



## EFFECT OF NATIVE BEAUVERIA BASSIANA VUILLEMIN ISOLATES ON EGG HATCHING OF TETRANYCHUS URTICAE KOCH (ACARI: TETRANYCHIDAE)

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**Abstract:** In this study, the effect of culture filtrates prepared at different doses of native *Beauveria bassiana* isolates (BIM-001, BY2, and IGÇ) on egg hatching of *Tetranychus urticae* Koch was determined. The adult females of *T. urticae* were transferred to bean leaves (4 cm) prepared according to the leaf disc method, as 10 individuals. After 24 hours, 20 eggs/leaf disc were prepared in each petri dish. Pure culture filtrates (1X) and other diluted doses (5X, 10X) were applied to leaf discs containing eggs for 10 seconds by spraying method. Observations were started 24 hours after the application and continued until the 7<sup>th</sup> day. Experiments were carried out with 5 replications for each dose of entomopathogen fungus isolates. The egg hatching of *T. urticae* was 19% at the pure culture filtrate dose of *B. bassiana* BIM-001 isolate (1X) 7 days after the application, and it was different and significant than the other isolates ( $P < 0.05$ ). Egg hatching rates of *T. urticae* for BIM-001, BY2, and IGÇ isolates were determined between 19-38%, 32-48%, and 36-53%, respectively. These rates were found to be 31-38%, 43-48%, and 46-53% at 5X and 10X doses of BIM-001, BY2, and IGÇ isolates. There was no significant difference in egg hatching rates of pure culture filtrates of *B. bassiana* BY2 and IGÇ isolates ( $P > 0.05$ ).

**Keywords:** Culture filtrate, Entomopathogenic fungus, Two-spotted spider mite

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### 1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important agricultural pest with a wide host range, including more than 1,400 plant species in different geographic regions (Afrotropic, Australasian, Nearctic, Neotropical, Eastern, and Palearctic) (Migeon et al., 2010; Vacante, 2016; Ghongade and Sood, 2021). *Tetranychus urticae* feeds on the sensitive, green parts of plants and causes a decrease in nutrients, stunting of plants, and insufficient chlorophyll in the leaves due to physiological changes (Budai, 2002). This pest reduces the chlorophyll content (55.26%) and the carotenoid content (79.3%) of the leaves (Hildebrand et al., 1986; Bosnyákné et al., 2017). In case of intense contamination, it is known that it reduces the area of photosynthesis activity and causes leaf fall (Gorman et al., 2002). There are many approaches including host plant resistance, cultural measures, biological and chemical control for the management of *T. urticae* in agricultural production areas (Sabelis and Van de Baan, 1983; Costello and Daane, 1998; James and Price, 2004; Van Leeuwen et al., 2015; Azadi Dana et al., 2018). The control of this pest is widely based on the use of acaricides and insecticides. It becomes more difficult to control due to its high reproductive potential, very short life cycle and archenotocous parthenogenesis, and

development of resistance to insecticides and acaricides (Luczynski et al., 1990; Nauen et al., 2000; Van Pottelberge et al., 2009; Van Leeuwen et al., 2010). *Tetranychus urticae* is one of the pests with the highest incidence of pesticide resistance among all arthropods (van Leeuwen et al., 2010). It is known that very intensive use of pesticides leads to outbreaks of *T. urticae* (Fraulo et al., 2008). In this context, biological control is becoming one of the most economical and environmentally friendly control methods for farmers (Cock et al., 2010). In biological control, the application of entomopathogenic fungi is increasing radically due to greater environmental awareness, food safety concerns, and the failure of conventional chemicals with an increasing number of insecticide-resistant species (Rai et al., 2014). Entomopathogenic fungi are known to regulate insect and mite populations in nature with epizootics and cause lethal infections (Burgess, 1981; McCoy et al., 1988; Shahid et al., 2012). Although there are an estimated 750 entomopathogenic fungal species in about 90 genera, most commercially produced fungi are species belonging to *Beauveria*, *Lecanicillium*, *Isaria* (Cordycipitaceae), and *Metarhizium* (Clavicipitaceae) that are taking place Hypocreales, which are relatively easy to mass produce (Roberts and Humber, 1981; Rai et al., 2014). In previous studies, the effects of different spore suspensions of



different isolates of *B. bassiana* on different developmental stages of *T. urticae* were generally investigated (Chandler et al., 2005; Örtücü and Albayrak İskender, 2017; Yanar et al., 2018; Yücel, 2021). Spore suspensions as well as culture filtrates of entomopathogenic fungi are known to have various effects on pests as insecticides or feeding deterrents (Kim et al., 2013). The culture filtrates may contain enzymes such as protease, chitinase, and lipase, which are important in the infection process with conidia (Yoon et al., 2013). From this point of view, the use of culture filtrates of entomopathogenic fungi in the control of harmful species has been also a matter of interest. The high reproductive potential and laying a large number of eggs of *T. urticae*, one of the harmful species that cause significant economic losses, make control more difficult. It was aimed to determine the effect of culture filtrates of three different isolates of *B. bassiana*, isolated from different provinces and hosts, on egg hatching of *T. urticae* in the current study.

## 2. Materials and Methods

Egg stages of *Tetranychus urticae* populations, and three different doses (1X, 5X, 10X) of culture filtrate of BY2 (Burdur, Yeşilova) and BIM-001 (Isparta, Center), IGÇ (Isparta, Center) isolates of *B. bassiana* were used. *Beauveria bassiana* BY2 was isolated from an individual belonging to Phlaeothripidae species collected from the wheat production area in Yeşilova, Burdur. BIM-001 isolate was isolated from potato beetle collected from potato production areas and also IGÇ isolate, on the other hand, was isolated from soil samples obtained outside the agricultural area in Isparta Center using *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae).

### 2.1. Plant Production

*Phaseolus vulgaris* L. (Fabaceae) plants were grown under climatic chamber conditions (25±2 °C temperature, 65±5% humidity, 16: 8 photoperiod). Bean seeds were sown in plastic pots with a diameter of 15 cm using a previously sterilized soil mixture (soil + organic matter).

### 2.2. Obtaining of *Tetranychus urticae* Eggs

In studies carried out to obtain eggs, individuals of *T. urticae* populations, which have been reared since 2018 at Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection were used. Adults were reared on common bean plants in climate rooms at 25±1 °C and 65±10% humidity. Then, these adults were taken to leaf discs (4 cm) to lay eggs for 24 hours.

### 2.3. Preparation of Culture Filtrates of Entomopathogenic Fungus Isolates

Three different isolates of *B. bassiana* included in the study were cultured on potato dextrose agar (PDA) plates for 14 days at 25 °C. One agar disc (1 cm) from each isolate, which was incubated for two weeks, was inoculated into 50 mL potato dextrose water (PDB) in

150 mL Erlenmeyer flasks and shaken at 25±1 °C and 200 rpm for 10 days. Then, the culture liquid of each isolate was passed through Whatman filter paper to remove the spores from the medium, and culture filtrates were obtained (Kim et al., 2013).

### 2.4. Method

The prepared bean leaf discs (4 cm) were placed on sterile water-saturated cotton and kept in plastic Petri dishes (9 cm). Then, 10 adult females were gently transferred to the leaf discs with a soft-tipped brush and allowed to lay eggs. Eggs were counted under a stereomicroscope 24 hours after the adult females were released and the number of eggs was adjusted to 20 eggs/leaf disc. Pure culture filtrate concentration (1X) and diluted concentrations (5X, 10X) of 3 different isolates of *B. bassiana* (BY2, BIM-001, and GÇ8) were prepared (Liu et al., 2008). The culture filtrate dose of each entomopathogenic fungus isolate was applied on the leaf discs with eggs for 10 seconds with the help of a modified apparatus that provides spraying at 4 atm pressure. After spraying, the petri dishes were transferred to the incubator under 25±2 °C, 65%±5% humidity, and 16:8 photoperiod conditions. Observations were started 24 hours after the application and continued until the 7<sup>th</sup> day. Experiments were carried out in plastic Petri dishes with 5 replications for each dose of entomopathogenic fungus isolate.

All percentage egg hatching values obtained from the study were calculated using the Abbott's formula [Corrected % = (1-n in T after treatment / n in Co after treatment) \*100], (n= mitet population, T= treated, Co= control) (Abbott, 1925). Then, one-way analysis of variance (One-Way ANOVA) Tukey multiple comparison test was performed on these data using the SPSS® 20.0 package program (P<0.05). In addition, the Paired Samples Test t-test was applied for paired comparisons in determining the time effect (Genç and Soysal, 2018).

## 3. Results

In experiments where pure culture filtrate dose (1X) was applied to BIM-001 isolate of *B. bassiana*, it was determined that only 19% of *T. urticae* eggs hatched 7 days after the application and 81% of the eggs in the experiment were not hatched. It was determined that the pure culture filtrate dose of BIM-001 isolate (1X) inhibited egg hatching significantly and was higher than other BIM-001 doses and culture filtrate doses of other *B. bassiana* isolates (P < 0.05). Egg hatching rates were 31% and 32% at the 5X dose of BIM-001 and 1X doses of BY2, respectively, and it was found that it inhibited egg hatching by 68-69%, higher than the other remaining doses (P > 0.05). In the study, the highest percentage of egg hatching occurred at the 10X dose of IGÇ with 53% (Table 1).

The effect of time after application on the hatching of *T. urticae* eggs to which all culture filtrate doses of different isolates of *B. bassiana* were applied was evaluated.

**Table 1.** The egg hatching rates of *Tetranychus urticae* Koch in which different culture filtrate doses of different isolates of *Beauveria bassiana* Vuillemin

Treatments	Doses	Egg hatching rates $\pm$ S. E. (%)
<i>Beauveria bassiana</i> BIM-001	1X	19 $\pm$ 2.00 <sup>a</sup>
	5X	31 $\pm$ 1.87 <sup>ab</sup>
	10X	38 $\pm$ 2.54 <sup>bc</sup>
<i>Beauveria bassiana</i> BY2	1X	32 $\pm$ 3.31 <sup>ab</sup>
	5X	43 $\pm$ 3.74 <sup>bcd</sup>
	10X	48 $\pm$ 3.67 <sup>cd</sup>
<i>Beauveria bassiana</i> IGÇ	1X	36 $\pm$ 2.54 <sup>bc</sup>
	5X	46 $\pm$ 2.00 <sup>cd</sup>
	10X	53 $\pm$ 3.67 <sup>d</sup>

<sup>a,b</sup>The difference between the values shown with separate letters in the same column was found to be statistically significant (P<0.05).

It was determined that there was no significant difference between egg hatching on the 3<sup>rd</sup> and 5<sup>th</sup> observation days (t= 0.972, P= 0.125), but there was a significant difference between the 3<sup>rd</sup> and 7<sup>th</sup> observation days in terms of egg hatching (t= 10.717, P= 0.125). 0.013). Again, a significant difference was found between the egg hatching rates detected on the 5<sup>th</sup> and 7<sup>th</sup> observation days of the study (t= 13.537, P= 0.001).

#### 4. Discussion

It is estimated that there are about 1000 species of entomopathogenic fungi known worldwide (Shang et al., 2015). More than 100 mycoinsecticides are commercially available worldwide and are used as biocontrol agents (Jaronski, 2010). They represent the majority of the current biopesticide market worldwide (Muñiz-Paredes et al., 2017; Bugti et al., 2018). Previous studies have noted the efficiency of some entomopathogenic fungi against *T. urticae*, such as *B. bassiana*, *Lecanicillium (Verticillium) lecanii*, and *M. anisoplia* (Chandler et al., 2005; Saranya et al., 2013; Bugeme et al., 2014; Zhang et al., 2014; Örtücü and Albayrak İskender, 2017; Elhakim et al., 2020). Sáenz-de-Cabezón Irigaray et al. (2003) reported that *B. bassiana* can be used as a mycoinsecticide on the adult and egg stages of *T. urticae*, which has a wide host range. In addition, studies were carried out to determine the lethal effect or egg hatching of spore suspensions of *B. bassiana* on *T. urticae* eggs. Negash et al. (2014) found that 82% and 65% mortality occurred in *T. urticae* eggs, respectively, seven days after applying 1x10<sup>8</sup> conidia/ml suspension of *B. bassiana* 9614 and 9609 isolates. In this study, it was determined that pure culture filtrates (1X) of *B. bassiana* BIM-001, BY2, and IGÇ isolates did not hatch in 81%, 68%, and 64% of *T. urticae* eggs, respectively. Bugeme et al. (2014) found the egg hatching rates of *T. urticae* to be 60.2%, 50.8%, 34.7%, 27.4%, 7 days after the application of 3x10<sup>5</sup>, 1x10<sup>6</sup>, 3x10<sup>6</sup>, and 1x10<sup>7</sup> conidia/ml concentrations of *B. bassiana* (ICIPE279) under laboratory conditions. Hassan et al. (2017) investigated the effects of 10<sup>6</sup>, 10<sup>7</sup>, and 10<sup>8</sup> spore/ml doses of 4 different isolates (B1, B2, B3, B4) of *B. bassiana* on *T. urticae* eggs. 7 days after applying suspensions of *B.*

*bassiana* isolates, the egg hatching rates of B1, B2, B3, and B4 isolates were 93.29, 81.67, 85.93, 80.2% for 10<sup>6</sup> spore/ml, 87.26, 58.75, 71.94, 36.38% for 10<sup>7</sup> spore/ml, 68.07, 33.9, 56.66, 25.2% for 10<sup>8</sup> spore/ml. In this study, egg hatching of *T. urticae* 7 days after the application varied between 19-38% in BIM-001 isolate, 32-48% in BY2 isolate, and 36-53% in IGÇ isolate. Doğan (2016) determined the mortality rates that occurred 7 days after applying the 1x10<sup>7</sup> conidia/ml suspension of *B. bassiana* to *T. urticae* eggs by the spraying method, as 11.8% in Petri trials and 14.8% in pot trials. Wu et al. (2020) reported that 1x10<sup>7</sup> conidia/ml suspension of *B. bassiana* GZGY-1-3 isolate caused 2.7-3.8% mortality in *T. urticae* eggs. In the mentioned studies, it was determined that different *B. bassiana* isolates caused low mortality rates in *T. urticae* eggs 7 days after the application of spore suspensions. In this study, it was found that the highest egg hatching rate was reached at the 10X dose of *B. bassiana* IGÇ isolate. In addition to these, an increase in death rates or a decrease in egg hatching occurred with the increase of spore concentrations in spore suspensions, in the other studies as well as an increase in egg hatching with the decrease of doses in this study.

#### 5. Conclusion

Culture filtrates of entomopathogenic fungi may contain secondary metabolites or compounds with different insecticidal activities (Kim et al., 2013). The use of secondary entomopathogenic fungal metabolites as the active component of mycoinsecticides is more effective and can be more easily integrated with other pest control methods (Gustianingtyas et al., 2020). Egg hatching rate of *T. urticae*, which is one of the important pests in agricultural production areas, is 19% in the culture filtrate application of *B. bassiana* BIM-001 isolate, and it can be considered promising for determining the effects of this pest on other developmental periods. In addition, it is thought that the different effects of different entomopathogenic fungal isolates on the same species can be determined by revealing the content of the culture filtrates in detail.

**Author Contributions**

All tasks made by the single author of the manuscript and the percentage of the author contributions is present below. The author reviewed and approved final version of the manuscript.

	A.U.Y.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C= Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study due to the use of research materials that did not fall under the definition of experimental animals (The Scientific and Technological Research Council of Türkiye, Animal Experiments Local Ethics Committee Directive, 2018, Article 3-c).

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