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Effects of local isolates of *Beauveria bassiana* (Balsamo) Vuillemin on the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae)

Cenk Yucel

Abstract

Background: The two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), is a widely distributed plant-feeding pest that causes significant yield losses in a wide range of crops. Newly developed or improved environmentally friendly biocontrol agents serve as an alternative to traditional pest control tools. Experiment of the effects of 2 local fungal isolates of *Beauveria bassiana* (BGF14 and BCA32) was carried out against *T. urticae* under laboratory conditions.

Results: Both tested isolates had lethal effect in a short time after application, and this effect increased as time progressed. BGF14 and BCA32 isolates caused *T. urticae* mortality rates ranging from 25.88 to 61.92 and 32.36 to 62.03% when applied at the concentrations between 1×10^5 and 1×10^8 conidia/ml, respectively. According to the Probit analysis performed on the effect of fungi on *T. urticae* adults, the LC_{50} values of BGF14 and BCA32 isolates on the 7th day after inoculation were 2.6×10^6 and 6.3×10^4 conidia/ml, respectively, and the LT_{50} values for both fungi applied at a concentration of 10^8 conidia/ml were 2.14 and 2.23 days, respectively.

Conclusions: The 2 isolates of *B. bassiana* (BGF14 and BCA32) had the potentials to suppress *T. urticae* population and can be recommended as promising biocontrol agent candidates for control of *T. urticae*.

Keywords: *Tetranychus urticae*, *Beauveria bassiana*, Local isolates, Biological control

Background

The two-spotted spider mite [*Tetranychus urticae* Koch (Acari: Tetranychidae)] is an economic mite species that infects more than 900 different plant hosts and causes significant damage to at least 150 of them (Mondel and Ara 2006). *T. urticae* causes severe damage to crops in greenhouses and vegetable and fruit gardens in Turkey and all around the world (Van Den Boom et al. 2003). Accordingly, different types of synthetic acaricides are used to prevent the damage caused by the mite and to prevent its spread to other fields (Kumral et al. 2010). Insect resistance to pesticide is a growing problem worldwide. Thus, the adoption of alternative control methods can minimize such problems. Nowadays, biocontrol agents such as entomopathogenic fungi (EPF) are widely used in integrated pest control (Faria and Wraight 2001). *Beauveria bassiana* (Hypocreales: Cordycipitaceae), for example, has been identified as a pathogen of several mites' species (Faria and Wraight 2007) that is highly effective in reducing mites' populations. Chandler et al. (2005) conducted greenhouse trials using a commercial isolate of *B. bassiana* (Naturalis L.) against *T. urticae* and reported that this fungus greatly reduced the number of mobile mites compared to the untreated control. Numerous studies have also been conducted to determine the possible use of EPF in biological control of mites (Gatarayiha et al. 2011).

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Among the alternative complementary and supplementary methods to overcome the issue of resistance to chemical control, use of entomopathogenic fungi (EPF) has occupied an important place in biological control methods. Currently, use of *B. bassiana* in bio-preparations has become common in the world.

In this study, the pathogenicity of two local isolates of *B. bassiana*, isolated from Turkey's nature, was investigated against *T. urticae* adults under laboratory conditions.

Methods

Mite culture

Tetranychus urticae was reared on bean plants (*Phaseolus vulgaris* L.) grown in pots placed in a climate room with temperature of 24 ± 1 °C, relative humidity of 65 ± 1, and 14:10 photoperiod.

Identification of fungus

Morphological identification of fungus

Two isolates of EPF were obtained from the body of insect cadaver. The fungus was morphologically identified under a light microscope according to its conidial features in accordance with the literatures (Samson et al. 1988; Goettel and Inglis 1997; Humber 1998). The isolates were developed on PDA (potato dextrose agar) by incubating them at 23±1 °C, including 12 h light (near ultraviolet light) 12 h dark for 10–12 days. The fungus colonies showed very quick growth by covering Petri dishes in 10 days. The colony color was first white, and then changed close to creamy white. The colonies had a white velvety appearance. Both isolates had an aerial miselium, intense wool-like miselium structure. It was observed that the fungus produced too many conidia. The conidia measured 2–2.5-μm diameter. The conidia occured in 3 days in Petri dishes (Fig. 1).

Fungal pathogens and preparation of the conidial suspensions

In this study, *B. bassiana* isolates were obtained from the fungal collection of the Plant Protection Research Center and Institute. The 2 isolates were obtained from a body of insect cadaver. BGF14 isolate was isolated from *Gonioctena fornicata* (Brüggmen) (Coleoptera: Chrysomelidae), while BCA32 was from *Cicadatra adanai* Kartal (Hemiptera: Cicadidae). Fungus cultures were maintained on Sabouraud dextrose agar (SDA) at 24 ± 1 °C for 21 days. Conidial suspensions were filtered through 3 layers of sterile cheesecloth to remove particles. The number of conidia per milliliter was counted using a hemocytometer. At the end of this period, the conidia were collected in sterile distilled water containing 0.02% Tween 80, and the conidial concentration of the stock culture was adjusted to a density of 1×10^8 conidia/ml.

Molecular identification of fungus

In the molecular studies, DNA extraction was carried out using 10–12-day-old culture with Qiagen Blood and Tissue Kit. PCR protocol was undertaken using ITS1 and ITS4 gene region. The positive isolates were sent to the sequencing facilities. The sequence results were opened software program called BIOEDIT, and nucleotide sequences were blasted in the NCBI website. As a consequence of blasted sequences that were showed (100%) homology with the other sequences; *B. bassiana*. The deposited accession numbers on the NCBI website of the 2 *B. bassiana* isolates are MW295632 and MW295633, respectively.

Bioactivity of entomopathogenic fungi

First, bean leaves were cut and placed on moist filter papers in a 3-cm Petri dish; then, 1 ml suspension of each of BGF14 and BCA32 with 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidial concentrations was sprayed on the leaves, using a spray tower device (Burkard Manufacturing Co. Ltd., Rickmansworth, Herts, UK) (Kumral et al. 2010). Petroleum jelly was applied to edges of the leaves that were left to air dry for 30 min. Afterwards, *T. urticae* females (1–2 days old females were tested) were placed on the leaves using a paint brush for each dose. The control group was sprayed with

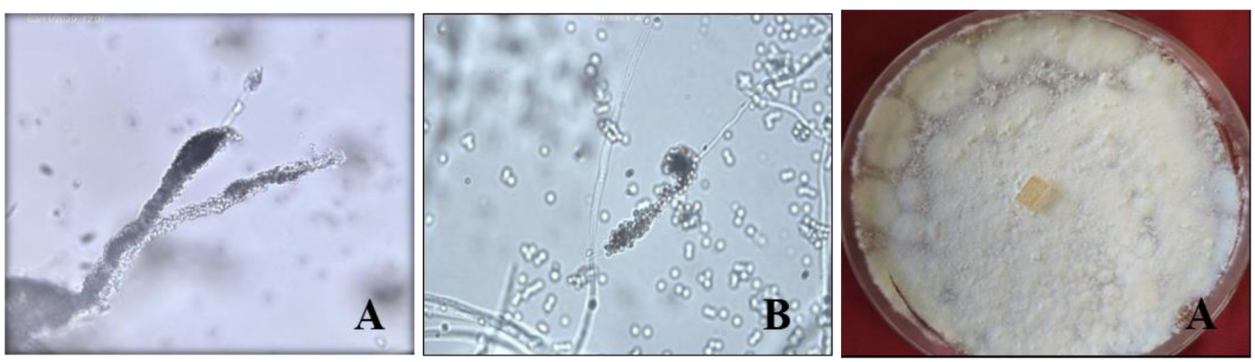

Fig. 1 Conidia of BGF14 (**a**) and BCA32 (**b**) isolates of *Beauveria bassiana* (×100 μm) and fungal growth in PDA media

water containing 0.02% Tween 80 on leaves, using a spray tower device. The experiments were carried out at room temperature of 24 ± 1 °C, RH of 65 ± 1%, and 14:10 photoperiod. The experiments were monitored after 3, 5, and 7 days post-application, and the ratios of dead and alive mites were recorded using a stereomicroscope. Each dose had 4 replicates (60 tested individuals), and the experiments were repeated 3 times for BGF14 and twice for BCA32.

Data analysis

The data obtained in the study was converted to measurements' percentage, transformed using arc-sin transformation, and then analyzed with analysis of variance (ANOVA). Percentage mortality was corrected by Abbott (1925). Comparisons between the percentage mortality were made using Duncan's multiple comparison test. The LC_{50} and LT_{50} values were calculated according to Finney (1971) in POLO-PC package program. All statistical analyses were done in SPSS 23.0 package program (IBM Corp, 2013).

Results

The first record of *T. urticae* mortality was recorded 3 days after treatment. On the 3rd day, concentrations of BGF14 and BCA32 isolates between 10^5 and 10^8 conidia/ml exhibited mortality rate ranging between 25.88 and 61.92% and between 32.36 and 62.03%, respectively. On the same day, mortality rates in the control treatments were 8.32% (for BGF14) and 11.67% (for BCA32). On the 7th day, the efficacy of the fungal isolates increased significantly to 48.08–82.50% mortality rates recorded for BGF14 and 67.92–90.95% for BCA32 between the tested concentrations, respectively. As for the control group, it was 23.38 % (BGF14) and 28.81% (BCA32) (Fig. 2).

After the application of *B. bassiana* BGF14 on the adults of *T. urticae*, it was observed that the fungus

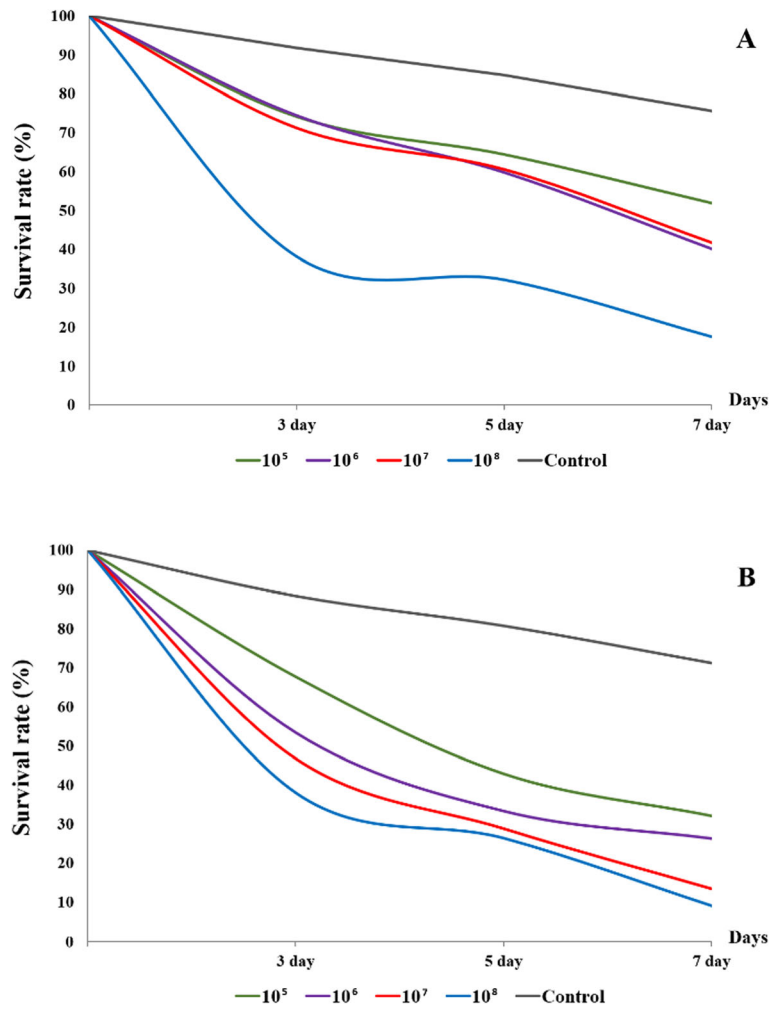

Fig. 2 Survival rate of *Tetranychus urticae* after application of BGF14 (**a**) and BCA32 (**b**) isolates of *Beauveria bassiana*

continued to develop, and its effect increased in the following days (Fig. 3). The pathogenicity of the (10^8 conidia/ml) application increased from 50% on the 3rd day to the highest of 76.98% as incubation progresses whereas the effect was less than 50% for the other tested concentrations, including 10^6 and 10^7 conidia/ml.

In the case of the isolate BCA32, fungal growth continued increasingly only after the 5th day. On the 3rd day, the fungus at 10^7 and 10^8 conidia/ml concentrations caused more than 50% mortality than the untreated control group. The highest effect was exhibited by 10^8 conidia/ml concentration on the 7th day with 87.27% mortality, followed by 10^7 conidia/ml concentration (81.09%), with non-statistical difference between the mortality by the two concentrations (Table 1).

Probit analysis was used to determine the LC_{50} and LT_{50} values of fungi in this study. The LC_{50} after 3 days of application with the *B. bassiana* isolate BGF14 to *T. urticae* populations was 1.1×10^7 conidia/ml, whereas it was low after 7 days of application with 2.6×10^6 conidia/ml. Concentration values of *B. bassiana* BCA32 were lower than BGF14. The LC_{50} value of the BCA32 isolate was 1.3×10^6 conidia/ml on the 3rd day and 6.3×10^4 conidia/ml on the 7th day (Table 2).

According to the data analysis, LT_{50} value of *B. bassiana* isolate BGF14 for the concentration of 10^8 conidia/ml was determined after 2.14 days, whereas similar effects were determined for the other treatments after 5.84–7.73 days. As for the other *B. bassiana* isolate, BCA32 had a short LT_{50} value of 2.23–4.27 days. The LT_{50} value was 2.23 days at the concentration of 1×10^8 conidia/ml (Table 3).

Discussion

The *T. urticae* is a global pest in greenhouse and field crops (Wekesa et al. 2011). In this study, the efficacy of 2 different *B. bassiana* isolates was evaluated against *T. urticae* under laboratory conditions.

Conidial concentrations and fungal isolates differed in adult viability: the greatest concentration the highest mortality of adults. According to Shi and Feng (2004), the mortality rate differed between the fungal isolates and conidial concentrations. In parallel with the results obtained in this study, 73.1% of *T. urticae* mites died within 10 days of applying *B. bassiana* (Shi and Feng 2009). Also, it has been reported that the efficacy of 5 different isolates of *B. bassiana* showed lethal effects ranging between 68.49 and 83.78% on the first day of application and 100% on the 4th day (Draganova and Simova 2010). Geroh et al. (2014) stated that on the 7th day, 0.3×10^8 conidia/ml concentration of *B. bassiana* was effective against *T. urticae* causing 63.31% mite mortality. In another study, Örtücü and Algur (2017) found that the pathogenicity effects of the 2 different isolates of *B. bassiana* on *T. urticae* was 24–60% on the 3rd day, 62.7–88% on the 5th day, and 90.7–100% on the 7th day. Shin et al. (2017) reported that 3 isolates of *B. bassiana* applied at the concentration of 10^8 conidia/

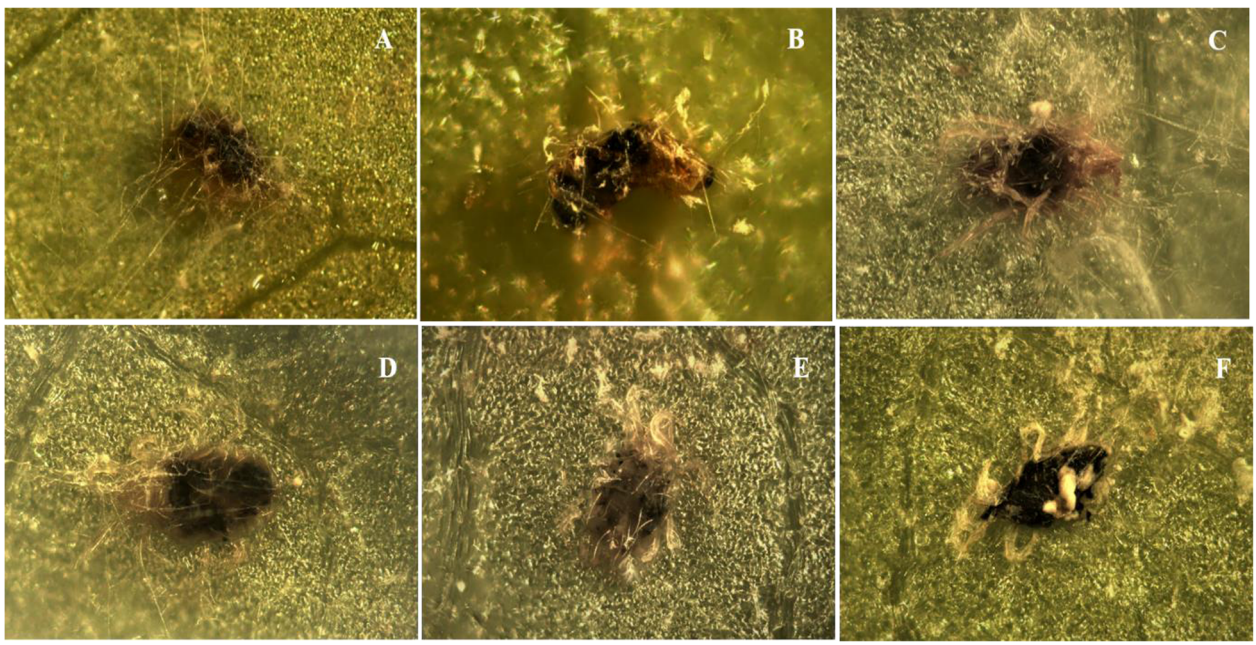

Fig. 3 *Tetranychus urticae* individuals infected with BGF14 isolate of *Beauveria bassiana* after 3 days (**a**), 5 days (**b**), and 7 days (**c**) and BCA32 isolate after 3 days (**d**), 5 days (**e**), and 7 days (**f**)

Table 1 Corrected mortality of BGF14 and BCA32 isolates of *Beauveria bassiana* against *Tetranychus urticae* adults

	Days	Control mortality ± SE	Corrected efficacy ± SE											
			Conidial suspensions at concentration (conidia/ml)											
			1×10^5			1×10^6			1×10^7			1×10^8			*F* ratio
BGF14	**3**	8.32±1.64	19.16±2.03	B*	b**	18.89±2.10	C	b	22.30±3.75	B	b	58.38±3.08	B	a	45.828
	5	15.17±2.01	24.01±2.24	B	b	29.39±4.10	B	b	28.63±3.49	B	b	62.05±3.60	B	a	26.181
	7	24.38±3.20	31.44±2.76	A	c	47.10±2.94	A	b	44.95±3.62	A	b	76.98±3.32	A	a	36.520
		***F* ratio**	6.854			20.433			10.413			8.699			$P \leq 0.05$
BCA32	**3**	11.67±1.43	24.44±4.77	B*	c**	40.72±3.95	B	b	48.21±4.99	C	ab	57.94±3.66	B	a	10.446
	5	19.28±2.20	46.87±3.69	A	b	58.68±3.75	A	a	64.24±2.80	B	a	67.37±3.44	B	a	6.890
	7	28.81±0.98	54.89±3.27	A	b	63.06±1.95	A	b	81.09±4.10	A	a	87.27±2.76	A	a	23.556
		***F* ratio**	15.892			12.560			16.355			20.515			$P \leq 0.05$

*Means within column bearing the same letter are not significantly different (Duncan's test, $p>0.05$)
**Means within lines bearing the same letter are not significantly different (Duncan's test, $p>0.05$)

ml caused 55–82% *T. urticae* mortality, whereas the different isolates used in a study by Yanar et al. (2018) had 32.5–72.5% effect on *T. urticae* when applied at 5×10^6 effect rate. Wu et al. (2020) also found that a 0.7×10^5 conidia/ml concentration of *B. bassiana* was highly effective with a mortality rate of 63.2–72.1% in *T. urticae* adults. Enzymes produced by each isolate may be correlated to the difference in virulence between the different fungal isolates of *T. urticae*.

This study revealed that the 2 native *B. bassiana* isolates (BGF14 and BCA32) and their metabolites had toxic effects on *T. urticae* adults. The LC_{50} value was high between treatments, and development of mycosis was observed after 3 days of application. These agree with the previous studies carried out by Geroh et al. (2015) who determined that 1×10^5–1×10^8 conidia/ml concentrations of *B. bassiana* applied to *T. urticae* adults showed 42–64% effects against the mite when the LC_{50} was 0.3×10^8 conidia/ml. Moreover, Elhakim et al. (2020) stated that *B. bassiana* caused 15 to 70% mortality against *T. urticae*, and its LC_{50} and LC_{90} values estimated 3.3×10^6 and 7.8×10^9 conidia/ml, respectively. It was also determined that *B. bassiana* caused 56.4–82.6% mortality against *Tetranychus evansi*, and the LC_{50} value was 1.1×10^7 conidia/ml on the 7th day (Wekesa et al. 2005). Chandler et al. (2005) stated that the LC_{25} value of *B. bassiana* was 8.65×10^7 6 days post-inoculation into *T. urticae* adults. Irigaray et al. (2003) reported that the commercial isolate of *B. bassiana* (Naturalis L.) had a LC_{50} value of 1.9×10^3 conidia/ml against *T. urticae* adults.

Furthermore, results of applying 2 different isolates of *B. bassiana* to *Tetranychus cinnabarinus* showed that the LT_{50} value was 3.6–5.8 days at the concentration of 1.5×10^3 conidia/ml (Shi et al. 2008). In another study, under greenhouse conditions, the LT_{50} value for a 1×10^8 conidia/ml concentration of *B. bassiana* against *Tetranychus kanzawai* was found to be 3.98–5.48 days, and the LT_{90} value was 9.58–15.78 days (Sanjaya et al. 2016). The LT_{50} value of 3 different isolates of *B. bassiana* after applied to *T. urticae* was 3.16–3.72 days (Örtücü and İskender 2017). These results are consistent with a previous study that the local strains used in this study had a high pathogenic effect against *T. urticae*. Evaluating the compatibility of EPF is a critical issue for the successful implementation of IPM programs to control pest mite species (Vergel et al. 2011).

Conclusion

Pathogenic potentials of 2 local isolates of *B. bassiana* against *T. urticae* were studied. In a relatively short period, BCA32 was highly efficacious and exhibited

Table 2 Median lethal concentration (LC_{50}) of *Beauveria bassiana* (BGF14 and BCA32 isolates) against *Tetranychus urticae* adults

EPF[a]	Days	χ^2	df	Slope±SE	LC_{50} (conidia/ml)	95% Confidence intervals
BGF14	3	28.25	45	0.359 ± 0.071	1.1×10^7	0.4×10^7–9.9×10^7
	5	25.65	45	0.388 ± 0.096	0.9×10^7	0.3×10^7–1.3×10^7
	7	20.87	45	0.353 ± 0.072	2.6×10^6	0.8×10^6–7.1×10^6
BCA32	3	17.02	29	0.270 ± 0.071	1.3×10^6	0.4×10^6–1.2×10^7
	5	12.05	29	0.219 ± 0.077	2.7×10^5	0.6×10^4–1.6×10^6
	7	8.95	29	0.355 ± 0.086	6.3×10^4	1.4×10^3–3.0×10^5

Entomopathogenic fungi

Table 3 Median lethal time (LT_{50}) values of BGF14 and BCA32 isolates of *Beauveria bassiana* against *Tetranychus urticae* adults

EPF[a]	Fungal conidial conc./ml	χ^2	df	Slope±SE	LT_{50} (days)	95% Confidence intervals
BGF14	10^5	6.48	33	1.602 ± 0.466	7.73	6.10–15.00
	10^6	12.93	33	2.389 ± 0.482	5.84	5.08–7.23
	10^7	16.35	33	2.002 ± 0.457	6.09	5.20–8.08
	10^8	15.11	33	1.648 ± 0.495	2.14	0.71–2.95
BCA32	10^5	6.57	21	2.839 ± 0.577	4.27	3.62–4.90
	10^6	7.18	21	2.847 ± 0.763	3.29	2.41–3.77
	10^7	11.36	21	2.615 ± 0.571	3.01	2.13–3.58
	10^8	8.48	21	1.946 ± 0.573	2.23	0.84–3.00

Entomopathogenic fungi

mortality rate that exceeded 50% at low concentrations. BGF14 also caused mortality rate more than 50% in high concentrations, but it lasted a long period to exhibit this compared to BCA32. Further studies are needed to determine the effectiveness of these 2 local isolates against *T. urticae* under field conditions and in greenhouses.

Abbreviations

EPF: Entomopathogenic fungi; BGF14: *Beauveria bassiana* [isolated from *Gonioctena fornicata* (Brüggmen)]; BCA32: *Beauveria bassiana* (isolated from *Cicadatra adanai* Kartal); DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; LT_{50}: Median lethal time; LC_{50}: Median lethal concentration; SDA: Sabouraud dextrose agar; PDA: Potato dextrose agar; NCBI: National Center for Biotechnology Information; ANOVA: Analysis of variance; ± SE: Positive or negative standard error

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Author's contributions

The author has developed and implemented this review article and written it. The author read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

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