) was purchased. The BIM-001 isolate was used at three different spore concentrations ($1x10^6$, $1x10^7$, $1x10^8$ spore mL^{-1}), yet, 12A isolate and Nostalgist used in the concentrations of $1x10^6$ spore mL^{-1} and $1x10^8$ spore mL^{-1}, respectively.

Methods

Preparation of *Beauveria bassiana* BIM-001 and *Fusarium subglutinans* 12A isolates

First, *B. bassiana* BIM-001 and *F. subglutinans* 12A isolates were cultured on potato dextrose agar (PDA) medium and incubated for 10 days at 25 ˚C in dark conditions. Then the spore suspensions of each isolate were diluted with sterile water, Tween 20 (0.1%) was added and the spores were counted via thoma slide under the microscope at 10× magnification (Nikon Eclipse E100). In this study, spore suspensions and concentrations were prepared according to method of Uzun et al. (2019). Spore viability was determined by measuring the rate of spore germination (Herlinda, 2010).

Application of entomopathogenic fungi to different life stages of *Leptinotarsa decemlineata*

The isolates of entomopathogenic fungi were sprayed with an apparatus capable of misting to different life stages of potato beetles with 1 atm pressure for 7 seconds (0.3 ml cm^2). The beetles were treated with sterile water only for the control group. The study was designed as a completely randomized plot design. The egg treatments were 10 replications and 10 eggs were used for each replication. The treatments were 5 replications for 1st, 2nd, 3rd, and 4th instar larvae and adults, and 10 individuals were used for each replication.

Egg treatments

The potato leaves obtained from rearing cages and containing a certain number of eggs were transferred to plastic Petri dishes (6 cm) containing a pre-moistened filter paper. The isolates were applied to the eggs by the spraying method as previously described.

Larvae and adult treatments

The different larvae stages and adult individuals of the Colorado potato beetle obtained from rearing boxes were transferred to plastic boxes (500 mL) with ventilation holes after being treated with the entomopathogenic fungus isolates depending on treatments in the study. Each of plastic boxes containing all life stages of the pest treated with all entomopathogenic fungus isolates separately were kept in 2 L plastic polyethylene bags for 48 hours. All experiments were conducted in the controlled chambers (26 ± 1 ºC temperature, 65 ± 5% humidity, and 16: 8 L: D photoperiod). The numbers of dead and alive organisms were determined until all died. Following applications, the observations were conducted on the 3, 5, 7, and 9th day for eggs and larvae, and 3, 5, 7, 14, and 21th days for the adults. The dead individuals were transferred to empty sterile. The re-isolation was made on the dead individuals were to determine whether the death was caused by entomopathogenic fungi. The beetles in the trials were fed with the potato plants materials (Agria variety) grown in the pots.

Statistical analysis

The SPSS 20.0 software was used for the statistical analyses of the data obtained. The one-way ANOVA analysis was performed to determine the effects of entomopathogenic fungus isolates on the mortality rates of *L. decemlineata* after arcsine transformation was applied to data and tested for normality before analysis. The p-value less than 0.05 was considered significant in all statistical tests.

RESULTS and DISCUSSION

Whereas the mortality rate of *L. decemlineata* adults treated with entomopathogenic fungus isolates was different from the control, however, the differences between them were not statistically significant (P > 0.05) in each (Table 1). The results revealed that entomopathogenic fungi used in the study showed limited lethal effects on *L. decemlineata* adults. The highest mortality rate (16%) was detected in the beetles treated with the spore concentrations $1x10^8$ spore mL^{-1} (P= 0.02, F= 1.793) and $1x10^6$ spore mL^{-1} (P= 0.03, F= 2.211) of BIM isolate. The mortality rates were 12% in $1x10^8$ spore mL^{-1} (P= 0.04, F= 3.014) of BIM isolate, and 8% and 4% in the Nostalgist and *F. subglutinans* 12A, respectively. Various studies in the literature have been reported the effectiveness of entomopathogenic fungi on the Colorado potato beetle adults varied. For instance, the mortality rates of *L. decemlineata* adults when treated with *B. bassiana* isolates ranged from 56% to 100%. Öztürk et al. (2015) determined that all adult beetles were killed with the use of three different *B. bassiana* isolates. It is known that entomopathogenic fungi of different genera and species have varying lethal effects on pests in certain families of Coleoptera. It has been reported that *B. bassiana*, *I. fumosorosea*, *I. farinosa*, *L. muscarium*, and *Paecilomyces farinosus* isolates caused death at the species belonging to Tenebrionidae belonging Coleoptera by 34% to 56% (Komaki et al., 2017). The study by Öz (2019) supported these results reporting that the lethal activity of three different isolates of *B. bassiana* was promising on *Sitophilus oryzae*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis*. However, it was determined that the mycopesticide and entomopathogenic fungi used in the current study did not show similar lethal effects on the adult of Colorado potato beetles, and the mortality rate was between 4 and 16%.

Table 1. Mortality rates (%) of *Leptinotarsa decemlineata* Say adults after 21 days in different applications
Çizelge 1. Leptinotarsa decemlineata Say erginlerinde uygulamalardan 21 gün sonra belirlenen ölüm oranları (%)

Treatments	N	Mortality Rates (%) ± S.E.
Control	50	0 ± 0.00 **b**
Nostalgist ($1x10^8$ spore mL^1)	50	8 ± 4.60 **a**
Fusarium subglutinans 12A ($1x10^6$ spore mL^{-1})	50	4 ± 5.23 **a**
Beauvaria bassiana BIM-001 ($1x10^6$ spore mL^{-1})	50	16 ± 7.14 **a**
Beauvaria bassiana BIM-001 ($1x10^7$ spore mL^{-1})	50	12 ± 5.12 **a**
Beauvaria bassiana BIM-001 ($1x10^8$ spore mL^{-1})	50	16 ± 3.36 **a**

a, b: Values followed by different letters in the same column are different (P <0.05)

The lethal effect of all fungus isolates was statistically different from the control for all larval stages. Although the effect of *B. bassiana* BIM-001 was different from those of *F. subglutinans and Nostalgist*, the difference was not statistically significant. In addition, the isolate concentration had no statistical effect on the mortality rate (P >0.05). The mortality rates were between 16% (the 1st larva stage subjected to $1x10^6$ spore concentration P= 0.02, F= 1.723) and 32% (the 4th larva stage subjected to $1x10^8$ spore concentration P= 0.04, F= 1.916) on the 3 days after the application. The highest mortality rate was recorded for the 3rd larva stage in the $1x10^8$ spore concentration on the 7 DAA. It changed between 70 and 90% in different spore concentrations for other larval stages. It was determined that all spore concentrations of *B. bassiana* BIM-001 isolate were effective against all larval stages, and provided 100% mortality on the 7 DAA. In addition, $1x10^8$ spore concentration of *B. bassiana* BIM isolate showed the

lethal effect in a relatively shorter time on the 3rd and 4th larvae, providing mortality rates of 90 and 94% on the 7 DAA, respectively. The lethal effects of *F. subglutinans* 12A and Nostalgist were not significantly different for the first two larval stages. Moreover, these two fungus isolates were found more effective for the 4th larva stage on the 9 DAA compared to the mortality rates detected on the other observation days. The mortality rates of *F. subglutinans* 12A and Nostalgist were 76 and 84%, respectively (Table 2).

Table 2. Mortality rates (%) determined after 9 days in different larval stages of *Leptinotarsa decemlineata* Say in different applications

Çizelge 2. Leptinotarsa decemlineata Say'in farklı larva dönemlerinde farklı uygulamalarda 9 gün sonunda belirlenen ölüm oranları (%)

Treatments	Mortality Rates (%) ± S.E. of Larvae			
	1st Stage	2nd Stage	3rd Stage	4st Stage
Control	0 ± 0.00 **Ca**	0 ± 0.00 **Ca**	4 ± 1.90 **Ca**	8 ± 1.19 C**a**
Nostalgist (1x10^8 spore mL^{-1})	12 ± 2.70 **Bb**	24 ± 8.20 **Bb**	60 ± 4.84 **Ba**	84 ± 5.78 **Aa**
Fusarium subglutinans 12A (1x10^6 spore mL^{-1})	16 ± 3.91 **Bb**	18 ± 2.21 **Bb**	64 ± 5.86 **Ba**	76 ± 5.56 **Ba**
Beauvaria bassiana BIM-001 (1x10^6 spore mL^{-1})	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**
Beauvaria bassiana BIM-001 (1x10^7 spore mL^{-1})	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**
Beauvaria bassiana BIM-001 (1x10^{8}spore mL^{-1})	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**	100 ± 0.00 **Aa**

A, B, C Values followed by different letters in the same column are different (P <0.05)
a, b, Values followed by different letters in the same row are different (P <0.05)

It was reported in previous studies that the different isolates of *B. bassiana* (1x10^8 spore mL^{-1}) caused 100% mortality on 7 days after application (DAA) at different larval stages of the potato beetle (2, 3, and 4th larval stages) (Öztürk et al., 2015). In the present study, the mortality rates of *B. bassiana* BIM isolate at three different spore concentrations in 1, 2, 3, and 4th larval stages were between 70 and 94% on the 7 DAA. It was observed that 100% mortality was reached 9 days after the application of isolates in all larval stages. Nostalgist containing *B. bassiana*, provided 50 to 84% mortality for the 4th larval stage after 7 and 9 days of the application, respectively. Similar to our results, Polat (2017) has also detected 90% mortality for the 3rd larval stage of *L. decemlineata* on the 10 DAA with the application of GOPT-552 isolate of *B. bassiana* (1x10^8 spore mL^{-1}). The lethal effect of *B. bassiana* isolates obtained from different hosts or geographical regions on *L. decemlineata* may vary. In addition, the methods used in the application of entomopathogenic fungus isolates affect the mortality levels. Güven et al. (2015) determined that *B. bassiana* BMAUM-001 isolate applied to 3rd larval stage of the Colorado potato beetle provided 72.7%, 64.5%, 67.7% mortality rates in the application methods of spraying, dipping, and using dry film, respectively. In the same study, the mortality rates of the three application methods were 83.6%, 92.9%, 90.8% for the BMAUM-002 isolate, and 83.6%, 59.7%, 79.2% for the BMAUM-003 isolate, respectively. Wraight and Ramos (2015) reported that conidiospores of the GHA isolate of *B. bassiana* caused 58.8% mortality when they were sprayed to the 2nd stage larva of *L. decemlineata* and up to 10% (on the 7 DAA) when applied directly to the leaf.

Although there are several studies in the literature exploring the lethal effect of *F. subglutinans* 12A against aphids and thrips (Erkiliç et al., 1999; Arıcı et al., 2012; Demirözer et al., 2015, 2016), to our knowledge, there is no information in the literature regarding its effectiveness on coleopteran species. It was found in the present study that the lethal effect of *F. subglutinans* 12A was 8% on *L. decemlineata* adults and 16% and 18% on the 1st and 2nd stage of the larvae, respectively. Moreover, *F. subglutinans* 12A resulted in the mortality rates of 64% and 84% for 3rd and 4th larval stages, respectively. Demirözer et al. (2010) reported that *F. subglutinans* 12A showed up to 70% lethal effect on the adults and 4th stage larvae *Chilocorus nigritus* (Fab.).

The effect of different isolates and different spore concentrations of entomopathogenic fungi on the egg hatching rate was also determined in the present study. The hatching rates of the eggs subjected to entomopathogenic fungus isolates were significantly different from the control. Additionally, egg hatching rates treated with the fungus isolates were lower compared the control for all observation days. On the 9th day after application, all eggs hatched at the control and the minimum egg hatching was seen in *B. bassiana* BIM-001 isolate that reduced the hatching rate by 55 to 60% (Table 3).

CONCLUSION

It could be concluded from the results of the current study that the entomopathogenic fungi had obvious lethal effects against the larval stages and eggs of *L. decemlineata*. On the other hand, they did not have a sufficiently high lethal effects on the adults of this pest. To reach more sound conclusion on effectiveness of these entomopathogenic fugal isolates against *L. decemlineata* as biocontrol agents further studies should be conducted to evaluate the efficacy of these isolates under *in vivo* conditions.

Table 3. Determined egg hatching rate (%) in all treatments and different observation times
Çizelge 3. Tüm uygulamalarda farklı gözlem zamanlarında belirlenen yumurta açılış oranı (%)

Treatments	Egg hatching rate (%) ± S.E. and observation times (Day)			
	3rd Day	5th Day	7th Day	9th Day
Control	34 ± 3.05 **Ca**	53 ± 3.00 **Ba**	92 ± 2.90 **Aa**	100 ± 0.00 **Aa**
Nostalgist ($1x10^8$ spore mL^{-1})	21 ± 2.33 **Db**	38± 2.90 **Cab**	50 ± 2.10 **Bb**	64 ± 3.05 **Ab**
Fusarium subglutinans 12A ($1x10^6$ spore mL^{-1})	11 ± 2.33 **Dbc**	26± 4.00 **Cb**	42 ± 5.21 **Bbc**	64 ± 4.76 **Ab**
Beauvaria bassiana BIM-001 ($1x10^6$ spore mL^{-1})	11 ± 1.79 **Cbc**	19± 2.76 **Cc**	29 ± 2.70 **Bc**	43 ± 3.00 **Ac**
Beauvaria bassiana BIM-001 ($1x10^7$ spore mL^{-1})	8 ± 2.49 **Cc**	22± 2.00 **Bc**	36 ± 2.20 **Abc**	45 ± 3.41 **Ac**
Beauvaria bassiana BIM-001 ($1x10^8$spore mL^{-1})	10 ± 2.58 **Cbc**	21± 3.48 **Bc**	34 ± 3.05 **Abc**	40 ± 3.33 **Ac**

A, B, C, D Values followed by different letters in the same row are different (P <0.05)
a, b, c Values followed by different letters in the same column are different (P <0.05)

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Researchers Contribution Rate Declaration Summary

The contribution of the authors is equal.

Conflict of Interest

The authors declare that there is no conflict of interest.

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