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Research Article

The Mortality Effects of Some Entomopathogenic Fungi Against *Helicoverpa armigera, Spodoptera littoralis, Tenebrio molitor* and *Blattella germanica* [1]

Engin KILIÇ [1,a] Özlem GÜVEN [2] Recep BAYDAR [3] İsmail KARACA [3]

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[1] Erzincan Binali Yildirim University, Faculty of Pharmacy, Department of Microbiology, TR- 24100 Erzincan - TURKEY
[2] Kahramanmaraş Sütçü İmam University, Faculty of Arts and Sciences, Biology Department, TR-46040 Kahramanmaraş - TURKEY
[3] Süleyman Demirel University, Agricultural Faculty, Department of Plant Protection, TR-32260 Isparta - TURKEY
[a] ORCID: 0000-0002-6838-5977

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Abstract

Laboratory bioassay studies were conducted to determine the effects of 9 isolates of *Beauveria bassiana* and one isolate of *Metarhizium anisopliae,* isolated from the soils in Isparta, Turkey, against *Helicoverpa armigera* (Hübner), *Spodoptera littoralis* (Hübner) (Lepidoptera: Noctuidae), *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) and *Blattella germanica* (L.) (Blattodea: Blattellidae). Concentrations of $2x10^7$ conidia/mL were applied on insects by hand sprayer. All treated insects were incubated at 26±1°C, 70±5% relative humidity for 12 days. All entomopathogenic fungi were infectious to all tested insects but infection rates were different. Isolates of *B. bassiana* and *M. anisopliae* showed very low infection rates on *B. germanica* (3.3-6.7%). Other insects had different mortality rates. *H. armigera, S. littoralis* and *T. molitor* had 53.3-73.3%, 56.7-66.7% and 73.3-80.0% infection rates, respectively. As all fungal isolates were not very effective on *B. germanica* under laboratory conditions but showed effectiveness on other insects.

Keywords: *Biological control, Entomopathogenic fungi, Helicoverpa armigera, Spodoptera littoralis, Tenebrio molitor, Blattella germanica*

Bazı Entomopatojenik Fungusların *Helicoverpa armigera, Spodoptera littoralis, Tenebrio molitor* ve *Blattella germanica'ya* Karşı Öldürücü Etkileri

Öz

Isparta topraklarından izole edilen 9 Beauveria bassiana ve bir *Metarhizium anisoplia* izolatının *Helicoverpa armigera* (Hübner), *Spodoptera littoralis* (Hübner) (Lepidoptera: Noctuidae), *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) ve *Blattella germanica* (L.) (Blattodea: Blattellidae) karşı laboratuvar şartlarında patojeniteleri belirlenmiştir. Fungal izolatlardan $2x10^7$ conidia/mL konsantrasyonları hazırlanarak böceklerin üzerine el spreyi ile uygulanmıştır. Uygulama yapılan böcekler, 12 gün boyunca 26±1°C'de,% 0±5 nispi nemde inkübe edilmiştir. Tüm entomopatojenik funguslar, test edilen tüm böceklere etki göstermiş, ancak enfeksiyon oranları farklılık göstermiştir. *B. bassiana* ve *M. anisopliae* izolatları, *B. germanica* (3.3-6.7%) üzerinde çok düşük enfeksiyon oranı göstermiştir. Diğer böceklerde ise farklı ölüm oranları görülmüştür. *H. armigera, S. littoralis* ve *T. molitor* sırasıyla %53.3-73.3, %56.7-66.7 ve %73.3-80.0 enfeksiyon oranlarına görülmüştür. Bütün fungal izolatlar *B. germanica* üzerinde laboratuvar koşullarında çok etkili olmamış, diğer böcekler üzerinde etkinlik göstermiştir..

Anahtar sözcükler: *Biyolojik mücadele, Entomopatojenik funguslar, Helicoverpa armigera, Spodoptera littoralis, Tenebrio molitor, Blattella germanica*

INTRODUCTION

Many researchers agreed on the importance of entomopathogenic fungi (EPF) for biological control of insect pests [1,2]. Many species of EPF are used to regulate insect pests in glasshouse and field crops, orchards, ornamental, stored products, and forest area. These biological control agents are also practiced for reduction of pest and vector

İletişim (Correspondence)
+90 446 224 53 44
enginfk@gmail.com

insects of veterinary and medical importance [3]. *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) and *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae) are the most common EPF found and grow naturally in soils throughout the world and act as a parasite on various insects species [4,5]. EPF are recognized to be an attractive alternative method to chemical pesticides. Several advantages of using EPF for pest control. They are safe for humans and other non-target organisms, no pesticide residues after spraying to target pests, and increases biodiversity in managed ecosystems [3].

The German cockroach, *Blattella germanica* (L.) can be a serious vector of some microorganisms such as pathogenic bacteria that contaminate the foods [6]. Mostly these insects can be observed in houses, apartments, restaurants, markets, hospitals, and bakeries. Because of rapid growing of these insects cause human health problem such as allergies, asthma, and other respiratory diseases. Chemical insecticides have been mostly used to control cockroaches but control failures due to insecticide resistance and chemical contamination of environment have led some researchers to focus on the other alternative control methods. Many researches have been conducted to effect of entomopathogens on cockroaches and some of them reported infections [7]. The most promising of these pathogens are EPF, such as *B. bassiana* [8] being pathogenic to cockroaches [9].

Spodoptera littoralis (Hübner) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), are the pests of field crops and *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) is pests at stored products [10]. EPF have been used and tested to control a wide range of insect pests including *Spodoptera* species [11-15]. The EPF, *M. anisopliae* and *Beauveria* spp. were infectious to *T. molitor* [16].

This study aimed to evaluate the mortality effect of locally isolated *B. bassiana* and *M. anisopliae* isolates against *H. armigera*, *S. littoralis, T. molitor,* and *B. germanica* under laboratory condition.

MATERIAL and METHODS

Insect Rearing

The German cockroach, *Blattella germanica* (L.) was collected from locally at some restaurant and cafe from Erzincan, Turkey and produced at laboratory conditions for bioassay study [17] *Helicoverpa armigera* was first collected in the field crops at Erzincan providence and maintained under laboratory conditions using semi-synthetic diet described by Singh and Rembold [18]. *Tenebrio molitor* reared at laboratory using the wheat bran as well as the straw were obtained from the common wheat *(Triticum aestivum* L.) planted in the Lunar Palace [19,20]. *Spodoptera littoralis* were kindly provided by Dr. Umut Toprak from Ankara University, Faculty of Agriculture, Plant Protection Department.

Fungal Species and Their Cultures

The EPF used in this study were isolated from agricultural soil [21] and *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) in Isparta, Turkey. Detail information of the EPF were presented in *Table 1*. Pathogenicity of these isolates were tested to *Galleria mellonella* (L.) in previous studies [21].

Fungal cultures were maintained on sterilized potato dextrose agar (PDA) at 25±2°C. Each EPF isolates of conidia were harvested from the surface of 2 to 3 week-old culture and suspended in 30 mL sterile distilled water with 0.3% Tween-80. Conidial suspensions were filtered through sterile muslin cloth to remove debris. Spore concentration was determined using a haemocytometer and final concentrations of $2x10^7$ conidia/mL were prepared for each fungi isolates [22].

The viability of conidia of each isolate was determined by inoculating $1x10^5$ conidia/mL^{-1} spore suspension on PDA and evaluating the germination after 24 h of incubation at 25±2°C. Percentage germination was determined by counting approximately 100 spores for each plate. The viability was above 90-95% for all isolates.

Application of EPF on Insects

The ten 3rd instar larvae of *H. armigera, S. littoralis T. molitor,* and five adult of *B. germanica* were placed in Petri plates (9 cm diameter) and covered with filter paper. The $2x10^7$ conidia/mL of the final concentration conidial suspension of each isolate were sprayed two times from 30 cm distance with hand sprayers on the insects placed in the petri plates. After drying in room temperature, the treated insects were transferred to new Petri plates with diet for each insect. Each assay consisted of 3 replicates with 10 insects. Control larvae and adult were treated with sterile distilled water with a 0.3% Tween-20. Insects were maintained in an incubator at 26±1°C, 70±5% relative humidity for 12 days.

Statistical Analysis

Larval and adult mortalities were corrected according to Abbott's formula and percent mortality rates were calculated. The data were subjected to ANOVA and subsequently to Tukey test to compare each treatment against controls. Data were analyzed using SPSS version 17.0 software (SPSS Inc., Chicago, IL).

RESULTS

This study was a preliminary virulence test of ten fungal isolates *(Table 1)* to investigate potential as a biocontrol agent against to *H. armigera, S. littoralis T. molitor,* and *B. germanica*. *Table 2* shows the infection rates of the ten EPF isolates ($2x10^7$ conidia/mL) on different larval stages of *H. armigera, S. littoralis* and *T. molitor* and adult form of *B. germanica* within 12 days post inoculation. Isolates of

Table 1. *Fungal isolates, species name, host and source of origin and geographic origin of the EPF isolates used for bioassay study*

Fungal Isolates[a]	Species Name	Host or Source of Origin	Geographic Origin
BMAUM-LDE-001	*Beauveria bassiana*	*Leptinotarsa decemlineata*	Isparta, Turkey
BMAUM -LDE-002	*B. bassiana*	*L. decemliniata*	Isparta, Turkey
BMAUM -K1-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUM -M3-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUIM-M1-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUM-M6-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUM -A4-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUM -A6-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUM -K6-001	*B. bassiana*	Soils	Isparta, Turkey
BMAUM -U3-002	*Metarhizium anisopliae*	Soils	Isparta, Turkey

[a] BMAUM: Biological Control Research and Development Center, A: Atabey, M: City Center, U: Uluborlu, K: Keçiborlu, LDE: Leptinotarsa decemlineata adult

Table 2. *Corrected percentage mortality of entomopathogenic fungi isolates (at spore concentration: 2x10^7 conidia/mL) on Helicoverpa armigera, Spodoptera littoralis Tenebrio molitor, and Blattella germanica (% ± SE) 12 days post inoculation*

No	Entomopathogenic Fungi Species	Isolate No[a]	Percentage Mortality of Insects at 12 Days[b] (%± SE)			
			B. germanica	*S. littoralis*	*H. armigera*	*T. molitor*
1	*B. bassiana*	BMAUM-LDE-001	3.3±3.3^a	54.2±5.7^b	63.9±1.4^d	77.7±0.1^b
2	*B. bassiana*	BMAUM-LDE-002	3.3±3.3^a	40.5±6.3^b	59.7±5.0^{cd}	77.7±6.4^b
3	*B. bassiana*	BMAUM-K1-001	3.3±3.3^a	44.6±9.8^b	51.9±1.9^{bc}	81.5±7.4^b
4	*B. bassiana*	BMAUM-M3-001	3.3±3.3^a	49.4±6.5^b	63.9±1.4^d	70.4±3.7^b
5	*B. bassiana*	BMAUM-M1-001	6.7±3.3^a	50.0±4.1^b	68.1±3.7^d	70.4±3.7^b
6	*B. bassiana*	BMAUM-M6-001	3.3±3.3^a	45.2±2.4^b	68.1±3.7^d	66.7±0.0^b
7	*B. bassiana*	BMAUM-A4-001	3.3±3.3^a	43.5±3.6^b	63.9±1.4^d	70.4±3.7^b
8	*B. bassiana*	BMAUM-A6-001	3.3±3.3^a	54.4±2.4^b	63.9±1.4^d	77.8±0.0^b
9	*B. bassiana*	BMAUM-K6-001	3.3±3.3^a	50.0±4.1^b	63.9±1.4^d	74.1±3.7^b
10	*M. anisopliae*	BMAUM-U3-002	6.7±3.3^a	40.5±6.3^b	44.0±3.6^b	70.4±3.7^b
11	Control	dH_2O/Tween 20	0.0±0.0^a	26.7±3.3^a	16.7±3.3^a	10.0±0.0^a

[a] BMAUM: Biological Control Research and Development Center, A: Atabey, M: City Center, U: Uluborlu, K: Keçiborlu, LDE: Leptinotarsa decemlineata adult;
[b] Means within columns with the same letter are not statistically different (Tukey's test at P≤0.05)

B. bassiana and *M. anisopliae* showed very low infection rates on *B. germanica* (3.3-6.7%). Other insects had different mortality rates. The entomopathogenic fungi used in the bioassay were all infectious to *H. armigera*, *S. littoralis* and *T. molitor*. Mortality rates were statistically similar for each isolates on each insect species *(Table 2)*.

DISCUSSION

Adults of *B. germanica* were not very susceptible to EPF isolates in this study. The treatments of EPF showed that mortality level was not significantly different as compared to the control (3.3%-6.7%). Susceptibility of *B. germanica* to EPF isolated from Argentina were shown by Gutierrez et al.[23]. The nymphs and adults of *B. germanica* and the smokybrown cockroach, *Periplaneta fuliginosa* Serville (Blattodea: Blattidae) were tested with isolates of *M. anisopliae* (CEP 085) and *B. bassiana* (CEP 077) using bait and direct contact methods. *Metarhizium anisopliae* caused 60 and 93% mortality in nymphs and adults of *B. germanica,* respectively and 80% mortality on adults by direct contact method. Results showed differences in susceptibility between the two species of cockroaches and between nymphs and adults of the same insects. They also indicated that application methods of EPF are also important factor for insect susceptibility. The other study conducted by Davari et al.[24] to evaluate the toxicity of *B. bassiana* (PTCC5197) and *L. muscarium* (PTCC 5184) against *B. germanica*. Both fungi species were toxic to German cockroach but *B. bassiana* was significantly more effective than *L. muscarium*.

Spodoptera littoralis (Hübner) is a widely distributed polyphagous pest for many economically important crops, such as cotton, tomato, lettuce, cabbage, and so on. It is difficult to control this pest because of its cryptic habitat and high rate of infestation [10, 25]. Several studies have been conducted to test EPF isolates for potential use as biological control agents. The potential of entomopathogenic fungi often vary among fungal species and strains [26-28]. Our study showed that all EPF isolates tested were infectious to *S. littoralis* but percentage mortality was low not exceeded 50% *(Table 2)*.

A lepidopteran insect pest, *H. armigera*, causes more than 50% loss in yield of important crops such as cotton, vegetables and sunflower. In recent years, due to high levels of insecticide resistance in *H. armigera,* yield loss became more vulnerable. To evaluate alternative methods for the control of this import agricultural pest, Revathi et al.[29] tested, *M. anisopliae, B bassiana,* and *Nomuraea rileyi* in field conditions. The *M. anisopliae* and *B. bassiana* isolates displayed 70% mortality and these species shows higher enzymes (chitinase, protease and lipase) production when compared with *N. rileyi* isolates. Other study also conducted to test susceptibility of third instar *H. armigera* to seven strains of three entomopathogenic fungal species (*M. anisopliae, B. bassiana* and *Paecilomyces fumosoroseus*) under laboratory conditions using the larval immersion method [30]. The mortality was ranging from 68 to 100% in treatments with *B. bassiana* and *P. fumosoroseus* strains. They concluded that all three fungal species, especially *P. fumosoroseus*, have a high potential for biocontrol of *H. armigera* larvae. The EPF isolates tested in this study were infectious to *H. armigera*, mortality ranging 44%-68% and *B. bassiana* isolates statistically more effective than *M. anisopliae* isolate.

In conclusion, **s**everal side effects of chemical pesticides such as development of resistance and negative impact on the environment has encouraged several researchers to investigate alternative control methods on important agricultural pests. In consequence, the development of biopesticides that are effective, biodegradable and no harmful side effect on the environment, turn out to be priority of these studies. Based on our studies, all entomopathogenic fungi isolates were infectious to *H. armigera*, *S. littoralis* and *T. molitor* larvae under laboratory conditions but not to *B. germanica* adults. Furthermore, more detailed studies will be conducted to test the control efficacy of these fungal isolates in greenhouses and different stages of insect pests.

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