



Isolation Entomopathogenic fungi From the Two-Spotted Spider Mite [*Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)] at Erzincan Province

Engin Kılıç^{1*}

^{1*} Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Microbiology, Erzincan, Turkey, (ORCID: 0000-0002-6838-5977), ekilic@erzincan.edu.tr

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Abstract

This study was conducted between 2015-2017. The infested with mites of the bean leaves were collected from the field. Then put in polyethylene bags, and brought to the laboratory. The mites on the bean leaves were transferred to the petri dish with Potato Dextrose Agar (PDA) by a brush at the laboratory. The petri dishes were incubated for growth and sporulation of the fungus (2 weeks in the incubator at 25±1°C). As a result, six entomopathogenic fungi (EPF) isolates were isolated from the cadaver of the *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) Four of them were *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes: Hyphomycetes) and two of them were *Metharizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes). After the identification of isolates, they have been coded and stored under refrigerator conditions for use in other studies at the Pharmaceutical Microbiology Laboratory of the Faculty of Pharmacy, Erzincan Binali Yıldırım University.

Keywords: *Beauveria bassiana*, *Metharizium anisopliae*, *Tetranychus urticae*, Erzincan

Erzincan İlinde İki Noktalı Kırmızı Örümcek [*Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)]'den Entomopatojenik Fungus İzolasyonu

Öz

Bu çalışma 2015-2017 yılları arasında yürütülmüştür. Kuru fasulye üretim alanlarından akarlarla bulaşık fasulye yaprakları toplanıp, polietilen torbabalara konularak laboratuvara getirilmiştir. Laboratuvarında bir fırça yardımıyla fasulye yapraklarındaki akarlar Patates Dekstroz Aagarlı (PDA) petrilere aktarılmıştır. Petrilere fungusların gelişimi ve sporulasyonu için inkübatöre konulmuştur (2 hafta-25±1°C). *Tetranychus urticae* Koch, 1836 (Acari:Tetranychidae) kadvralarından 6 adet entomopatojenik fungus (EPF) izolatu elde edilmiştir. Bunlardan 4'ü *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes: Hyphomycetes) ve 2'si *Metharizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) olduğu teşhis edilmiştir. Teşhisten sonra izolatu kodlanmış ve daha sonraki çalışmalar için Erzincan Binali Yıldırım Üniversitesi, Eczacılık Fakültesi, Farmasötik Mikrobiyoloji Laboratuvarında buzdolabı şartlarında depolanmıştır.

Anahtar Kelimeler: *Beauveria bassiana*, *Metharizium anisopliae*, *Tetranychus urticae*, Erzincan

1. Introduction

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae) is the most important pest species in the family Tetranychidae (Balci et al., 2020). It frequently occurs in the glasshouse and outdoor crops. It recorded on more than 200 host plants species (Morris, H., and Waterhouse, D. F., 2001.) In world, farmers largely rely on synthetic acaricides to control this pest. However, there are some problems (such as mite resistance, environmental contamination etc.) related to the use of synthetic acaricides in controlling *T. urticae*. Thus, non-chemical control measures are being developed as alternatives to synthetic acaricides for the control of the two spotted spider mite pest. One of them is the biological control using EPF.

EPF are the most common pathogens associated with spider mites (Maniania, et al., 2016). Several reports on the association between epf and *T. urticae* have been reported (Tamai et al., 2002; Irigaray et al., 2003). *B. bassiana* and *M. anisopliae* have

been reported to cause mortality in mite species (Rossi-Zalaf and Alves, 2006), including 5 the two-spotted spider mite, *T. urticae* (Alves et al., 2002; Tamai et al., 2002; Wekesa et al., 2005)

The main purpose of this study is to isolate EPF from the two-spotted spider mite *T. urticae*, which is a very important pest on agricultural crops.

2. Material and Methods

2.1. Geographical Origin of Collected Fields of Mites

In this study, the infested leaves with mites were collected from the bean production fields in Erzincan province. They were put them in polyethylene bags and brought to the laboratory into ice box. (Table 1).

Table 1. Geographical origin of collected of mites

Research Area	Geographical Origin of Collected of Mites	Country
Bean Production Fields	Erzincan -City Center	Turkey
	Erzincan -Kemah	
	Erzincan -Üzümlü	
	Erzincan-City Center	

2.2. Isolation and Identification of Fungi

In this study, the infested with mites were collected from the bean production fields and then putting in polyethylene bags and brought to the laboratory. The mites on the bean leaves were transferred to the petri dish with PDA by a brush at the laboratory. (Ocak et al., 2007). The petri dishes were incubated for growth and sporulation of the fungus (2 weeks in the incubator at 25 ± 1 °C). To identify mold colonies that have completed their development, macroscopic structures of molds and microscopic structures using the cellophane tape method were examined. The fungi were identified using morphological characteristics of reproductive structures with the aid of relevant taxonomic literature (De Hoog, 1972; Samson et al., 1988. Hasenekoğlu 1991) Diagnosed pure cultures were placed on horizontal agar containing PDA medium and stored at + 4 °C after two weeks incubation at 25 °C.

3. Results and Discussion

3.1. Isolation fungi from *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)

In this study, *B. bassiana* and *M. anisopliae* were isolated from *T. urticae* collected from bean leaves in the different localities of Erzincan (Table 2).

Table 2. Fungal material isolated from *T. urticae*

Fungi Species	No	Code of Isolates	Hosts	Geographical Origin of Isolates	Country
<i>B. bassiana</i>	1	BbEMRKZakar	<i>T. urticae</i>	Erzincan - City Center	Turkey
	2	BbEMRKZakar			
	3	BbEMRKZakar		Erzincan -Kemah	
	4	BbEMRKZakar			
<i>M. anisopliae</i>	1	MaEÜZLakar		Erzincan -Üzümlü	
	2	MaEMRKZakar	Erzincan - City Center		

3.2. Identification of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes: Hyphomycetes)

In this study, *B. bassiana* was isolated from *T. urticae* collected from bean leaves (Table 2). *B. bassiana*: In PDA, the colonies are 8–23 µm in 8 days, velvety powdery, cinnamon, the colony is white then yellowish, colorless under colony, no exudate and odor, sunken hives are transparent, flat-walled, 1.5–3 µm wide, overhead hives flat wall 1–2 µm wide, creepers and climbers carry swollen lateral cell groups on them, measuring 3–6 x 3–5 µm. These cells, branch out to form smaller and swollen cells, conidiogen cells develop in the form of an ellipse or semi-cylindrical, lateral cells in small groups or individually or directly through hyphae, 1.5–6 µm in size, spherical or sometimes oval. Shaped elongated base parts measuring 3–6 x 2.5–3.5 µm, terminal cells 1 µm wide in the shape of a cluster, up to 20 µm long, geniculate, denticles up to 1 µm long as the cluster, cones transparent, flat-walled. There are spherical, sometimes apiculate bases, measuring 2–3 x 2–2,5 µm.

3.3. Identification of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes)

M. anisopliae, an anamorphic fungus that belongs to the phylum Ascomycota, is the most intensively studied species of the genus *Metarhizium* (Liu et al., 2001). According to Alves, 1998 and Jackson and Jaronski, 2009, the reproductive structures of *M. anisopliae* comprise conidiophores and conidia. Leveduriform structures or blastospores and appressoria are produced by *M. anisopliae* through mycelial differentiation. Blastospores can function in certain cases as reproductive units and are produced in submerged cultures and the hemolymph of insect hosts. The appressoria, formed at the extremity of the hyphae, may be involved in fungus pathogenicity and have the function of initiating epicuticular and procuticle penetration of the insect tegument. The production of microsclerotia by isolates of *M. anisopliae* has been observed after cultivation in liquid media with different concentrations of carbon and carbon-nitrogen.

4. Conclusions and Recommendations

B. bassiana causes a disease known as "white muscadine" in insects. The spores of this fungus germinate when they come into contact with the upper skin layer of insects and grow directly from the upper skin into the bodies of their hosts. The fungus reproduces rapidly in the body by producing toxins and drying the insect's food. Therefore, unlike bacterial and viral pathogens of insects, the only contact is sufficient for infection of *Beauveria* and other fungus pathogens. The host does not need to eat themselves. Fungi; when it kills its host, it grows outward, covering the beetle with a layer of white mold between the softer parts of the soft skin. This fine downy mildew produces millions of new infective spores released into the environment. It is common for pathogenic fungi to harm the population of oribatids and other mites that make up most of the secondary animal decomposers that contribute to the formation of humus in the soil. There is many more research for biological control of some parasite mites (Kaaya & Hassan 2000; Shaw et al., 2002; Sáenz-de-Cabezón, 2003; Irigaray et al., 2003).

According to (Shi & Feng, 2004), *Tetranychus cinnabarinus* eggs can be killed by *B. bassiana*. Barreto et al., 2000, *B. bassiana* isolates tested on *Mononychellus tanajoa*, one of Tetranychid mites parasitic on cassava, were found to be effective. Investigated *B. bassiana*'s entomopathogenic activity against *Psoroptes ovis* (Acari: Psoroptidae) mite, which is parasitic in rabbits. After the mites were immersed in a solution containing 10^7 – 10^9 conidia in 1 ml, the fungus was sported on the body surface of the mite and all of the mites were covered with mycelium. There was no decrease in ovulation due to fungal infection, but both the rate of egg-laying and the life span of the hatched larvae were significantly reduced. According to the results of this study, it was emphasized that *B. bassiana* has a high level of pathogenic activity against *Psoroptes* species and that it should be more important to use as a biological control agent against *Psoroptes* species that are parasites in rabbits, sheep, and cattle (Lekimme et al., 2006). *B. bassiana* isolate obtained from *Varroa destructor* mites, a parasite of honey bees, has been used in the treatment of honey bees (*Apis mellifera*) parasitized with these mites and successful results have been obtained (Meikle et al., 2006). Most studies on acar-fungi are related to Tetranychids and Eryophyids (Geest et al., 2000). Studies on other mite groups are very few.

When the existing literature is reviewed, it could be said that the fungus isolated in the present study is an important natural enemy of the mentioned mites and may cause changes in the mite populations by carrying the mites with various body parts. Recently, EPF known to be harmless for humans and mammals in the soil to use biological control have begun to be discovered. Some of them were recorded in this study. The present study and previous studies show these this fungus species can be used in the biological control of harmful mites. However, more studies are needed in the future.

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