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Original article (Orijinal araştırma)

Efficacy of spore suspension and culture filtrate of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolates on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) in different conditions

Beauveria bassiana (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) izolatlarının spor süspansiyonu ve kültür filtratının farklı koşullarda *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) üzerindeki etkinliği

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Abstract

In this study, the effectiveness of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolates on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) was determined with Petri and pot experiments in laboratory and climate room conditions by applying spore suspension and culture filtrate in Isparta University of Applied Sciences of in 2022. In the Petri experiment, the spore suspensions ($1x10^6$, $1x10^7$, and $1x10^8$ spore/ml) and culture filtrates (1X, 5X, 10X) of BIM-001 and BY2 isolates of *B. bassiana* were sprayed in the form of mist for 10 seconds at a speed of 3 m/s onto leaf discs in Petri which included ten adult individuals. In the pot experiment, the same treatments were conducted with the same application method in the Petri experiment on single-leaf plants in pots. In the Petri and pot experiments, the difference between the mortality rates in the 10^8 spore/ml dose of BIM-001 and BY2 (64.00±4.52 and 42.00±2.49%, 58.00±2.91 and 41.00±2.77%, respectively) was significant on the 7th observation day. In the pure culture filtrate treatments, the mortality rates in the BIM-001 and BY2 were 73.00±2.13 - 68.00±3.59% and 60.00±3.65 - 57.00±5.17% respectively in the Petri and pot experiments. BIM-001 and BY2 culture filtrate in both Petri and pot experiments (1X) applications were not statistically significant. The BIM-001 isolate of *B. bassiana* was more effective than the BY2 isolate treatment against adult females of *T. urticae* both in vivo and in vitro. In conclusion, it is thought that the culture filtrate of *B. bassiana* BIM-001 isolate is considered to have potential for the control of two-spotted spider mites.

Keywords: Biocontrol, culture filtrate, entomopathogenic fungus, *Tetranychus urticae*

Öz

Bu çalışmada, 2022 yılında Isparta Uygulamalı Bilimler Üniversitesi'nde *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) izolatlarının *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) üzerindeki etkinliği spor süspansiyonu ve kültür filtratı uygulanarak petri ve saksı denemeleri yoluyla laboratuvar ve iklim odası koşullarında belirlenmiştir. Petri denemesinde, *B. bassiana*'nın BIM-001 ve BY2 izolatlarının spor süspansiyonları ($1x10^6$, $1x10^7$ ve $1x10^8$ spor/ml) ve kültür filtratları (1X, 5X, 10X) petri içinde yaprak diskleri üzerinde bulunan ergin bireylere 3 m/s hızla 10 saniye mistleme şeklinde püskürtülmüştür. Saksı denemeleri petri denemelerindeki aynı uygulama yöntemiyle saksıdaki tek yapraklı bitkiler üzerinde gerçekleştirilmiştir. Petri ve saksı denemelerinde BIM-001 ve BY2'nin 10^8 spor/ml dozundaki ölüm oranları arasındaki fark (sırasıyla %64.00±4.52 ve %42.00±2.49, %58.00±2.91 ve %41.00±2.77) 7. gözlem gününde anlamlıydı. Saf kültür filtratı uygulamalarında BIM-001 ve BY2'deki ölüm oranları petri ve saksı denemelerinde sırasıyla %73.00±2.13 - 68.00±3.59 ve %60.00±3.65 - 57.00±5.17 olarak bulunmuştur. Hem petri hem de saksı denemelerinde BIM-001 ve BY2 kültür filtratı (1X) uygulamaları istatistiksel olarak anlamlı bulunmamıştır. *Beauveria bassiana* BIM-001 izolatı *T. urticae*'nin ergin dişilerine karşı hem in vivo hem de in vitro koşullarda BY2 izolatından daha etkili olmuştur. Sonuç olarak *B. bassiana* BIM-001 izolatının kültür filtratının iki noktalı kırmızı örümcek kontrolü için potansiyele sahip olduğu düşünülmektedir.

Anahtar sözcükler: Biyokontrol, kültür filtratı, entomopatojen fungus, *Tetranychus urticae*

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Introduction

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) is a polyphagous and cosmopolitan agricultural pest causing economic losses worldwide (Migeon et al., 2010; Lagziri et al., 2015). It is known that it feeds on field plants, ornamental plants, annual and perennial plants (Sim et al., 2003; de Carvalho Ribeiroa et al., 2019; Assouguem et al., 2022). *Tetranychus urticae* has the ability to develop rapid pesticide resistance and is one of the most pesticide-resistant species among arthropods (Knowles, 1997; Van Leeuwen et al., 2008; Van Leeuwen et al., 2010). The rapid development and high fecundity of this species lead them to very quickly reach population levels that cause economic losses (Sato et al., 2005; El-Saiedy & Fahim, 2021). Entomopathogenic fungi can be preferred as an alternative to chemical insecticides. The microbial control agents have numerous advantages over conventional chemical insecticides. These can be listed as minimal adverse effects for humans and other non-target organisms, reduction of pesticide residues, and increased biodiversity in the ecosystem (Abd El-Ghany, 2015). Entomopathogenic fungi have an important role in the control of spider mite populations and they can be included in biological control programs as an alternative to synthetic acaricides currently in use (Maniania et al., 2008). There are thought to be about 750 fungi species that cause infections in insects or mites (Abd El-Ghany, 2015). *Beauveria* is one of the most widely recognized and encountered entomopathogenic fungi, due to its widespread distribution, and easy identification. *Beauveria*'s broad host range and wide variation in virulence against different hosts make it preferred for the biological control of pests (Rehner, 2005). Besides 707 insect species, 13 mite species have been reported among the hosts of *B*. *bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) (Li, 1988; Goettel et al., 1990; Zimmermann, 2007). In order to decide the most virulent isolate, pathogenicity of different isolates against the target pest must be determined (Zimmermann, 2007). Recent studies have recorded the effectiveness of different isolates of *B*. *bassiana* against *T*. *urticae* (Chandler et al., 2005; Draganova & Simova, 2010; Örtücü & Albayrak İskender, 2017; Yucel, 2021). These studies investigated the lethal effect of different spore concentrations on *T*. *urticae*. Entomopathogenic fungi contain secondary metabolites that manipulate the host's immune mechanisms and cause death. This feature increases *B*. *bassiana*'s potential for pest control (Zibaee et al., 2011). About 33.9% of mycoinsecticide formulations were based on *B*. *bassiana* (Faria & Wraight, 2007). However, the killing speed needs to be increased, which is important limiting their use as mycoinsecticide (St Leger & Wang, 2010). It has been supported by different studies that culture filtrates of entomopathogenic fungi cause faster death than spore suspensions (Namara et al., 2017; Herlinda et al., 2020). As we mentioned before, *T*. *urticae* can reach devastating population levels very quickly due to its features. For this reason, it is thought that important to ensure the rapid death of this pest. In this context, this study aimed to determine the effects of *B*. *bassiana* isolates on *T*. *urticae* adults in culture filtrates as well as spore suspensions. In this study, the effect of culture filtrates and spore suspensions of local *B*. *bassiana* isolates on *T*. *urticae* was determined both in vivo and in vitro conditions.

Materials and Methods

Mite culture

As study materials bush bean (Atlantis, Arzuman), *Phaseolus vulgaris* L., 1753 (Fabales: Fabaceae), and the population of *T*. *urticae* were produced in the climate chamber (25±2ºC temperature, 65±5% humidity, and 16: 8 L: D photoperiod) in 2022. When plants have 3-4 leaves, two-spotted spider mites (red form) were transferred to these plant leaves. Then, bean plant leaves infected with mites are cut off and transferred to non-contaminated plants to ensure the continuity of the population. The *T*. *urticae* population was obtained from vegetable greenhouses in Antalya (Türkiye) in 2018. The identification of *T*. *urticae* was done according to Jeppson et al. (1975) and Bolland et al. (1998).

Fungal isolates

The other materials were *B*. *bassiana* BIM-001 isolated from *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) in potato areas Isparta/Center (37°50'16.77" N 30°32'17.61" E, 1017 m, 23.08.2018) and *B*. *bassiana* BY2 isolated from Phlaeothripidae (Thysanoptera) species in Burdur-Yeşilova wheat areas (37°30'52.3" N 29°45'45" E, 1191 m, 14.06.2017). These entomopathogenic fungi isolates were identified by Prof. Dr. Evrim Arıcı Şenkaynağı.

Preparation of culture filtrates of entomopathogenic fungus isolates

Two different entomopathogenic fungi isolates were produced in potato dextrose agar (PDA) for 14 days at 25°C. These isolates were incubated for two weeks. An agar disc (1 cm) from each isolate was transferred into potato dextrose water (PDB, 50 ml) and shaken at 25±1°C and 200 rpm for 10 days. Then, these prepared liquids were passed through Whatman filter paper, and culture filtrates were obtained (Kim et al., 2013).

Preparation of spore suspensions of entomopathogenic fungus isolates

Two different isolates of *B*. *bassiana* (BY2 and BIM-001) were cultured on potato dextrose agar (PDA) medium at 25±1°C for 15-30 days. At the end of the incubation period, 10 ml of sterile distilled water with 0.02% Tween 80 was poured into the Petri dishes, and spores were harvested with a glass spreader. The spore suspension was passed through sterile cheesecloth. Spore counts of these spore suspensions were performed under light microscope on Thoma slides and spore concentrations were prepared to 1×10^6, 1×10^7, and 1×10^8 spore/ml for each isolate (Polat et al., 2022).

Petri experiments

Leaf discs (4 cm diameter) placed upside down on a cotton layer in Petri dishes (9 cm diameter) with a few holes onto the lid for ventilation. A vaseline ring was formed around the leaf disc (Al-Azzazy et al., 2020). Ten adult females of *T*. *urticae* were transferred to leaf discs. Each petri dish was one replication and 10 replications were used for each spore suspension and culture filtrate. Petri dishes were transferred to an incubator (Siemens, Touch) at 25±2°C, 65±5% RH, and 16: 8 (L:D) photoperiod.

Spore suspension application

Three different spore suspensions, $1x10^6$, $1x10^7$, and $1x10^8$ of the isolates BIM-001 and BY2 of *B*. *bassiana* were sprayed on leaf discs with adult individuals (a replicate) (Draganova & Simova, 2010) in the form of mist for 10 seconds (0.25 ml) at a speed of 3 m/s (at 4 atm pressure) in Petri dishes. Entomopathogenic fungus spore suspensions and culture filtrate doses were applied by mounting glass material that sprays the liquid coming under pressure at the end of the motorized insect aspirator. Leaf discs were checked 24 hours, 3, 5, and 7 days after application (DAA), and the number of dead mites was recorded on all observation days.

Culture filtrate application

Three different concentrations, 1X (pure culture filtrate), 5X, and 10X of the isolates BIM-001 and BY2 of *B*. *bassiana* were sprayed on leaf discs with adult individuals (a replicate) (Shin et al., 2011) in the form of mist for 10 seconds (0.25 ml) at a speed of 3 m/s (at 4 atm pressure) in Petri dishes. Observations were made in the same way as in the spore suspension application.

Pot experiments

Bean plants grown in pots were left with one leaf (Doğan, 2016). Each plant was considered a replicate. A leaf disc (4 cm diameter) was created by making a vaseline ring on the leaf of each plant in the pots. In single-leaf plants, ten adult females of *T*. *urticae* were transferred into the vaseline ring on the leaves with the help of a brush, and then entomopathogenic fungus concentrations were applied at 4 atm pressure for

10 seconds (0.25 ml). The applications performed above in Petri dishes were also carried out on these plants for all three spore suspensions of each fungus isolate. Applications made in Petri dishes were also carried out on a single-leaf plant in vivo conditions for both spore suspension and culture filtrate application. Pot experiments were carried out at 25±1°C, 60±10% relative humidity, and 16: 8 [L: D] photoperiod.

Distilled water containing 0.02% Tween 80 was used in the control application in Petri and pot experiments. BIM-001 and BY2 isolates of *B*. *bassiana* were re-isolated from all dead individuals in both Petri and pot experiments (Meng et al., 2017).

Statistical analysis

The percentage of mortality was calculated using the raw data of the number of dead individuals obtained. The mortality rates calculated by counting the dead individuals in the applications on each observation day were first subjected to the homogeneity test (Levene's), and the Shapiro-Wilk's normality test was applied. The obtained mortality rates were transformed using angular transformation. Then, one-way analysis of variance (One-Way ANOVA) was performed on these data. Tukey's (HSD) multiple comparison test was applied to determine similar and different groups. Statistical analyses were performed with the SPSS® 20.0 package program. The median lethal time (LT50) values of the most effective applications were calculated using the Excel program. For each application, a regression equation was drawn in Excel, and LT50 values were calculated with the help of the regression coefficient.

Results and Discussion

Petri experiments

In the Petri experiment, there was no mortality in *T*. *urticae* adults 24 hours after the application of spore suspensions of *B*. *bassiana* BIM-001 and BY2. However, deaths were observed at the 3 DAA (day after application) and the death was significantly different from the control for doses of BIM-001 and 10^8 spore/ml dose of BY2. The highest mortality was detected in the 10^8 spore/ml dose of BIM-001 (16.00±3.40%) and determined to be significant from all other treatments ($F_{6,63}$= 6.071, *p< 0.001*). On the 5 DAA, 10^8 spore/ml dose of BIM-001 (46.00±4.00) were statistically different from each other ($F_{6,63}$= 28.924, *p< 0.001*). On the last observation (7 DAA), the difference between the mortality rate in 10^8 spore/ml of BIM-001 and the treatment 10^8 spore/ml of BY2 (64.00±4.52% and 42.00±2.49%, respectively) was statistically significant and the 10^8 spore/ml of BIM-001 was significant and different from other applications ($F_{6,63}$= 51.019, *p*< 0.001). Mortality rates in spore suspension applications were found to be significant and different from the control treatment at the 5 and 7 DAA (Table 1).

Table 1. Mortality rates caused by spore suspension treatments of BIM-001 and BY2 in the petri experiment

Treatments	Mortality rates (%)±Std. err.							
	1 DAA	3 DAA		5 DAA		7 DAA		
BIM-001-10^8	0.00	16.00±3.40	a*	46.00±4.00	a	64.00±4.52	a	
BIM-001-10^7	0.00	12.00±2.49	ab	34.00±3.40	b	49.00±2.33	b	
BIM-001-10^6	0.00	10.00±2.11	ab	21.00±2.33	c	32.00±1.33	cd	
BY2 10^8	0.00	14.00±1.63	ab	28.00±2.49	bc	42.00±2.49	bc	
BY2 10^7	0.00	9.00±2.33	abc	20.00±2.58	c	33.00±3.00	cd	
BY2 10^6	0.00	6.00±1.63	bc	18.00±2.00	c	27.00±2.60	d	
Control	0.00	0.00±0.00	c	0.00±0.00	d	2.00±1.33	e	

* Different letters in the same column indicate significantly different mortality rates in the treatments (P ≤ 0.05).

Twenty-four hours after application, the mortality was not observed in *T*. *urticae* adults in the treatments of culture filtrate. On the 3 DAA, the mortality rates were not different significantly for pure culture

filtrate treatment of BIM-001 and BY2 (30.00±2.58% and 28.00±3.89%, respectively), but these treatments were statistically different from other treatments ($F_{6,63}$= 13.849, p< 0.001). On the 5 DAA, the highest mortality rate was determined in the 1X culture filtrate treatment of BY2 (52.00±5.33%) ($F_{6,63}$= 27.913, p< 0.001). The mortality rates in the BIM-001 and BY2 1X culture filtrate (73.00±2.13% and 68.00±3.59%, respectively) treatments were found to be statistically different from the other treatments at the 7 DAA ($F_{6,63}$= 60.082, p< 0.001). In addition, mortality rates in both BIM-001 and BY2 treatments were significant and different from the control treatment on all observation days (Table 2).

Table 2. Mortality rates caused by culture filtrate treatments of BIM-001 and BY2 in the petri experiment

Treatments	Mortality rates (%)±Std. err.			
	1 DAA	3 DAA	5 DAA	7 DAA
BIM-001-10^8	0.00	30.00±2.58 a*	49.00±2.33 ab	73.00±2.13 a
BIM-001-10^7	0.00	15.00±2.69 b	35.00±4.28 b	54.00±4.52 b
BIM-001-10^6	0.00	12.00±2.49 b	23.00±3.35 c	36.00±2.67 c
BY2 10^8	0.00	28.00±3.89 a	52.00±5.33 a	68.00±3.59 a
BY2 10^7	0.00	19.00±2.77 ab	32.00±2.91 c	40.00±3.33 c
BY2 10^6	0.00	12.00±3.27 b	21.00±2.77 c	30.00±3.33 c
Control	0.00	0.00±0.00 c	0.00±0.00 d	2.00±1.33 d

* Different letters in the same column indicate significantly different treatment mortality rates ($P \leq 0.05$).

The 10^8 spore/ml dose of BIM-001 caused the highest mortality rate between the spore suspensions of BIM-001 and BY2 isolates, and the LT50 value was determined as 5.76 days (Figure 1a). In culture filtrate treatments of BIM-001 and BY2 isolates,1X culture filtrate of BIM-001 caused the highest mortality rate and the LT50 value was 5.09 days (Figure 1b).

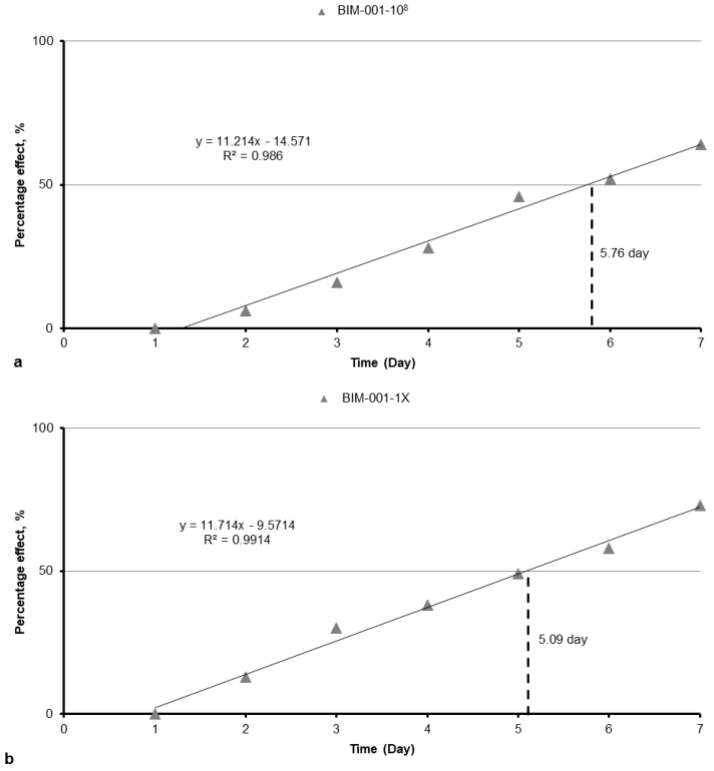

Figure 1. The median lethal time for a) 10^8 spore/ml and b) pure culture filtrate of *Beauveria bassiana* BIM-001 in the Petri experiment.

Pot experiments

In the pot experiment, the mortality in *T. urticae* adults was not detected in the treatments of different spore suspensions of BIM-001 and BY2 isolates 24 hours after application. While the highest mortality occurred in 10^8 spore/ml treatment of BY2 (9.00±1.80%) at the 3 DAA, it was not different from 10^8 spore/ml treatment of BIM-001 ($F_{6,63}$= 2.955, p= 0.013). On the 5 DAA, there was no statistical difference between the mortality rates of *T. urticae* in the10^8 spore/ml treatment of BIM-001 (32.00±2.91%) and BY2 isolates (24.00±1.63%) ($F_{6,63}$= 21.520, p< 0.001). On the 7 DAA, it was determined that the mortality rate in the 10^8 spore/ml treatment of BIM-001 (58.00±2.91%) was statistically different compared with the 10^8 spore/ml treatment of BY2 (41.00±2.77%). In addition, it was found that spore suspension treatments of both *B. bassiana* isolates were significant and different from the control treatment ($F_{6,63}$= 54.195, p< 0.001) (Table 3).

Table 3. Mortality rates caused by spore suspension treatments of BIM-001 and BY2 in the pot experiment

Treatments	Mortality rates (%)±Std. err.							
	1 DAA	3 DAA		5 DAA		7 DAA	
BIM-001-10^8	0.00	7.00±2.13	a*	32.00±2.91	a	58.00±2.91	a
BIM-001-10^7	0.00	6.00±2.21	ab	25.00±2.24	ab	42.00±3.27	b
BIM-001-10^6	0.00	4.00±1.63	ab	17.00±1.53	bc	29.00±2.33	cd
BY2 10^8	0.00	9.00±1.80	a	24.00±1.63	abc	41.00±2.77	b
BY2 10^7	0.00	8.00±2.00	a	21.00±3.14	bc	30.00±2.98	c
BY2 10^6	0.00	4.00±1.63	ab	15.00±2.24	c	19.00±1.80	d
Control	0.00	0.00±0.00	b	0.00±0.00	d	0.00±0.00	e

* Different letters in the same column indicate significantly different treatment mortality rates ($P \leq 0.05$).

In the pot experiment, the mortality was not observed 24 hours after the application of the culture filtrate treatments. On the 3 ($F_{6,63}$= 17.087, p< 0.001) and 5 DAA ($F_{6,63}$= 20.280, p< 0.001), the mortality rates in pure culture filtrate (1X) of BIM-001 were statistically significant from control and other applications. On the last observation day, it was determined that there was no significant difference between the mortality rates in the 1X culture filtrate of BIM-001 and BY2 (60.00±3.65% and 57.00±5.17%), and they were significant and different from other treatments. There was no significant difference in mortality rates between the treatments of the 10X culture filtrate (33%) and 10^8 spore/ml of BY2 (41%) ($F_{6,63}$= 29.386, p< 0.001). It was determined that mortality rates in culture filtrate treatments of BIM-001 and BY2 were significantly different from the control on the 3, 5, and 7 DAA (Table 4).

Table 4. Mortality rates caused by culture filtrate treatments of BIM-001 and BY2 in the pot experiment

Treatments	Mortality rates (%)±Std. err.							
	1 DAA	3 DAA		5 DAA		7 DAA	
BIM-001-10^8	0.00	29.00±2.33	a*	47.00±3.67	a	60.00±3.65	a
BIM-001-10^7	0.00	15.00±1.67	c	33.00±3.00	abc	41.00±4.82	b
BIM-001-10^6	0.00	10.00±2.11	cd	24.00±2.67	c	31.00±3.48	b
BY2 10^8	0.00	26.00±3.06	ab	39.00±3.14	ab	57.00±5.17	a
BY2 10^7	0.00	17.00±3.00	bc	31.00±4.82	bc	45.00±3.42	ab
BY2 10^6	0.00	12.00±2.91	c	22.00±3.89	c	33.00±3.00	b
Control	0.00	0.00±0.00	d	0.00±0.00	d	0.00±0.00	c

* Different letters in the same column indicate significantly different treatment mortality rates ($p \leq 0.05$).

The 10^8 spore/ml treatment of BIM-001 caused the highest mortality rate among the different spore suspensions of BIM-001 and BY2 isolates and the LT50 value was found as 6.70 days (Figure 2a). The pure culture filtrate (1X) application caused the highest mortality rate among culture filtrate treatments of BIM-001, and the LT50 value was determined as 5.68 days (Figure 2b).

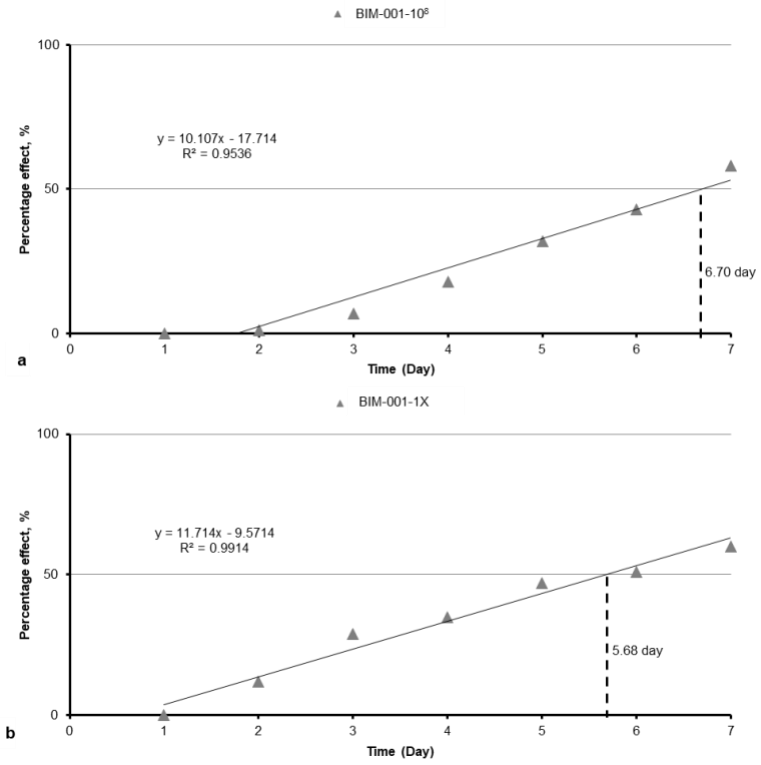

Figure 2. The median lethal time for a) pure culture filtrate and b) 10^8 spore/ml of *Beauveria bassiana* BIM-001 in the pot experiment.

The mode of action of entomopathogenic fungus is known to infect the insect body after the fungal spores are released. The fungal spores initially spread outside the host body and penetrate the host. Then, death occurs in the host in 4-7 days depending on the number of spores (Sharma & Sharma, 2021). Draganova and Simova (2010) investigated the effects of five isolates of *B*. *bassiana* on *T*. *urticae* by spraying conidial suspensions at 10^6, 10^7, and 10^8 conidia/ml concentrations. Isolates of *B*. *bassiana* caused mortality in the range of 80-100% at 5 days after the application. In a different study, Örtücü & İskender Albayrak (2017) determined the efficacy of *B*. *bassiana* isolates on *T*. *urticae* at three different doses ($1x10^6$, $1x10^7$ and $1x10^8$ conidi ml^{-1}) on *T*. *urticae*. PaF04 isolate was more effective than PaF09 and PaF76 isolates. For this isolate, 80.7±4.2%, 92±3.5%, and 100% mortality occurred in mites until the 5th day of $1x10^6$, $1x10^7$, and $1x10^8$ conidia ml^{-1} concentrations, respectively. In this study, BIM-001 and BY2 isolates of *B*. *bassiana* reached 18-46% mortality at 10^6, 10^7, and 10^8 spore/ml on the 5 DAA, while it remained in the range of 27-64% on the 7 DAA. Geroh et al. (2014) found that *B*. *bassiana* (strain ITCC-4668) isolate caused 63.31% and 59.49% mortality at 0.3×10^8 and 0.3×10^7 conidia ml^{-1} on the 7 DAA on *T*. *urticae*. Doğan (2016) determined that 80.3±3.8% mortality occurred 7 days after the application of $1x10^7$ conidiospore/ml dose of *B*. *bassiana* isolate to *T*. *urticae* adults under Petri conditions. Doğan (2016) found that the mortality rate was 45.8±1.4% on 7 days after the applied $1x10^7$ conidiospore/ml dose of *B*. *bassiana* isolate to *T*. *urticae* adults under pot conditions. Yeşilayer (2018) determined that mortality rates occurring 3, 5, and 7 days after $1x10^8$ conidi/ml dose of *B*. *bassiana* were applied 10.00±0.00%, 16.66±2.10% and 16.66±2.10%, respectively. Ali et al. (2020) reported that the mortality rates were 39.2% and 78.65% on

the *T*. *urticae* 7 days after *B*. *bassiana* B1 and B2 isolates (1×10^8 spore/ml) were applied. Yucel (2021) investigated the lethal effects of *B*. *bassiana* BGF14 and BCA32 isolates (1×10^6, 1×10^7, and 1×10^8 spore/ml) on *T*. *urticae*. On the 7 DAA, at 10^6, 10^7, and 10^8 spore/ml doses of BGF14 isolate occurred 47.10±2.94%, 44.95±3.62% and 76.98±3.32% mortality, and these mortality rates were 63.06±1.95%, 81.09±4.10%, and 87.27±2.76% for BCA32 isolate. In the current study, the mortality rates were observed at 49.00±2.33-64.00±4.52% and 33.00±3.00-42.00±2.49% at 10^7 and 10^8 spore/ml doses of BIM-001 and BY2 isolates on the 7 DAA in Petri treatments, these mortality rates were 42.00±3.27 -58.00±2.91% and 30.00±2.98-41.00±2.77% in pot treatments.

In culture filtrate applications, since the spores are removed, the spores do not come into contact with the host, and toxin production steps do not occur to kill the host. Therefore, direct host death and disease initiation occur more rapidly when culture filtrate is applied (Gustianingtyas et al., 2020; Herlinda et al., 2020). The enzymes such as chitinases, lipases, and proteases containing culture filtrates facilitate the infection process by disrupting the cuticle of the host (Hanan et al., 2020). Namara et al. (2017) reported that the spore-free culture filtrate renders the host more susceptible to a pathogen. Entomopathogenic fungi include secondary metabolites with antimicrobial, insecticidal, and cytotoxic activities (Gibson et al., 2014). Zibaee et al. (2011) reported that the secondary metabolites in the culture filtrate were toxic. *Beauveria bassiana* produces beauvericin, bassianolides, bassianin, and bascianolone as secondary metabolites (Kanaoka et al., 1978; Grove & Pople, 1980; Quesada-Moraga & Vey, 2004; Wang & Xu, 2012). The maximum efficacy on the pest is provided by the production of metabolites in the culture filtrate (Hanan et al., 2020). It is necessary to determine the effects of entomopathogenic fungi against *T*. *urticae* in order to reveal this activity. Yun et al. (2017) compared the effects of culture filtrates, air conidia, and blastospores of *B*. *bassiana* 2R-3-3-1 against *T*. *urticae*. On the 7 DAA, treatment with entomopathogenic fungus culture filtrate alone caused 83.3±2.7% mortality, while combined applications with air conidia (94.3±3.1%) or blastospores (98±2%) increased these mortality rates. However, the LT50 values of the treatments were determined as 3.96, 2.49, and 3.35 days for combined applications with entomopathogenic fungus culture filtrate alone, air conidia, or blastospores, respectively, and were not significantly different. In the present study, pure culture filtrates of BIM-001 and BY2 isolates caused 73.00±2.13% and 68.00±3.59% mortality in Petri dishes trials, while the mortality rates were detected as 60.00±3.65% and 57.00±5.17% in pot trials. The LT50 values in 10^8 spore/ml and 1X culture filtrate of BIM-001 were 5.76 and 5.09 days in Petri dishes, while it was 6.70 and 5.68 days in pot trials. As a result of this study, the BIM-001 isolate of *B*. *bassiana* was found the most effective treatment against *T*. *urticae* adult females both in vivo and in vitro conditions. These results suggest that the culture filtrate of *B*. *bassiana* BIM-001 isolate could have the potential for the control of two-spotted spider mites.

Zibaee et al. (2009) reported that secondary metabolites are more easily applicable in the field. The culture filtrate, which contains toxic secondary metabolites, has an important potential as a "mycoinsecticide active ingredient". The culture filtrate of entomopathogenic fungus has the advantages comparatively more stable than the culture containing conidia, longer storage time, and also there is no problem of nozzle clogging when the culture filtrate is sprayed (Soesanto et al., 2019). The results from this study support that culture filtrate applications of *B*. *bassiana* isolates are effective on *T*. *urticae* in both Petri dishes and pot experiments. In addition, it is thought to be important to evaluate the potential of achieving efficacy on also the different life stages of *T*. *urticae* in future studies.

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