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Can *Beauveria bassiana* reduce the root lesion nematode, *Pratylenchus thornei*, infection on wheat?

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Abstract

Background: Limited number of control methods against root-lesion nematodes has increased the search for alternative management strategies. Due to secondary metabolites such as toxins and enzymes secreted by the entomopathogenic fungus, *Beauveria bassiana*, the number of studies on nematicidal potentials on many plant parasitic nematodes has increased. Almost no work was done on the nematicidal activity of *B. bassiana*, which is widely used as a bio-insecticide, on the root lesion nematode, *Pratylenchus thornei*, commonly found on wheat in Turkey. In the present study, it was aimed to determine the pathogenicity of two native *B. bassiana* isolates (BIM-001 and BY2) obtained from Turkey against *P. thornei* on wheat under controlled conditions.

Results: Spore suspension of *B. bassiana* isolates affected *P. thornei* soil, root, final nematode density and reproduction rate at different degrees according to spore concentration. The soil, root, total nematode density, and reproduction rate in the control on İkizce cv. were 1234.0 adult + larvae/500 g soil, 1105.0 adult + larvae/per pot, 2341.0 (soil + root density) and 5.8 (PF(final nematode density/PI (initial inoculum density)), respectively. It was determined that these parameter values were at the lowest at 10^8 spore/ml than other concentrations in both isolates of *B. bassiana*. In 10^8 spore/ml concentrations of BIM-001 isolate, while *P. thornei* soil, root, total nematode density, and reproduction rate on İkizce were 641.7 adult + larvae/500 g soil, 930.9 adult + larvae/per pot, 1572.6 (soil + root density) and 3.9 (PF/PI), respectively, these parameter values were found to be 645.2 adult + larvae/500 g soil, 849.0 adult + larvae/per pot, 1492.2 (soil + root density) and 3.6 (PF/PI) at 10^8 spore/ml concentrations of BY2 isolate. It was observed that the reproduction rate of *P. thornei* decreased at 10^8 spore/ml concentration of BIM-001 and BY2 isolates compared to the control.

Conclusion: Native *B. bassiana* isolates of BIM-001 and BY2 reduced *P. thornei* on wheat and the concentration was important for the pathogenicity of *B. bassiana*. While the reproduction rate of *P. thornei* at 10^8 and 10^7 spores/ml concentrations in both isolates decreased than the control. At 10^6 spore/ml concentration it was found similar to the control. Even though *B. bassiana* is known as an effective biocontrol agent against insects and some plant-parasitic nematodes, more detailed studies should be done on its effect on *P. thornei*.

Keywords: *Beauveria bassiana*, Wheat, *Pratylenchus thornei*, Root lesion nematode, Virulence

Background

The root lesion nematode, *Pratylenchus thornei* (Sher et Allen) (Tylenchida: Pratylenchidae), is known as a parasite of the roots of cereals and legumes in many countries around the world causing economic losses (Castillo and Vovlas 2007; Thompson et al. 2021). This nematode species is commonly found in cereal production areas

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in Turkey (Göze Özdemir et al. 2021). This nematode is polycyclic and completes its life cycle in about 45 days under optimum conditions (Larson 1959). Therefore, population densities increase exponentially during the production season (Thompson 2015). While *P. thornei* feeds and multiplies in the root cortex, it also disrupts the function of the root. It causes characteristic lesions that inhibit the plant's ability to absorb water and nutrients, and cause yield loss in susceptible crops (Tan et al. 2013). In addition, these lesion areas constitute areas of infection for many fungal agents (Mallaiah et al. 2014). It is suggested that root lesion nematodes can be controlled with methods such as ensuring field hygiene, cultivation resistant or tolerant wheat varieties, or rotation with non-host crops (Owen et al. 2010). The absence of licensed nematicides for the control of nematodes in cereals also limits the possibilities of controlling this pest group and increases the search for alternative and sustainable control methods (Thompson et al. 2021). As a result of these searches, it has been reported that studies on the effects of different fungi on plant-parasitic nematodes have increased due to the ability to derive compounds from toxins, enzymes, or metabolites (Göze Özdemir and Arıcı 2021).

Beauveria bassiana (Balsamo.-Criv.) Vuillemin 1912 (Hypocreales/Cordycipitaceae) is a widely known entomopathogenic fungus worldwide and there are many isolates commercially used for the control of various arthropod species (Mwaura et al. 2017). *Beauveria*, besides insects, also produces organic compounds that affect nematode behavior (Hummadi et al. 2021). These compounds offer the potential to be used as fumigants to eliminate the free-living stages of plant-parasitic nematodes (Khoja et al. 2021). The mechanism of antagonism of *B. bassiana* includes antibiosis, competition and induced systemic resistance (Devi 2019). Basically, chitinases, lipases, and proteases are the most important of all the enzymes produced by *B. bassiana* (Amobonye et al. 2020). However, in different studies, it has been determined that it has the ability to produce other enzymes such as amylase, asparaginase, cellulase, galactosidase (Petlamul and Boukaew 2019). In different studies, the presence of beauvericin, bassianolide, bassiacridin, and oosporein toxins in *B. bassiana* culture supernatants have been reported (Ortiz-Urquiza et al. 2010). There are different studies showing that fermented filtrate products from *B. bassiana* have potential in the control of important plant-parasitic nematodes such as *Ditylenchus destructor* Thorne, 1945, *Meloidogyne hapla* Chitwood, 1949, *M. incognita* (Kofoid and White 1919) Chitwood, 1949, *Heterodera avenae* (Wollenweber 1924) and *Pratylenchu*s sp. (Youssef et al. 2020).

It is known that the use of resistant-tolerant varieties has priority in the control of root lesion nematodes in wheat worldwide and a different alternative method has not been recommended yet. The lack of any detailed study on the effect of *B. bassiana*, which is widely used as a bio-insecticide in pest control, on Root lesion nematodes led to the planning of this study. This study aimed to reveal the pathogenicity of two native *B. bassiana* isolates obtained from Turkey against *P. thornei* in wheat under controlled conditions.

Materials and methods

Materials

The study was carried out with the SK11 isolate of *P. thornei* which was obtained from the wheat production area of Şarkikaraağaç district (Turkey) in Isparta Province (Göze Özdemir et al. 2021) and whose mass production in the laboratory continued according to Zuckerman et al. (1985)'s carrot disk method. BIM-001 of *B. bassiana* isolated from *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) in potato fields in Isparta (Sarı, 2020) and BY2 isolate isolated from *Haplothrips* sp. in Burdur wheat fields were used in the study (Uzun, 2020). The wheat material of the study was İkizce cultivar, which was found to be susceptible to root lesion nematode in previous studies conducted in Isparta Province (Söğüt et al. 2011).

Preparation of nematode inoculum

After mass production of *P. thornei*, carrot disks in 9 cm diameter Petri dishes were cut into small pieces, sterile water was added to cover the Petri dish. After two days, the nematodes that got into the water were taken with a tape measure and the precipitation process was carried out. The obtained nematode suspension was counted using a binocular microscope and approximately 400 *P. thornei* (adult + larvae) were transferred into Eppendorf tubes with 1 ml of sterile water (Kepenekçi et al. 2018).

Preparation of the fungal inoculum

Beauveria bassiana BIM-001 and BY2 isolates were cultured on Potato Dextrose Agar (Sigma-Aldrich, Germany) medium in 10 cm diameter Petri dishes and incubated at 25 °C for 10 days in dark conditions. After ten days, distilled water was poured onto the fungus culture dishes and the dishes were rubbed with a spreader. Then, a filter was placed on the beaker and distilled water + spores were poured onto the filter. Spore suspension concentrations 10^6, 10^7, 10^8 spores/ml were prepared

by counting the spores on the thoma slide (Youssef et al. 2020).

Determination of pathogenicity of *Beauveria bassiana* against *Pratylenchus thornei* on wheat

The study was carried out in 500 cc plastic pots with 500 g of autoclaved soil mixture (68% sand, 21% silt, and 11% clay) in a climate room with 25 ± 2 °C and 60 ± 5% proportional humidity under controlled conditions. Three wheat plants in pot were considered as one replica. Experiments were set up separately for *B. bassiana* BIM-001 and BY2 isolate. The study was carried out with 10 replications of both entomopathogenic fungus isolates according to a randomized plot design for each suspension concentration (1×10^6, 1×10^7, 1×10^8 spores/ml). One week after sowing the seeds, 400 *P. thornei* individuals (larvae + adult) were inoculated in each pot with the help of plastic pipettes into the holes drilled to 2–3 cm soil depth around the root zone (Kepenekçi et al. 2018). Five ml of the spore suspensions were prepared immediately after the nematode inoculation was applied to each pot with the help of a measuring tape. Only wheat plants treated with *P. thornei* were used as control. Experiments were terminated after 9 weeks. At the end of this period, plant and root length (cm) and fresh weight (g) of wheat plants were recorded. *P. thornei* individuals were obtained from soil and root using the Baermann funnel method (Hooper et al. 2005). Then, nematodes counts were made under a binocular microscope at 10X magnification, and after the total nematode density was obtained, the reproduction rates were calculated with the formula; Reproduction rates = Final nematode density (PF)/number of inoculated nematodes (PI).

Statistical analysis

SPSS (version 20.0) program was used for the statistical analysis of the data obtained in the study and analysis of variance (ANOVA) was performed to test the differences between the means. "Tukey" test was used when variances were homogeneous to determine the means of different groups.

Results

In the study, the mean of soil nematode density was lower than the control plants that were only treated with *P. thornei* in all concentrations of *B. bassiana* BIM-001 isolate. However, it was determined that there was non-significant difference between the concentrations of BIM-001 isolate in terms of mean of soil nematode density ($P \geq 0.05$). The mean root nematode density of 10^8 (930.9 ± 45.9/per pot) and 10^7 (960.0 ± 68.7/per pot) spores/ml concentrations of BIM-001 isolate was lower than the control (1105.0 ± 111.2/per pot), but the difference between them was non-significant ($P \geq 0.05$). The highest mean of total nematode density and reproduction rate was determined at 10^6 spore/ml concentration of BIM-001 isolate and control. Although the mean of total nematode density and reproduction rate parameters of 10^8 spore/ml concentrations of BIM-001 isolate were lower than 10^7 spore/ml concentrations, non-significant difference was found between them ($P \geq 0.05$). However, it was determined that they were lower than the control (Table 1).

The mean soil nematode density of 10^8, 10^7, and 10^6 spores/ml concentrations of *B. bassiana* BY2 isolate was lower than the control. It was determined that the lowest mean of *P. thornei* soil, root, total nematode density and reproduction rate was found at 10^8 spore/ml

Table 1 Effect of *Beauveria bassiana* BIM-001 and BY2 isolates on soil, root, total nematode density and reproduction rate of *Pratylenchus thornei*

Parameters					
Isolates	Spore Concentration	Soil nematode density (500 g soil)	Root nematode density (per pot)	Final nematode density (soil + root)	Reproduction rate (PF/PI)
*Mean ± Standard error**					
BIM-001	10^8	641.7 ± 27.1^{b}	930.9 ± 45.9^{b}	1572.6 ± 36.2^{b}	3.9 ± 0.0^{b}
	10^7	717.4 ± 43.0^{b}	960.0 ± 68.7^{b}	1819.0 ± 65.6^{b}	4.5 ± 0.1^{b}
	10^6	859.0 ± 76.9^{b}	1429.6 ± 77.1^{a}	2147.0 ± 109.2^{a}	5.3 ± 0.2^{a}
	Control	1234.0 ± 71.6^{a}	1105.0 ± 111.2^{b}	2341.0 ± 53.3^{a}	5.8 ± 0.1^{a}
BY2	10^8	645.2 ± 35.0^{C}	849.0 ± 97.7 B	1492.2 ± 99.7^{C}	3.6 ± 0.2^{C}
	10^7	769.6 ± 36.3 BC	908.0 ± 111.3^{AB}	1809.8 ± 92.5 B	4.4 ± 0.2 B
	10^6	901.7 ± 72.2 B	1270.7 ± 78.1^{A}	2046.7 ± 65.8^{AB}	5.0 ± 0.1^{AB}
	Control	1234.0 ± 71.6^{A}	1105.0 ± 111.2^{AB}	2341.0 ± 53.3^{A}	5.8 ± 0.1^{A}

Different lowercase letters in the same column indicate significantly different means for isolate BIM-001, while uppercase letters in the same column indicate significantly different means for isolate BY2 ($P \leq 0.05$)

concentration of BY2 isolate. An inverse correlation was determined between the spore concentration density of the BY2 isolate and the total nematode density, and reproduction rate values. The lowest mean of total nematode density (1492.2 ± 99.7/soil + root) and reproduction rate (3.6 ± 0.2) were found at the BY2 concentration of 10^8 spore/ml, while these values were found at the highest concentration of 10^6 spore/ml of 2046.7 ± 65.8/soil + root and 5.0 ± 0.1, respectively, and there was a statistical difference between them ($P \leq 0.05$) (Table 1).

Another result obtained in the study was that the plant and root growth parameters were determined higher than the control in wheat treated with BIM-001 and BY2 isolates. Plant and root height and plant and root fresh weight reached the highest values at 10^8 spore/ml concentration of BIM-001 isolate compared to other concentrations. In the BY2 isolate, there was non-statistical difference between 10^8 and 10^7 spore/ml concentrations in plant and root wet weight parameters, while the values were higher at 10^8 spores/ml in plant and root length parameters and the statistical difference was found between 10^8 and 10^7 spore/ml concentrations in terms of these parameters ($P \leq 0.05$) (Table 2). Although the root nematode density was determined higher than the control at BIM-001 and BY2 10^6 spore/ml concentration, there was non-significant difference in root length and wet weight parameters between the control at this concentration (Tables 1, 2).

Discussion

In this study, the effect of concentrations of *B. bassiana* isolates on *P. thornei* varied. While the reproduction rate of *P. thornei* at 10^8 and 10^7 spore/ml concentrations in both isolates decreased than the control, at 10^6 spore/ml concentration was found to be similar to the control. Unfortunately, it was determined that the *P. thornei* density in the roots increased at 10^6 spore/ml concentration of both isolates, and accordingly, the total nematode density and reproduction rate increased. Interestingly, a positive effect on nematode reproduction was determined at low concentration. The reason for this was that the concentration of 10^6 spore/ml compared to the other concentrations could not infect *P. thornei*. Since this concentration cannot reach the nematode in the soil, it is thought that the nematode density increases. Secondly, *B. bassiana* remained at a low density in the soil, substances such as the secreted enzyme or toxin may not have developed enough to kill the nematodes (Petlamul and Boukaew 2019). Therefore, the study should be repeated using higher concentrations with the same or different isolates. It is known that nematicidal activity and endophytic abilities may vary depending on the characteristics of the different fungal strains used (Karabörklü et al. 2022). In addition, detection and monitoring of *B. bassiana* in wheat root tissues was found to be necessary to improve our knowledge of the endophytic colonization of *B. bassiana*. Also, there are studies based on increasing nematode development of *B. bassıana.* Hanel (1994) hypothesized that the increased nematode numbers after the application of *B. bassiana* to the soil were due to the indirect effect of *B. bassiana*. He noted these indirect effects as higher nutrient supply for plant nematodes, changes in soil microflora, and increased nutrient availability for plants. Mwaura et al. (2017) found that ten ml of a *B. bassiana* spore suspension at a concentration of 5×10^7 inoculated tuber and soil together with *Ditylenchus destructor* and *D. dipsaci* in the greenhouse caused a higher nematode reproduction in potatoes. Yerukala et al. (2021) reported that *B. bassiana* in Rutgers tomato cultivar increased the number of gall and egg masses formed by *M. incognita* in the roots.

Table 2 Effects of single application of BIM-001 and BY2 isolates on *Beauveria bassiana* on wheat growth

Parameters					
Isolates	Spore Concentration	Plant length (cm)	Plant wet weight (g)	Root length (cm)	Root wet weight (g)
*Mean ± Standard Error**					
BIM-001	10^8	50.9 ± 1.2^{a}	2.7 ± 0.1^{a}	24.9 ± 0.8^{a}	2.1 ± 0.1^{a}
	10^7	44.8 ± 1.0^{b}	2.0 ± 0.1^{b}	18.6 ± 0.8^{b}	1.6 ± 0.1^{b}
	10^6	37.9 ± 0.7^{c}	$1.5 \pm 0.0^{b}c$	16.2 ± 0.9^{b}	0.8 ± 0.1^{c}
	Control	35.8 ± 1.1^{c}	1.3 ± 0.0^{c}	17.3 ± 0.7^{b}	1.1 ± 0.0^{c}
BY2	10^8	48.1 ± 0.8^{A}	2.4 ± 0.1^{A}	24.0 ± 0.7^{A}	1.7 ± 0.0^{A}
	10^7	42.1 ± 0.8^{B}	2.1 ± 0.1^{A}	20.3 ± 1.1^{B}	1.5 ± 0.0^{A}
	10^6	36.9 ± 0.7^{C}	1.6 ± 0.0^{B}	17.7 ± 0.9^{B}	1.0 ± 0.0^{B}
	Control	35.8 ± 1.1^{C}	1.3 ± 0.0^{B}	17.3 ± 0.7^{B}	1.1 ± 0.0^{B}

Different lowercase letters in the same column indicate significantly different means for isolate BIM-001, while uppercase letters in the same column indicate significantly different means for isolate BY2 ($P \leq 0.05$)

Additionally, few studies with different plant-parasitic nematode species support the nematicidal effect of *B. bassiana* (Ghayedi and Abdollahi 2013; Kepenekçi et al. 2017; Youssef et al. 2020; Karabörklü et al. 2022). The present study results showed that 10^8 and 10^7 spores/ml of BIM-001 and BY2 isolates negatively affected *P. thornei* soil and root density according to control. However, the most effective concentration on *P. thornei* was 10^8 spore/ml in both isolates. While the reproduction rate of *P. thornei* was found to be 5.8 in the control, the reproduction rates of BIM-001 and BY2 isolates in 10^8 spore/ml concentration were found to be 3.9 and 3.6, respectively. Although nematode reproduction rate was not suppressed at a highly, these results showed that *B. bassiana*, an entomopathogenic fungus, could control *P. thornei* infection on wheat to a certain extent. Maybe the effect on nematode control will be higher when working with higher concentrations. In addition, Göze Özdemir et al. (2022) found that *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 after 72 h in vitro. The high nematicidal effect found as a result of the study carried out in vitro, unfortunately, could not be found in the experiment carried out on wheat under controlled conditions. It should not be overlooked that there was no direct contact between *P. thornei* and *B. bassiana* in the soil as in the in vitro study. Consequently, *B. bassiana* 10^8 and 10^7 spores/ml concentration applications reduced the reproduction of *P. thornei* on wheat than in the control. It was determıned that these isolates showed nematicidal effect on *P. thornei.* This may be due to secondary metabolites of *B. bassiana* during the infection (Amobonye et al. 2020).

Another result of the study, when *B. bassiana* was applied to the soil contaminated with *P. thornei* at concentrations of 10^8 and 10^7 spores/ml, it was determined that the height and fresh weight of wheat plants and roots increased than the control. *B. bassiana* isolate (MARD 92) was identified to have endophytic property which enables it to be established within plant tissues and increases its field efficacy in controlling some pests (Khudhair et al. 2016). Beserra et al. (2019) reported that *B. bassiana* application in soybean positively affected vegetative development and reduced parasitism of *P. brachyurus* in roots. According to Kepenekci et al. (2017), tomato growth and yield increased with increasing dose applications of *B. bassiana* (F-56 and F-63) isolates in tomato roots infected with *M. incognita*.

Conclusions

In the present study, the application of native *B. bassiana* isolates BIM-001 and BY2 inoculum density was found important for biocontrol efficacy. Also, as concentration densities above 10^8 spore/ml are considered more promising in control to root lesion nematodes, reassessment with different concentrations can be done. *B. bassiana* BIM-001 and BY2 isolates may be used in the control of root lesion nematode on wheat which is an important plant parasitic nematode group in Turkey and the world. Therefore, detailed research is needed on the factors responsible for the pathogenicity of *P. thornei* in the presence of the endophytic *B. bassiana* on wheat. The results of this study may guide the bionematicide recording of BIM-001 and BY2 isolates.

Author contributions

FGGO and AUY prepared the materials, conducted the experiment, and ultimately collected the data. FGGO, AUY and OD wrote the manuscript, read and approved the final manuscript.

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Declarations

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Competing interests

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