J. Glob. Innov. Agric. Sci., 2022,10(1):37-41.
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/10.975
http://www.jgiass.com

Determination of the efficacy of two local *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912 (*Hypocreales: Cordycipitaceae*) isolates (Bb-1 and Bv-1) against root-knot nematodes

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Root-knot nematodes cause economic damage to various agricultural products, especially vegetables globally. Due to the adverse effects of pesticides on the environment and human health, entomopathogenic fungi are one of the biological control agents used in pest control. In this study, the effects of two different local isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912 (Hypocreales: Cordycipitaceae) (Bb-1 and Bv-1) at 10^6, 10^7, and 10^8 CFU ml^{-1} concentrations were investigated against *Meloidogyne incognita* race 1 (Kofoid and White, 1919) and *Meloidogyne javanica* (Treub, 1885) (Tylenchida: Meloidogynidae) on tomato plants. Sixty days after the nematode inoculation, the number of egg packs formed in each plant, plant height (cm), wet plant weight (g), dry plant weight (g), and wet root weight (g), and root dry weight (g) were determined. According to the results, *Beauveria bassiana* Bb-1 and Bv-1 isolates significantly reduced the reproductive ability of root-knot nematodes. In both isolates, 10^8 CFU ml^{-1} concentrations were more effective against *M. javanica* and *M. incognita* race1 than other concentrations. Bb-1 isolate is more effective than Bv-1 isolate against *M. javanica.* On the other hand, the Bv-1 isolate is more effective than another isolate against *M. incognita* race 1. Entomopathogenic fungi (Bb-1 and Bv-1) are a promising long-term biocontrol strategy in agriculture against root-knot nematodes.
Keywords: Biological control; entomopathogenic fungus; root-knot nematodes; tomato.

INTRODUCTION

The number of harmful organisms limiting agricultural production has increased in recent years. In addition to abiotic factors such as climate change, pests, diseases and nematodes are among the biotic factors causing yield losses in agricultural fields (Anonymous, 2014; Perry *et al.*, 2009). Among these, root-knot nematodes constitute an important group as around 2000 plants worldwide are susceptible to nematode infection, which causes approximately 5% production loss (Sasser and Carter, 1985). Because the ability of plants damaged by nematodes to carry nutrients in the soil is reduced, symptoms such as yellowing and small leaf formation are seen on the upper parts of the plants. Root-knot nematodes cause damage to annual plants such as tomatoes, peppers, eggplants, cucumbers, beans, potatoes, sugar beets, cotton, tobacco, carrots, spinach, and perennials such as figs, mulberries, bananas, peaches, and plums (Whitehead, 1998; Topp *et al.*, 1998). Species such as *M. incognita*, *M. arenaria* and *M. javanica* are among the most common RKNs (Lunt *et al.*, 2014).
Several nematicides are used effectively to control nematode damage in agricultural products (van Berkum and Hoestra, 1979). Chemical nematicides used to control nematodes are not sustainable due to their adverse effects on the environment and human health, so alternative control methods such as biocontrol have gained importance in recent years. Microbial control agents are effective against pests, environmentally friendly and economical in the long term. Many results have been reported in studies on entomopathogenic fungi to control nematodes (Chen and Dickson 2004).
Beauveria bassiana, which is the most widely used species among entomopathogenic fungi, has also been reported to affect some pests such as trunk worms, aphids, mites, and whiteflies (Biswas *et al.*, 2012; Yücel, 2021). Species of the Beauveria have been reported to make the metabolites bassiacridin, bassianin, oosporein beauverolides, beauvericin, tenellin and bassianolide (Strasse *et al.*, 2000). Beauvericin produced by *B. bassiana* has been reported to be used against the larvae of some insect pests (Dingra and Sinclair, 1985). Moreover, beauvericin provided that have nematicidal activity against root-knot nematodes (Kepenekci *et al.*, 2017; Hamill *et al.*, 1969; Liu *et al.*, 2008). In recent studies, *B.*

Yağci, M. 2022. Determination of the efficacy of two local *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912 (*Hypocreales: Cordycipitaceae*) isolates (Bb-1 and Bv-1) against root-knot nematodes. Journal of Global Innovations in Agricultural Sciences.10:37-41.
[Received 14 Nov 2021; Accepted Jan 2022; Published 28 Mar 2022]

bassiana is effective against many plant-parasitic nematodes, such as root-knot nematodes (Ekanayake *et al.*, 1994; Liu *et al.*, 2008; Zhao *et al.*, 2013, Zhang *et al.*, 2020*).*
This study aimed to determine the effect and nematicidal potentials of two local isolates of *B. bassiana* (Bb-1 and Bv-1) against the *M. incognita* race 1 and *Meloidogyne javanica* on tomato under controlled conditions.

MATERİALS AND METHODS

***Rearing of root-knot nematodes*:** Egg masses belonging to *M. incognita* race 1 *and M. javanica* were obtained from the roots of the tomato (*Solanum lycopersicum* cv. Tueza F1) plants placed in a climate room (25±2ºC and 65% RH with a 16:8 h (L:D) photoperiod). For the reproduction process, 10-15 egg masses were inoculated into the root zone of the sensitive plant at a depth of 2 cm (Mistanoğlu *et al.*, 2016). The harvested roots were washed 60 days after the inoculation, and the egg masses were collected one by one under the binocular microscope and then left to incubate at 25ºC. The J2s were extracted from the eggs using a Baermann funnel (Hooper, 1970). Later, the larvae were counted under a light microscope (Leica DM 300, Wetzlar, Germany).
***Production of fungal cultures*:** In the study, the effect of two local isolates of entomopathogenic fungus *B. bassiana* (Bb-1 and Bv-1) were used to determine against root-knot nematode. The Bb-1 isolate was obtained from the Plant Protection Department, Faculty of Agriculture, Ege University, and the Bv-1 isolate was obtained from Plant Protection Department, Faculty of Agriculture, Eskişehir Osmangazi University. *B. bassiana* isolates from stock cultures were inoculated into 10 Petri dishes containing PDA (potato dextrose agar) and incubated at 25°C for ten days. After incubation, 10-15 ml of sterile distilled water was added to each petri dish, and the spore and micelle mass were scraped from the agar surface by a simple laboratory spatula and passed through cheesecloth. 0.05% Tween-80 was added to the obtained suspension and mixed on a magnetic stirrer until homogeneous (Youssef *et al.,* 2020). Then, counts were made on the Thoma slide to get the spore concentrations of 10^6, 10^7, and 10^8 CFU ml^{-1}. On the same day, after counting, the suspensions were applied to the root area of the tomatoes.
***Effect of fungus isolates on root-knot nematodes*:** A mixture of soil (80% sand, 15% soil, and 5% clay) was sterilized at 121°C. After sterilization, the soil mixture was put into plastic pots with 500 ml to be used in the trials (Robbins and Barker, 1974; Chen *et al.*, 1995). Tomato seedlings grown in the climate room were transplanted when they reached the 3-4 leaf stage. Ten days after the seedlings were transplanted, four holes 2 cm deep were drilled around the roots and 1200 J2 of *M. incognita* race 1 and *M. javanica* were inoculated into the holes with a pipette (Figure.1). Next, Bb-1 and Bv-1 isolates of *Beauveria bassiana* 10^6, $10^{7,}$ and 10^8 cfu (spore) ml^{-1} were pipetted into other holes on the tomato root region (Oclarit *et al.*, 2009). *Beauveria bassiana* suspensions were applied to the soil simultaneously with the nematodes in the study. Only water was used in the negative (-) control, and only the nematode larvae were added to the positive (+) control in the experiments. The experiments were carried out in five replicates for each concentration of entomopathogenic fungi. Sixty days after application, the plants were removed from the soil and their roots were washed under water. Roots were examined under a binocular microscope and egg masses were counted. The length (plant height) of the upper parts of each plant was measured. Then tomato roots are dyed with red food coloring and the upper parts of the plant are weighed and recorded on a precision scale. These parts were weighed again after drying in the oven at 70°C for 48 hours (Mohammad *et al.*, 2007). The study was conducted at the Nematology Laboratory.
***Statistical Analysis*:** The number of egg masses of root-knot nematodes in each plant root, plant height (cm), wet and dry weight (g) of the plant, wet and dry root weight (g) were measured at the end of the experiments. The parameters obtained in the experiments (number of egg masses belonging to root-knot nematodes in each plant root, plant height (cm), wet and dry weight of the plant (g), wet and dry root weight (g)) were evaluated using the SPPS 20 statistical analysis program (ANOVA) and Duncan test was used for comparison of the groups ($P \leq 0.05$).

RESULTS

The Bb-1 isolate of *B. bassiana* significantly reduced the number of egg masses in tomato roots. When the results are evaluated in terms of *M. javanica* on egg mass formation, the highest effect was seen at 10^8 cfu ml^{-1} with 89.80 egg masses, while the lowest effect was seen at 10^6 cfu ml^{-1} with 142.80. In the positive control, the number of egg masses was 147.60. When the upper parts of the plant were evaluated, the longest plant height was 37 cm for 10^7 cfu ml^{-1} followed by 10^6 and 10^8 cfu ml^{-1}. The positive and negative control group were shorter than plants treated with *B. bassiana*. There was no statistically significant difference between the applications in the upper part wet weight of the plant and the control group. The highest dry weight of the plant upper parts was observed at 0.74 g for 10^6 cfu ml^{-1}, followed by 0.67 g for 10^7 cfu ml^{-1} and 0.57 g for 10^8 cfu ml^{-1}, respectively. Root wet weight results were 4.66 g for 10^6 cfu ml^{-1}, 3.96 g for 10^7 cfu ml^{-1}, and 3.27 g for 10^8 cfu ml^{-1} with. When evaluated by root dry weight, the highest weight was seen at 10^6 cfu ml^{-1} with 0.61 g, followed by 10^7 cfu ml^{-1} with 0.52 g and 10^8 with 0.42 (Table 1).
Considering the results in terms of the effect of the entomopathogenic fungus *B. bassiana* Bb-1 on the egg mass formation of *M. incognita* race 1on the plant roots, the highest effect was seen at 10^8 cfu ml^{-1} with 80.60 egg masses, followed by 10^7 and 10^6 cfu ml^{-1} with 86.20 egg.

Table 1. Virulence of different concentrations (10^6, 10^7 ve 10^8 cfu ml^{-1}) of *B. bassiana* Bb-1 isolates on *M. javanica* the egg pack and plant growth parameters.

	Meloidogyne javanica Concentrations				
Parameters	**+ K**	**10^6**	**10^7**	**10^8**	**-K**
Plant height (cm)	28,00±4,40 b	34,00±4,80 ab	37,00±6,20 a	32,20±4,30 ab	30,60±2,08 ab
Number of egg mass	147,6±13,50 a	142,8±9,44 a	92,2±8,19 b	89,8±11,43 b	-
Wet plant weight (g)	4,02±1,70 a	5,65±0,90 a	5,27±0,50 a	4,51±1,30 a	4,02±0,10 a
Dry plant weight (g)	0,51±0,07 b	0,74±0,22 a	0,67±0,11 ab	0,57±0,15 ab	0,51±0,06 b
Wet Root weight (g)	3,72±1,14 ab	4,66±0,91 a	3,96±0,36 ab	3,27±0,95 b	1,90±0,18 c
Dry root weight (g)	0,35±0,05 c	0,61±0,20 a	0,52±0,05 ab	0,42±0,07 bc	0,17±0,17 d

Table 2. Virulence of different concentrations (106, 107 ve 108 cfu ml-1) of *B. bassiana* Bb-1 isolates on *M. incognita* race 1 the egg pack and plant growth.

	Meloidogyne incognita race 1 Concentrations				
Parameters	**+ K**	**10^6**	**10^7**	**10^8**	**-K**
Plant height (cm)	34,40±6,50 a	29,20±4,26 a	33,80±8,04 a	29,0±3,16 a	30,60±2,08 a
Number of egg mass	157±15,2 a	86,20±7,39 b	86,20±13,91 b	80,60±11,92 b	-
Wet plant weight (g)	3,46±1,25 b	3,67±1,04 b	5,29±1,06 a	4,81±0,55 ab	4,02±0,1 a
Dry plant weight (g)	0,54±0,08 a	0,51±0,10 a	0,54±0,20 a	0,54±0,13 a	0,51±0,06 a
Wet Root weight (g)	3,15±0,44 a	3,51±0,76 a	3,43±0,59 a	3,69±0,76 a	1,90±0,18 b
Dry root weight (g)	0,31±0,05 b	0,41±0,12 b	0,39±0,02 b	0,54±0,07 a	0,17±0,17 c

Table 3. Virulence of different concentrations (10^6, 10^7 ve 10^8 cfu ml^{-1}) of *B. bassiana* Bv-1 isolates on *M. javanica* the egg pack and plant growth parameters.

	Meloidogyne javanica Concentrations				
Parameters	**+ K**	**10^6**	**10^7**	**10^8**	**-K**
Plant height (cm)	28±4.47 a	30.40±5.41 a	30.80±2.77 a	33.80±2.38 a	30.66±2.08 a
Number of egg mass	147.00±13.5 a	114.0±8.57 b	105.60±13.55 b	108.2±13.98 b	-
Wet plant weight (g)	4.02±1.71 a	3.96±0.71 a	4.50±0.47 a	5.03±1.11 a	4.02±0.15 a
Dry plant weight (g)	0.51±0.07 b	0.57±0.15 b	0.59±0.06 b	0.83±0.20 a	0.51±0.06 b
Wet Root weight (g)	3.72±1.14 ab	3.79±0.61 a	2.79±0.28 bc	3.52±0.25 ab	1.90±0.18 c
Dry root weight (g)	0,35±0,05 a	0,40±0,11 a	0,45±0,04 a	0,46±0,11 a	0,17±0,17 b

In positive control, the number of egg masses was determined as 157. There was no statistical difference in the effect of concentrations on the egg masses. It was seen that the applications made were effective when compared with the positive control. When the effects of *B. bassiana* on plant height were evaluated, the longest length was determined at 10^7 cfu ml^{-1} with 33,80 cm, followed by 10^6 and 10^8 cfu ml$^{-1,}$ but they were all in the same group statistically. In terms of upper part dry plant weight (g) and root wet weight (g), there was no statistically significant difference between the groups, but the highest value in both parameters was found at 10^8 cfu ml^{-1} (Table 2).

When the effect of the other isolate of *B. bassiana* Bv-1 used in the study on the egg mass formation of *M. javanica* on tomato roots was examined, it was found that there was no statistically significant difference among the concentrations in the applications. Besides, compared to the positive control, Bv-1 isolate decreased the egg mass formation. It was also observed that in terms of plant height (cm) and wet weight (g) parameters, the differences between the treatment concentrations (10^6, 10^7, and 10^8 cfu ml^{-1}) were not significant. The upper plant part dry weight (g) and root wet weight (g) were highest for 108 and 106 cfu ml-1. There was no statistical significance in the dry root weight parameter (Table 3).

About the egg mass formation of *M. incognita* race 1 on tomato roots after Bv-1 treatment was examined, the highest number of egg masses was observed at 10^8 cfu ml^{-1} with 75.6 egg mass and 10^7 with 94.6, and 10^6 cfu ml^{-1} with 119.4. When the height of the plants in the application (cm), the wet and dry weight of the plant (g), and the root wet weight (g) was examined, it was seen that there was no difference between the applications and the controls (Table 4).

Table 4. Virulence of different concentrations (10^6, 10^7 ve 10^8 cfu ml^{-1}) of *B. bassiana* Bv 1 isolate on *M. incognita* race 1 the egg pack and plant growth parameters.

	Meloidogyne incognita race 1				
	Concentrations				
Parameters	**+ K**	**10^6**	**10^7**	**10^8**	**-K**
Plant height (cm)	34,40±6,50 a	32,80±5,63 a	34,00±2,54 a	36,60±4,33 a	30,66±2,08 a
Number of egg mass	157,60±15,20 a	119,4±12,97 b	94,60±17,30 c	75,60±9,65 d	-
Wet plant weight (g)	3,46±1,25 a	4,65±1,19 a	4,20±0,38 a	4,48±0,83 a	4,02±0,15 a
Dry plant weight (g)	0,54±0,08 a	0,54±0,08 a	0,61±0,18 a	0,60±0,05 a	0,51±0,06 a
Wet Root weight (g)	3,15±0,44 a	3,17±0,56 a	3,01±0,30 a	3,65±0,83 a	1,90±0,18 b
Dry root weight (g)	0,31±0,05 b	0,35±0,06 ab	0,37±0,06 ab	0,41±0,06 a	0,17±0,17 c

DISCUSSION

Few studies have investigated the effects of *Baeuveria bassiana* on root-knot nematodes. In the Study by Di Zahao *et al.* (2013), the effects of 10^5 *B. bassiana* isolates on *M. incognita*, *Aphelenchoides besseyi*, *Heterodera glycines*, *Caenarhabditis* sp. were examined and nine isolates were found to have high nematicidal activity against *M. incognita* second-stage larvae. The culture of *B. bassiana*'s different isolates had various activities against the same nematode. Moreover, Liu *et al.* (2007) examined the effect of *B. bassiana* on the egg mass formation in tomato roots after infection by *M. hapla* and found that the increase in mortality rate was directly proportional to the increase of fungus concentrations in applications. Also, they discovered that parameters like the plant height, plant weight, etc. were higher for fungal treatments than the positive control. Yaussef *et al.* (2020) were studied the effect of three concentrations of *B. bassiana* (10^6, 10^7, 10^8 cfu ml^{-1}) on *M. incognita* juveniles and found that 75% mortality occurred in 10^8 cfu ml^{-1}. Egg mass formation was reduced by 68% at 10^8 concentrations.
Moreover, they found that fungus applications increased plant growth compared to control. Devi and Bora (2018) found that *B. bassiana* at 50% culture filtrate concentration caused 67.66% of juvenile death after 72 hours. Pant and Pandey (2014) determined that *B. bassiana* decreased the number of root-knot nematodes and increased plant growth. Ahmed (2016) investigated the virulence of two entomopathogenic fungi species (*B. bassiana* and *Isaria tenuipes*) on *M. javanica* populations in cucumber and pepper plants and found that both fungi significantly decreased the nematode gal and egg mass indices in treated plants compared to control plants and the highest plant height was in pots treated with *B. bassiana.*

***Conclusions*:** In recent years, studies on alternative control methods to pesticides in pest control have gained momentum, and biological control studies that are reliable in terms of environment and human health have gradually gained importance. With this study, promising results have been obtained in the use of *B. bassiana* isolates against root-knot nematodes, which are an important pest group in Turkey and the world, and while fungus treatments reduced the formation of egg mass in the roots, generally, the growth parameters (plant height (cm), wet and dry plant weight (g), wet and dry root weight (g) were increased compared to the control. However, *Beauveria* isolates, which were effective in this study and other studies so far, are thought to be helpful in biological control programs in the control of root-knot nematodes. However, more work in field conditions needs to be done.

***Authors Contributions statement*:** M. Yağcı: Supervision, Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, review & editing

***Conflict of interest*:** I have no conflict of interest

***Acknowledgments*:** The author would like to thank Professor Gülay TURHAN (Ege University, Faculty of Agriculture, İzmir, Turkey) and Associate Professor Filiz ÜNAL (Eskişehir Osmangazi University, Faculty of Agriculture, Eskişehir, Turkey) for providing the entomopathogenic fungi material.

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