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Assessment of nematicidal activity of *Beauveria bassiana* (Bals.-Criv.) vuill on *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae)

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Abstract

In this study, nematicidal activity of two different isolates (BY2 and BIM-001) of the *Beauveria bassiana* (Bals.-Criv.) Vuill was investigated on *Pratylenchus thornei* Sher et Allen) (Tylenchida: Pratylenchidae) using culture filtrates and spore suspensions. Three spore suspensions ($1x10^6$, $1x10^7$, $1x\ 10^8$ spore/ml) and four culture filtrate concentrations (1X, 10X, 20X, 50X) were tested in the study. Depending on the treatment; 2 ml of spore suspensions or culture filtrates in different concentrations of both isolates and 400 larvae+adults from *P. thornei* were transferred to 6 mm petri dishes. The dead nematodes were counted under a light microscope after 24 and 72 hour and their mortality rate (%) were calculated. The nematicidal effect of culture filtrates of *B. bassiana* BY2 and BIM-001 isolates on *P. thornei* was found to be higher than the spore suspension. It was determined that the 1X concentration of culture filtrate of *B. bassiana* BY2 reached 100% mortality rate on *P. thornei* after 24 hour. After 72 hours, 10X (99.0%) concentrations of BY2 isolate and 1X (100%) and 10X (93.2%) concentrations of BIM isolate showed similar nematicidal activity with the commercial nematicide Velum (97.6%). On the other hand, after 72 hour, *P. thornei* mortality rate was 75.5% and 64.1%, respectively, at a concentration of 10^8 spore/ml of *B. bassiana* BY2 and BIM-001 isolates. This study will contribute to the development of a new control method as an alternative to the use of crop rotation and resistant cultivars in the control of *P.thornei*.

Keywords

Culture Filtrate, Nematicidal Effect, Nematicidal Compounds, Entomopathogenic Fungi, Biological Control

Introduction

Root lesion nematodes (*Pratylenchus* spp.) are migratory plant-parasitic nematodes and cause lesions and necroses in the roots of the plants (Davis et al., 2011). These nematodes lead to significant economic losses that are common in fruits, vegetables and field crops worldwide (Smiley and Nicol, 2009). They damage cells in stems, thus plants cannot properly absorb water and nutrients from the soil, resulting in symptoms similar to nutrient and water deficiency (Castillo and Vovlas, 2007; Kumar et al., 2018). In addition, root lesion nematodes indirectly cause economic losses by helping many soil-borne diseases and pests enter the plant (Smiley et al., 2004; Göze Özdemir, 2020). *Pratylenchus thornei* Sher & Allen 1953 is an economically important pest found in grains and legume roots in many countries of the world (Smiley and Nicol, 2009; Ganguly and Pandey, 2012; Thompson et al., 2017; Mokrini et al., 2019; Kumar et al., 2018). *Pratylenchus thornei* and *P. neglectus* were widely reported in cereal production areas in Turkey (Dababat et al., 2019; Yavuzaslanoğlu et al., 2012, 2020; Göze Özdemir et al., 2021;). It has been reported that cysts and root lesion nematodes in wheat in the Central Anatolian Plateau of Turkey cause about 50% yield loss (Nicol and Ortiz Monastterio, 2004). Field hygiene, use of resistant and tolerant varieties and non-host crop rotation are more preferred for the control of *P. thornei* as in the management of other plant-parasitic nematodes. In addition, cereals cultivation in very large areas makes chemical control less economical. In recent years, biological control of plant parasitic nematodes has been

reported as an important management strategy (Timper, 2014; Li et al., 2015; de Oliveira, 2021). A large number of microorganisms are known as antagonists of plant parasitic nematodes (Akhtar and Malik, 2000). Some soil-borne pathogens have promising potential in the control of plant parasitic nematodes (Anke and Sterner, 1997). Culture filtrates of many entomopathogenic fungi are known to be effective against plant parasitic nematodes, and this nematicidal effect is thought to be caused from toxic metabolites produced by the fungus (Caroppo et al., 1990; Liu et al., 2008; Göze Özdemir and Arıcı, 2021).

Beauveria bassiana (Bals.-Criv.) Vuill belonging to entomopathogenic fungi (Hypocreales: Cordycipitaceae) has great potential in the control of many insects and arthropods (Feng et al., 2004; Pu et al., 2005; Hatting et al., 2012; Demirozer et al., 2016; Canassa et al., 2019; Uzun et al., 2021). It has been known that many species of Beauveria secrete secondary metabolites such as bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein (Strasse et al., 2000; Quesada and Vey, 2004). These secreted toxins act as a thrombocyte inhibitor in the insect body (Butt et al., 2001). The culture filtrate of *B. bassiana* isolate has been determined to have high nematicidal effect on *Ditylenchus destructor*, *Meloidogyne incognita* (J2), *M. hapla* (J2), *Heterodera glycines* (J2), *Aphelenchoides besseyi* and *Caenarhabditis* sp. (Liu et al., 2007; 2008; Zhao et al., 2013).

There are very few studies about the nematicidal effect of *B. bassiana* on root lesion nematodes. Therefore, this study aimed to determine the nematicidal activity of different spore and culture filtrate concentrations of two different *B. bassiana* isolates (BY2 and BIM-001) originated from Turkey against the root-lesion nematode *P. thornei* under *in vitro* conditions.

Materials and Methods

Materials

Beauveria bassiana BIM-001 isolate obtained from *Leptinotarsa decemlineata* Say in Isparta potato fields and BY2 isolate obtained from Haplothrips sp. in Burdur wheat fields were used in the study (Sarı, 2020; Uzun, 2020;). Different spore (10^6, 10^7, 10^8 spore/ml) and culture filtrate concentrations (1X, 10X, 20X and 50X) of these isolates were used. Entomopathogenic fungal isolates were kept in the Integrated Control Laboratory of the Plant Protection Department in Isparta University of Applied Sciences (ISUBU) and renewed periodically to prevent the loss of pathogenicity.

The *Pratylenchus thornei* isolate (SK11) was obtained from a wheat field in Sarkikaraağaç in Isparta, Turkey and identified in previous studies (Göze Özdemir, 2020; 2021). Mass production of *P. thornei* was carried out in carrot discs and renewed every four months in the Nematology Laboratory of the Plant Protection Department in ISUBU (Zuckerman 1985). Nostalgist (*B. bassiana* strain Bb-1, Agrobest Grup Ltd. Şti, Turkey) and Velum (Fluopyram, Bayer Group Co. Ltd.) commercial preparations were used in positive control applications. The distilled water was used as negative control.

Preparation of the spore suspension

Beauveria bassiana BIM-001 and BY2 isolates were cultured on potato dextrose agar (PDA) and incubated for 10 days at 25˚C under dark conditions. Spore suspensions (10^6, 10^7, 10^8 spore/ml) were prepared by counting spores in a hemocytometer.

Preparation of the culture filtrate

Beauveria bassiana BIM-001 and BY2 isolates were cultured in 250 ml flasks containing 50 ml PDB (potato dextrose broth agar) and sterilized at 121 °C for 20 min. Then, 1 cm^2 pieces of 2-week-old fungus colony grown in Potato Dextrose Agar (PDA) were taken into each erlenmeyer flask and incubated at 25 ° C in the dark for 10 days. The flasks were manually shaken every day during the incubation period. After ten days, the culture filtrate was first passed through two layers of filter paper (Whatman No. 1) to remove fungus spores and micelles, then re-filtered through a 0.45 1m pore size filter. Obtained filtrate were used as the pure (1X) culture solution. Other concentrations (10X, 20X and 50X) were prepared by diluting the pure culture with sterile distilled water (Liu et al., 2008). They were kept in the refrigerator at 4° C until used in the experiment.

Nematode inoculum

The carrot discs in which *Pratylenchus thornei* was produced were transferred to 120 mm petri dishes. Then they were cut into small pieces, and sterile distilled water was added to cover the petri dish. After six hours, the nematodes were extracted using the Baermann funnel method (Mudiope et al., 2004). Adult and larvae of *P. thornei* population were counted under the light microscope at 10X magnification. Then, *P. thornei* nematode density to be used in the study was adjusted and the inoculum was kept in the refrigerator at 4° C in eppendorf tubes containing pure water.

Nematicidal effect bioassays

The treatments carried out to determine the nematicidal effects of spore and culture filtrates at different concentrations of two *B. bassiana* isolates on *P. thornei* were carried out in plastic petri dishes (6 cm diameter). All treatments were conducted according to a randomized plot design with five replications. The petri dishes were kept at 25 ± 1°C and 60% relative humidity during the treatments. Depending on the treatment group; 2 ml of spore suspensions or culture filtrates of different concentrations of both isolates and 50 microliters (400 larva+adults) from *P. thornei* inoculum were transferred to petri dishes (Liu et al. 2008; Kepenekçi et al. 2018). As a positive control treatment, the nematicidal effects of commercial plant protection products Nostalgist and Velum (at maximum field application doses of 250 ml/da and 120 ml/da, respectively) on *P. thornei* were investigated with the same method. In the negative control, sterile distilled water was used. After 24 and 72 h, dead and living *P. thornei* individuals were counted under the light microscope at 20X magnification and the results were recorded. During counting, the nematode individual that did not move when touched with a thin needle (38X0.30 mm) was considered dead (Cayrol et al., 1989; Zhao et al., 2013). Mortality rates were calculated from the obtained values (Liu et al., 2008).

Statistical analysis

All data obtained in the study were statistically analyzed by the SPSS 20.0 program. Analysis of

variance (ANOVA) was performed to test the differences between the means of mortality rates. Means of mortality rates at different observation times were compared with Tukey's HSD test at a significance level of $p \leq 0.05$.

Results and Discussion

Nematicidal effect bioassays of spore concentrations

The mortality rates at 10^6, 10^7 and 10^8 spore/ml concentrations of BY2 isolate of *B. bassiana* were 9.5%, 20.9% and 42.8% respectively after 24 h whereas these rates in BIM-001 isolate were 5.9%, 16.7% and 35.1%, respectively. It was found that the mortality rates were found higher even at the lowest concentration of *B. bassiana* isolates than the control at 24 h after the application. At the highest concentration of both isolates, the mortality rates at 24 h were found to be below 50%. There were significant differences between the mortality rates for each application dose of *B. bassiana* isolates compared to the control after 24 h. The mortality rates of Nostalgist (*B. bassiana* strain Bb-1), commercial bioinsecticide, were 22.8% and 27.9% at 24 and 72 h, respectively. After 72 h, the nematicidal effect of Nostalgist (22.8%) was higher than *B. bassiana* 10^6 spores/ml concentration when compared to BIM-001 (15.2%) and BY2 (20.0%) isolates, but lower than 10^7 and 10^8 spore/ml concentrations of both isolates. The mortality rates of BIM-001 isolate at 10^7 and 10^8 concentrations were 35.9% and 64.1%, respectively while those of BY2 isolate at the same concentrations were 41.7% and 75.5%, respectively after 72 h. The nematicidal effect of *B. bassiana* BY2 isolate was determined to be higher than BIM-001 and a statistically significant difference was found between each spore suspension concentrations of BIM-001 and BY2 isolates ($p \leq 0.05$). It was observed that the mortality rate of *P. thornei* increased directly proportional to the spore concentration in both isolates of *B. bassiana* at 24 and 72 h. The mortality rates of the commercial nematicide Velum (Fluopyram) were 92.2% and 97.6% at 24 and 72 h, respectively. The mortality rates of both isolates at a concentration of 10^8 spore/ml were over 50% at 72 h after application. A statistically significant difference was found between BIM-001 (64.1%) and BY2 (75.5%) isolates of *B. bassiana* at 10^8 spore/ml concentration and Velum (97.6%) in terms of mortality rate ($p \leq 0.05$). It was determined that the nematicidal effect of *B. bassiana* differs according to the isolates, spore concentrations of the entomopathogenic fungus and the time passed after the application (Table 1).

Nematicidal effect bioassays of culture filtrate

The mortality rates of 1X, 10X, 20X and 50X concentrations of *B. bassiana* culture filtrate were 100.0%, 46.5%, 23.1% and 14.7% respectively in BY2 isolate after 24 h, whereas these rates were 85.3%, 48.1%, 20.3% and 10.9% in BIM-001 isolate. It was determined that the mortality rate decreased with the dilution of the culture filtrate. The pure culture filtrate concentration of BY2 isolate (1X) killed all *P. thornei* adult+larvae stages in 24 h. In BIM-001 isolate, the mortality rate was 85.3% at 1X concentration in 24 h, while it was 100% after 72h. It was observed that the nematicidal effect increased over time at other culture filtrate concentrations. The mortality rates of *B. bassiana* BY2 culture filtrate in 1X, 10X, 20X and 50X concentrations were 100.0%, 99.0%, 46.4% and 27.8%, respectively, whereas these rates were 100.0%, 93.2%, 36.5% and 22.1% in BIM-001 isolate. The mortality rates were found to be below 50% at 20X and 50X culture filtrate concentrations of both isolates. However, the nematicidal effect of *B. bassiana* BY2 was higher considering the low culture filtrate concentrations. The difference between 1X concentration of BY2 isolate (100%) and Velum (92.2%) was statistically significant at 24 h, therefore the nematicidal effect of BY2 was higher than the commercial nematicide Velum ($p \leq 0.05$). On the other hand, this difference was not statistically significant for 1X concentration of BIM-001 isolate ($p \geq 0.05$). After 72 h, the nematicidal effect of 10X concentration of BY2 isolate was similar to that of 1X concentration. Also, there was no statistically significant difference between the mortality rates at 1X and 10X concentrations of BIM and BY2 isolates ($p \geq 0.05$). The nematicidal effect of Nostalgist was similar to the effect of 50X culture filtrate concentration of *B. bassiana* BIM-001 and BY2 at 72 h. However, the nematicidal effects of 1X, 10X and 20X concentrations of the culture filtrate in both isolates were higher than Nostalgist at 72 h. The mortality rates of Nostalgist and 50X concentration of the two isolates were significantly higher than the control (the pure water) (Table 2).

It was determined that the nematicidal effect of Turkish isolates named BY2 and BIM-001 of *B. bassiana* obtained from different insects reached 75.5% and 64.1% mortality, respectively, at 72 h in vitro. The mortality rate occurred at 10^8 spores/ml concentration of *B. bassiana* on *P. thornei* was higher than Nostalgist which was a commercial bioinsecticide containing *B. bassiana*. There are very few studies about the nematicidal effect of *B. bassiana* on root lesion nematodes. Kepenekci et al. (2017) investigated the effect of 10^6, 10^7 and 10^8 cfu/ml-1 suspensions of two Turkish *B. bassiana* isolates (F-56 and F-63) in the tomato field infested with *Meloidogyne incognita* and *M. javanica*, then they reported that 10^8 cfu/ml-1 suspension in each two isolates showed a nematicidal effect and positively affected the yield.

The nematicidal effect of the culture filtrates of *B. bassiana* BY2 and BIM-001 isolates on *P. thornei* was higher than the spore suspensions of the two isolates. The culture filtrates of BIM-001 and BY2 had a high nematicidal effect (1X, 100%) on *P. thornei* at 72 h *in vitro*. Since the culture filtrate is a fermented product, it is thought that the nematicidal effect may have increased with the synergistic or antagonistic effect of the secondary compounds in its content and the possibility of higher amount of enzymes and toxins (Ciancio, 1995; Nitao et al., 1999; Lopez-Llorca et al., 2006; Kim et al., 2013). Extracellular hydrolytic protease and chitinase enzymes produced for cuticle penetration and digestion by fungi used as biocontrol agents are effective on nematodes (Morton et al., 2004; Huang et al., 2004). Regaieg et al. (2010) reported that *M. incognita* eggs exposed to *Verticillium leptobactrum* culture filtrates deteriorated due to the defect in the chitin layers and appeared collapsed. Chitinase and proteinase enzymes purified from *V. suchlasporium* were found to break

down the shells of *Globodera pallida* eggs (Tikhnov et al., 2002).

In the present study, the mortality rates (%) of *B. bassiana* BY2 and BIM-001 isolates on *P. thornei* were found to be 100% and 85.3%, respectively, at 1X culture filtrate concentration after 24 h and the nematicidal effect of BY2 isolate was higher than Velum (92.2%). In terms of the nematicidal effect, it was found that Velum (97.6%), 10X (99.0%) concentration of BY2 isolate and 1X (100%) and 10X (93.2%) concentrations of BIM isolate were in the same statistical group after 72 h. Lu et al. (2016) found that the culture filtrate of four out of 10 *B. bassiana* isolates had high virulence against *Pratylenchus* sp. and mortality rates of the BD-B173, BD-B180, BD-B061-3 and BD-B315 isolates were 97.20%, 96.50%, 91.16% and 90.32%, respectively after 24 h. Zhao et al. (2013) determined that the culture filtrate of nine *B. bassiana* isolates completely killed the *Caenarhabditis* sp. within 48 h while caused 15-96% mortality on *M. incognita*, 15-65% on *Aphelenchoides besseyi*, and 51-100% on *Heterodera glycines*. Liu et al. (2008) stated that 1X (96.5%) and 5X (88.9%) concentrations of *B. bassiana* culture filtrate showed high antagonistic activity against J2 of *M. hapla*, and 1X concentration achieved the same mortality as Aldicarb (96.5%). Chen at al. (1996) reported that although the parasitization rate of *H. glycines* eggs of *B.bassiana* was low, larvae hatching was suppressed in parasitized eggs. Sun et al. (2006) found that the parasitization rate of eggs and females of *B. bassiana* was 100% on *Meloidogyne* spp. It was observed in previous studies that the nematicidal effect of *B. bassiana* may differ according to the nematode species.

Table 1. Mortality rates (%) of spore suspension concentrations of different *Beauveria bassiana* isolates on *Pratylenchus thornei*

	Observation Times*			
	24 h		72 h	
	Entomopathogenic Fungus isolates		Entomopathogenic Fungus isolates	
	BY2	BIM-001	BY2	BIM-001
Treatments	Mean of Mortality rate%± Standart error			
10^6 spore/ml	9.5±0.3 d A	5.9±0.6 e B	20.0±0.9 e A	15.2±0.8 e B
10^7 spore/ml	20.9±0.7 c A	16.7±0.7 d B	41.7±1.3 c A	35.9±1.7 c B
10^8 spore/ml	42.8±1.1 b A	35.1±1.1 b B	75.5±1.3 b A	64.1±2.1 b B
Nostalgist®(Positive control)	22.8±0.8 c	22.8±0.8 c	27.8±1.1 d	27.8±1.1 d
Velum® (Positive control)	92.2±1.0 a	92.2±1.0 a	97.6±1.1 a	97.6±1.1 a
Distilled water (Negative control)	0.9±0.1 e	0.9±0.1 f	1.1±0.1 f	1.1±0.1 f

*Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means differ significantly ($p \leq 0.05$).

Table 2. Mortality rates (%) of culture filtrate concentrations of different *Beauveria bassiana* isolates on *Pratylenchus thornei*

Treatment	Observation Times*			
	24 h		72 h	
	Entomopathogenic Fungus isolates		Entomopathogenic Fungus isolates	
	BY2	BIM-001	BY2	BIM-001
	Mean of Mortality rate%± Standart error			
1X	100.0±0.0 a A	85.3±3.7 a B	100.0±0.0 a A	100.0±0.0 a A
10X	46.5±1.4 c A	48.1±0.8 b A	99.0±0.7 a A	93.2±2.8 b A
20X	23.1±0.9 d A	20.3±0.7 c B	46.4±2.0 b A	36.5±0.8 c B
50X	14.7±0.5 e A	10.9±1.1 d B	27.8±0.9 c A	22.1±1.4 d B
Nostalgist® (Positive control)	22.8±0.8 d	22.8±0.8 c	27.8±1.1 c	27.8±1.1 d
Velum® (Positive control)	92.2±1.0 b	92.2±1.0 a	97.6±1.1 a	97.6±1.1 ab
Distilled water (Negative control)	0.9±0.1 f	0.9±0.1 e	1.1±0.1 d	1.1±0.1 e

*Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means differ significantly ($p \leq 0.05$).

Conclusion

The inoculum method of *B. bassiana* was found important for the nematicidal effect on *P. thornei*, and the culture filtrate method had higher potential than spore suspension. In addition, there was no statistically significant difference between 1X and 10X concentrations of both entomopathogenic fungal isolates (BY2 and BIM-001) and Velum after 72 h under laboratory conditions. These concentrations show similar results with Velum, a chemical nematicide, suggesting that *B. bassiana* BY2 and BIM-001 isolates are promising in the control of *P. thornei* under *in vitro* conditions. It is thought that re-evaluation of the obtained results for culture filtrate concentrations in this

study would be beneficial for application in open field studies for both isolates

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

FGGO and AUY participated in the nematicidal effect bioassays and collected of data. OD performed statistically analysis of these data. FGGO, AUY and OD wrote the manuscript, read and approved the final manuscript.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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