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Orijinal araştırma (Original article)

Mortality effects of *Isaria farinosa* (Holm.) and *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) on *Aelia rostrata* Boh. (Hemiptera: Pentatomidae) [1]

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Summary

The effects of entomopathogenic isolates, *Isaria farinosa* (Holm.) and *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales), were investigated on the adult stages of *Aelia rostrata* Boh. (Hemiptera: Pentatomidae) under of 70% and 95% relative humidity and with $1x10^6$ and $1x10^8$ conidial concentration (ml^{-1}). These experiments were conducted in a climatic chamber with 27±1°C and 16 h. light and 8 h. dark photoperiod, and the mortality percentages were determined in the 6th, 9th and 12th days of incubation. The result of experiments showed that both of the entomopathogens were more effective in 95% R.H. and $1x10^8$ conidial concentrations (ml^{-1}). At 70% R.H. and $1x10^8$ conidial concentrations (ml^{-1}), while *I. farinosa* caused 70% mortality in the 12th day of incubation, *B. bassiana* caused 100% mortality in the 9th day of incubation. It was concluded that *B. bassiana* isolate was more effective than *I. farinosa* isolate on wheat stink bug, *A. rostrata*.

Key words: *Aelia rostrata*, *Beauveria bassiana*, *Isaria farinosa*, entomopathogen

Anahtar sözcükler: *Aelia rostrata*, *Beauveria bassiana*, *Isaria farinosa*, entomopatojen

Introduction

Wheat stink bugs (*Aelia* spp.) are in the main pests of wheat. Lodos (1982) recorded 11 *Aelia* species in Turkey. The author stated that *Aelia rostrata* Boh. (Hemiptera: Pentatomidae) was the most important species. The bug causes damages on wheat in Greece, Hungary, France, Spain, Italy, Australia and old

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Yugoslavia. (Bullmann & Faber, 1958; Tadic, 1970; Campanella et al.,1978; Stavraki, 1978; Benedek, 1979; Gallego & Sanchez-Boccherini, 1980). The pest causes epidemics and has been included in pest management programmes (Memişoğlu et al., 1994). The pest caused 34% to 93%, yield losses in wheat between 1989 and 1991 in Ankara province (Memişoğlu et al., 1994).

Aelia rostrata overwinters as adult life stage under dry leaves or soil of surrounded of stub of *Pinus* spp., *Acantholimon venustum*, *Quercus* spp., *Astragalus acucilaris*, *A. microcephalus*, *Thymus* sp. on the mountains or hills named overwintering area. It can be found in all directions of the hills, but it prefers north side. Generally, it is found more densely at 1500-1700 m altitudes (Memişoğlu et al., 1996). During this period, several living organisms or environmental factors effect the population of *A. rostrata* (Kocatürk et al., 1994). Especially entomopathogenic fungi take part in the dormant stage, because of weakness of the pests after long time hibernation (Kocatürk et al., 1994). One of the main factors for the high level of the mortality was entomopathogenic fungi (Kocatürk et al., 1994). Another reason of the mortality in the hibernation area is the parasitoids belonging to Diptera and Nematoda (Memişoğlu et al., 1996). *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) were assumed as the most common entomopathogenic fungus causing the mortality (Kocatürk et al., 1994). Entomopathogenic fungi grow well under 20-25^{o}C and high relative humidities (Ferron, 1977). In addition to *B. bassiana*, the species belonging to, *Isaria* (formerly *Paecilomyces*) and *Verticillium* genera were isolated from related species, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) cadavers (Parker et al., 2003). Among the entomopathogenic fungi, *B. bassiana* (Balsamo) Vuillemin was dominant one. Another entomopathogenic genus, *Isaria*, includes two important pathogens on sunn pests. These are *Isaria farinosa* and *Isaria fumosorosea* (Parker et al., 2003). *Isaria farinosa* (Holm.) (Sordariomycetes: Hypocreales) is a ubiquitous insect parasite and also has an unlimited host range. The fungus recorded on wide range of insect hosts, including Lepidoptera, Diptera, Hemiptera, Coleoptera, Hymenoptera and Arachnida (Zimmermann, 2008). Furthermore, the entomopathogen can be isolated from soil, preferably forest soils and wood, but probably of insect origin in the habitats (Domsch et al., 1980). *I. farinosa* has been used for biological control experiments with various insects. Its pathogenicity to *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), *Pyrausta nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae) has received particular attention (Domsch et al., 1980). *I. farinosa* was also isolated from *Saissetia coffeae* (Walker, 1852) (Hemiptera: Coccidae) in Sri Lanka (Evans & Hywel-Jones 1997). The pathogenic activity of the fungus was studied on sunn pest (*E. integriceps*) (Parker et al., 2003) and *Ips sexdentatus* (Draganova et al., 2006). The entomopathogen was firstly isolated from sunn pests in South-East region of Turkey by (Parker et al., 2003; Anonymous, 2004). The fungus was tested on a lot of insect pests as a biological control agent (Domsch et al., 1980; Hayden et al., 1992).

The aim of the study was to determine and compare the effectiveness of two entomopathogenic fungi on *A. rostrata* under different relative humidity and inoculum concentrations.

Materials and Methods

Insect culture

Adult *A. rostrata* individuals, collected from the over-wintering areas in Ankara districts were used in the tests. The insects were maintained in insectarium with 27±1 ^{o}C, 60±10 relative humidity and 16 h. light and 8 h. dark photoperiods. After the bugs were adapted to the laboratory condition, the insect culture was fed daily with fresh graminea plants (*Festuca* sp. and *Lolium* sp.).

Fungal culture

The entomopathogenic fungi, *I. farinosa* and *B. bassiana* were isolated from *Aelia* sp. cadavers collected from overwintering areas in Ankara district. The cadavers observed under stereoscopic microscope for fungal growth and a piece of fungal mat transferred to water agar (WA) by a sterile needle. The cultures incubated for 5 days 25°C for 5 days under fluorescent and black light lamps, 12L:12D photoperiod. Mycelial tips were cut from the edge of the 5-day-old fungal colonies and transferred to 2% malt extract agar (MEA) (Merck) and incubate 10 days under the same conditions. Single spore cultures were prepared and stored less than 3°C on the PDA (Merck) slants and under -80 °C in cryogenic tubes containing 10% glycerol. Identifications were made according to morphological characters under light microscope (Samson et al., 1974, Domsch et al., 1980).

Inoculum preparation

The fungi were cultured on Malt Extract Agar for 14 day under 27 ^{o}C. Ten ml sterile distilled water containing 0.02% Tween 20 was added to each Petri plate, and gently rubbed with a sterile spatula to harvest conidia. Conidia suspensions were filtered through Whatman No. 5 and adjusted to 1x10^{6} and 1x10^{8} conidia ml^{-1} using Neubauer hemocytometer.

Inoculations of entomopathogens on *Aelia rostrata* Boh.

Five adults of the pest were placed in Petri plates (90x15 mm) which contain filter paper in the bottom and ventilation holes on the covers. Conidia suspensions were applied as 2 ml to each Petri plate by a Potter spray tower (Burkard, Rickmansworth, Hertz UK) with fine droplet spray nozzle (0.25 mm diameter) to the Petri plates containing *A. rostrata*. The spray pressure was 8 lb/in^{2} (approximately 0.56 kg/cm^{2} or 0.55 bar.). This procedure resulted in the deposition of approximately 850 ± 20 (n = 10) spores mm^{2} for 1x10^{6} conidia ml^{-1}. The spray tower was washed with 70% ethanol and sterile distilled water after each application of entomopathogenic fungus suspension for the disinfection of

the apparatus. Only sterile water containing 0.02 % Tween 20 was sprayed to control Petri plates. The Petri plates were incubated in growth chambers containing 27±1 ^{o}C, 16 h light: 8 h dark photoperiod. Humidity was adjusted to 70 and 95% R.H. by means of room humidifier. The humidity adjustment was performed by means of digital hygrometer (Lae Electronic Co. Inc. Italy) with 1% precision. Eight replications were used for each conidial concentration-relative humidity combination. Dead individuals on which the fungal sporulation observed, were counted under stereoscopic microscope and percent mortality was calculated for per Petri plate.

Data analysis

Mortality data were normalised using arcsine transformation (Anscombe transformation) (Zar, 1999). Transformed mortality data were analysed by means of Repeated Measurement Anova (P= 0.05) and Duncan's Multiple Range Test (P=0.05). All statistical analyses were carried out using Minitab 15 software version (Minitab Inc., State College, PA, USA) .

Results and Discussion

The result of variance analysis indicated that the interactions among the incubation time (days), humidity levels and fungal isolate applications were statistically significant (F= 2,323, df= 8, p= 0,023) (Table 1). The effects of *I. farinosa* and *B. bassiana* on *A. rostrata* under 70% R.H. can be seen on Table 1. *B. bassiana* caused 82.5% mortality in $1x10^6$ conidial concentrations at 9th day of incubation, mortality was reached to 100% in $1x10^8$ conidial concentration (ml^{-1}). At the same incubation time, *I. farinosa* caused 22.5% and 45% mortality in $1x10^6$ and $1x10^8$ conidial concentrations (ml^{-1}), respectively. *B. bassiana* caused significantly higher mortality in all two conidial concentrations (ml^{-1}) at 9th day of incubation. While *I. farinosa* presented significant mortality in only high conidial concentration, the mortality in low conidial concentration of *I. farinosa* was not different from the control. In the 12th day of incubation, *B. bassiana* caused 95% mortality in $1x10^6$ conidia ml^{-1}. The mortality caused by *I. farinosa* could reach to 52.5% and 70% in $1x10^6$ and $1x10^8$ conidial concentrations (ml^{-1}), respectively.

When the effects of conidial concentrations on the mortality were compared, high conidial concentrations were more effective for two entomopathogens. At 6th day of incubation, any mortality was seen in the Petri plates inoculated with $1x10^6$ conidia ml^{-1} concentration of *B. bassiana*, but 42.5% mortality was detected in the Petri plates inoculated with $1x10^8$ conidia ml^{-1} concentration of the same entomopathogen. In the same way, while all the bugs were died in $1x10^8$ conidial concentration (ml^{-1}) in the 9th day of incubation, 82,5% of the bugs were died in $1x10^6$ conidial concentration (ml^{-1}), the differences were statistically significant (p=0.05); however any significant difference in the mortality

percentage between conidial concentration of *B. bassiana* was seen the 12th day of incubation. Similarly, the difference between the mortalities in two conidial concentrations of *I. farinosa* was significant in 6th and 9th days of incubation. In the 12th day, the differences were not statistically significant.

Table 1. Percent mortalities of the *Aelia rostrata* Boh. adults inoculated with two different conidial concentrations of *Beauveria bassiana* (Balsama) Vuillemin and *Isaria farinosa* (Holm.) under 70% relative humidity

Incubation Time (day)	n	*Beauveria bassiana*		*Isaria farinosa*		Control
		$1x10^6$ conidia ml^{-1}	$1x10^8$ conidia ml^{-1}	$1x10^6$ conidia ml^{-1}	$1x10^8$ conidia ml^{-1}	
6th	8	0±0 b**C*	42.5±9.59 aB	2.5±2.5 bC	22.5±5.9 aB	0.0±0 bB
9th	8	82.5±8.81 bB	100.00±0 aA	22.5±2.5 dB	45.00±5.00 cB	22.50±2.50 dA
12th	8	95.00±3.27 aA	100.00±0 aA	52.5±5.26 bA	70.00±3.78 bA	25±3.27 dA

* Within columns, means followed by same capital letters do not differ significantly (Duncan's multiple range test p=0.05)

** Within rows, means followed by the same small letter do not differ significantly (Duncan's multiple range test p=0.05)

Significant differences were also detected between the effects of two entomopathogens. The entomopathogens did not have any significant effect on the mortality rate at $1x10^6$ conidial concentration (ml^{-1}) in 6th day. As a contrast, both fungi caused significant mortality at $1x10^8$ conidial concentration (ml^{-1}) in the same incubation time, the difference between them was not significant. In the 9th day of incubation, *I. farinosa* could not cause any significant mortality rate at $1x10^6$ conidial concentration (ml^{-1}) when compared with control the control, but in the same conidial concentration, *B. bassiana* killed 82.5% of the bugs. In the same incubation day, inoculation of *B. bassiana* in the concentration of $1x10^8$ conidia ml^{-1} resulted 100% mortality, but the mortality was 45% in same concentration of *I. farinosa*. The difference was statistically significant (p=0.05). The mortality rates were significantly high in the *B. bassiana* inoculated Petri plates in two conidial concentrations after 12th day of incubation period (p=0.05).

The effects of *I. farinosa* and *B. bassiana* on *A. rostrata* under 95% R.H. can be seen on Table 2. In 95% relative humidity, the effects of the entomopathogens were similar to those which in 70% humidity level. Both of the pathogens caused significant raise in the mortality when applied at $1x10^8$ conidial concentrations (ml^{-1}) in 6th day of incubation. The mortality difference between two isolates was not statistically significant at the conidial concentration (p=0.05). In $1x10^8$ conidial concentrations (ml^{-1}), while *B. bassiana* caused 100% mortality in 9th day of incubation, *I. farinosa* could cause 45% mortality. The mortality in $1x10^6$ conidial concentration (ml^{-1}) of *I. farinosa* was not significantly high from that of control (p=0.05), the mortalities

were raised to 50 and 75.0% in $1x10^6$ and $1x10^8$ conidial concentrations of the fungus respectively in 12^{th} day of incubation.

Table 2. Percent mortalities of the *Aelia rostrata* Boh. adults inoculated with two different conidial concentrations of *Beauveria bassiana* (Balsama) Vuillemin and *Isaria farinosa* (Holm.) under 95% relative humidity

Incubation time (day)	n	*Beauveria bassiana*		*Isaria farinosa*		Control
		$1x10^6$ conidia ml^{-1}	$1x10^8$ conidia ml^{-1}	$1x10^6$ conidia ml^{-1}	$1x10^8$ conidia ml^{-1}	
6^{th}	8	7.08±3.48 b** B*	30.0±8.45 aB	7.5±3.66 bC	25.0±3.27 aC	2.5±2.5 bA
9^{th}	8	100.0±0 aA	100.00±0 aA	24.58±3.39 cB	45.00±3.27 bB	12.50±3.66 cA
12^{th}	8	100.0±0 aA	100.00±0 aA	50.0±3.78 cA	75.0±5.00 bA	17.5±2.50 dA

* Within columns, means followed by same capital letters do not differ significantly (Duncan's multiple range test p=0.05)

** Within rows, means followed by the same small letter do not differ significantly (Duncan's multiple range test p=0.05)

As a result of this study, both *B. bassiana* and *I. farinosa* were found pathogenic to wheat stink bug adults. There are limited studies focused on the effectiveness of entomopathogenic fungi on *A. rostrata*. Memişoğlu et al. (1996), recorded 80-95% mortality in the *A. rostrata* population. The authors attributed the results to meteorological factors and entomopathogenic fungus, *B. bassiana*. Kocatürk et al. (1994), stated that *B. bassiana* was the primary factor that determine the population density of *A. rostrata* and that mortality was reached to 100% in some overwintering areas. The authors also applied the fungus to *A. rostrata* adults. The pathogen caused up to 77.7% mortality in 20 days. In this study, *B. bassiana* caused 100% mortality in 9^{th} day of incubation. The reason of the high effect of the pathogen may be the regular humidity level in our study. Since the authors kept the inoculated pests in wire nets, the humidity might have not constant in their research. The difference may also be resulted from the variations in the conidial concentrations and the application methods.

The high pathogenic effects of *B. bassiana* was demonstrated on different pests and predators in Pentatomidae family (Tsuda et al., 1996; Poprawski et al. 1997; Sosa-Gomez & Moscardi, 1998; Ihara et al., 2001; Santos et al., 2002; Todorova et al., 2002; El-Zoghby, 2003; Tsutsumi et al., 2003; Holtz et al., 2009). The *B. bassiana* isolate showed high pathogenic activity on *A. rostrata* in our study.

Inspite of *I. farinosa* has been isolated from *Aelia* sp. (Anonymous, 2009), there is any record on the effectiveness of *I. farinosa* on species belonging to Pentatomidae family. However, effectiveness of two entomopathogenic fungi, *B. bassiana* and *I. farinosa*, has been compared on some other insect species belonging to other families and orders. Draganova et al. (2006), investigated the effectiveness of *B. bassiana* and *I. farinosa* on *Ips sexdentatus* Boerner and *Ips acuminatus* Gyll (Coleoptera: Scolytidae) and they recorded that *B. bassiana* caused higher mortality than *I. farinosa* on these species; furthermore,

B. bassiana was found more effective on *Nezara viridula* L. (Hemiptera. Pentatomidae) than *Isaria* sp. (Leite et al., 1987).

In another study, pathogenicities of three species of entomopathogenic fungi, *Beauveria bassiana*, *I. farinosa* and *I. fumosorosea*, against *Bemisia argentifolii* (Gennadius, 1889) (Hemiptera: Aleyrodidae) were measured and compared. The results indicated that *P. fumosoroseus* and *B. bassiana* strains were highly virulent. *I. farinosa* were also pathogenic; however, LC_{50} were relatively high (Wraight et al., 1998).

Puterka (1999), reviewed the research on entomopathogenic fungi in orchard systems and presents research on a mycoinsecticidal approach to an important pest of pear, the pear psylla. In this study, *B. bassiana* and *I. fumosorosea* were used as entomopathogenic isolates. *B. bassiana* caused nymphal mortality higher than 60%, whereas *I. fumosorosea* gave a rise to approximately 40% mortality. Based on the results of the research, author stated that, a mycoinsecticidal approach to pear psylla management could be a useful component in an integrated pest management program for pear.

In our study, *B. bassiana* isolate caused 100% mortality in the 9^{th} day of incubation whereas the mortality percentage caused by *I. farinosa* isolate was 45% at that time and the mortality reached to 75% in 12^{th} day of incubation; thus, pathogenic activity of *B. bassiana* isolate is higher than *I. farinosa* isolate. According to these laboratory results, especially *B. bassiana* isolate may be promising biological control of *Aelia* spp. If the resistance development of *Aelia* spp. to insecticides (Ünal et al., 1994) and adverse effects of pesticides on environment and human health is taken in the consideration, microbial control by using these fungi may be used in the integrated *Aelia* spp. management programs as an ecologically safe method.

Özet

Isaria farinosa (Holm.) ve *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales)'nın *Aelia rostrata* Boh. (Hemiptera: Pentatomidae) üzerine etkileri

Entomopatojen türler, *Isaria farinosa* (Holm.) ve *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales)' nın %70 ve %95 nem, $1x10^6$ ve $1x10^8$ konidi yoğunluğunda (ml^{-1}) *Aelia rostrata* Boh. (Hemiptera: Pentatomidae) erginleri üzerine etkileri incelenmiştir. Denemeler 27±1 °C'de 16 saat aydınlık 8 saat karanlık koşulları içeren iklim odalarında yürütülmüş, inkubasyonun 6, 9 ve 12. günlerinde bireylerdeki ölüm oranları belirlenmiştir. Entomopatojenlerin her ikisi de $1x10^8$ konidi ml^{-1} yoğunlukta ve %95 nemde daha yüksek etkiye sahip olmuşlardır. Funguslardan *B. bassiana*, *I. farinosa*' ya oranla daha yüksek ölüme neden olmuştur. Yüzde 70 nemde ve $1x10^6$ konidi ml^{-1} yoğunlukta *I. farinosa* inkubasyonun 12. gününde %70 ölüme neden olurken, *B. bassiana* inkubasyonun 9. gününde %100 ölüme neden olmuştur. Yüzde 95 nemde

ise *B. bassiana* inkubasyonun 9. gününde her iki konidi yoğunluğunda da %100 ölüme neden olurken, *I. farinosa* ise inkubasyonun 12. gününde, $1x10^8$ konidi ml^{-1} yoğunlukta ancak %75 ölüme neden olmuştur.

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