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Short Communication: Biological control of *Meloidogyne javanica* by *Pasteuria penetrans* and *Trichoderma harzianum* on tomato plants

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Abstract. *Saleh HM, Shafeeq AF, Khairi MA. 2023. Short Communication: Biological control of* Meloidogyne javanica *by* Pasteuria penetrans *and* Trichoderma harzianum *on tomato plants. Biodiversitas 24: 847-851.* This study was to determine the efficiency of *Pasteuria penetrans* and *Trichoderma harzianum* to control root-knot nematodes on tomato plants. To assess the biocontrol efficiency and the threshold level of spores/J2 for mass propagation of *P. penetrans*, four different levels of spores load on second-stage juveniles (J2) (1-3, 4-8, 9-14 and 15-25 spores/J2) were tested, Results showed that levels 9-14 and 15-25 spore/J2 achieved the best significant reduction in root gall index and the number of eggs/g root which were 2.4, 1.8, 1726 and 563, respectively as compared to the other treatments and control. It was also observed that 15-25 spores/J2 were more efficient (0.05) in reducing the number of eggs, females in the root and the number of J2 in 250 g of soil compared with other treatments. The addition of 2 g of bacterial inoculum at 3 and 7 days before transplanting gave the best result in reducing root gall index and the number of eggs/g root as it reached 2.0, 161 eggs and 1.4, 78 eggs compared with other treatments. The best day for the application of *T. harzianum* to reduce infection of root-knot nematodes was 7 days before transplanting at a level of 4 g as compared with other treatments. A significant reduction was observed in the root gall index (2.0) and the number of eggs (885 eggs) compared to the treatment of level of 2 gm of the fungal inoculum and *M. javanica* treatment, which were 2.8, 1352 eggs and 4.6, 11317 eggs.

Keywords: Biological control, *Meloidogyne javanica, Pasteuria penetrans, Trichoderma harzianum*, tomato

INTRODUCTION

Nematodes attack plants, primarily in the form of soilborne root pathogens, causing a 12% loss in agricultural yields worldwide (Askary 2012). Furthermore, nematode management is frequently difficult. The estimated global loss due to nematodes is around USD 157 billion per year (Singh et al. 2014). Chemical control of plant-parasitic nematodes has been a preferred method for many years (Kepeneki et al. 2017). However, these chemicals caused serious damage to the ecosystem (Rani et al. 2021). Plant-parasitic nematode biological control agents are typically very specific natural enemies of the target plant pest nematodes. Farmers have been able to use these natural agents in the soil around their crop plants once they have been commercialized to limit the damage caused by harmful plant-parasitic nematodes (Bastakoti et al. 2017; Ciancio 2018; Singh et al. 2022).

Bacterium (*Pasteuria penetrans*, an obligate parasite of root-knot nematodes) is one of the best-studied biological controls of nematodes *Meloidogyne* spp.(Saleh et al. 1999; Akyazi and Dickson 2014). *Pasteuria penetrans* is an endospore-forming bacterium that lives in the soil until it encounters a suitable nematode host (Cho et al. 2013). *Pasteuria* spores stick to the nematode cuticle (outer surface), infect the nematode, and grow inside the nematode body. *Pasteuria penetrans* has been identified as one of the most promising biological agents for root-knot nematode control (Stephan et al. 2002; Stirling et al. 2017). *Lycopersicon esculentum* is one of the crops commonly grown in tropical and subtropical regions, and it is the second most consumed crop after the potato crop. China is the main producer of tomatoes, as its production constitutes about 31% (Navyashree et al. 2020). Tomato crop is infected with more than one pathogen caused by a group of fungi, bacteria and viruses, as well as other pests, including the root-knot nematode *Meloidogyne* spp. (Singh et al. 2017).

Pasteuria penetrans is widely distributed throughout the world and contributes to natural nematode control, particularly of root-knot nematodes (Baidoo et al. 2017; Stirling et al. 2017). *Pasteuria penetrans* attachment to root-knot nematode, *Meloidogyne* spp., juveniles (J2) (Akyazi and Dickson 2014; Singh et al. 2014). Endospores of *Pasteuria* spp. are resting propagules that are highly resistant to adverse conditions such as high temperature or desiccation and an infective propagule. The parasite multiplies within the host and eventually initiates sporulation. Sporulation occurs inside the host, usually after consuming a portion or all of its body content, resulting in a significant reduction in fecundity and reproductive capacity (Singh et al. 2014). *Pasteuria penetrans* increased tomato yield by 43.79% over nematode-inoculated plants. The

reproduction parameters of nematodes, such as root galls, egg masses, and J2/100 mL, varied between plants inoculated with nematodes and those treated with nematode + *Pasteuria*. There was an 88.54% reduction in nematode reproduction factor between inoculated nematode plants and nematode + *Pasteuria* treated plants (Kamran et al. 2014). Some *Trichoderma* species have been used as biocontrol agents against nematodes and soilborne diseases (Herrera-Parra et al. 2018).

The genus *Trichoderma* may also promote plant growth and colonize root surfaces and cortex, suggesting that it is parasitic on nematode stages (Szabó et al. 2012, 2013; Izuogu and Abiri 2015; Ghazanfar et al. 2018), *Trichoderma* fungi are valuable industrial enzymes in support of 'zero-waste' technology for converting agro-industrial biomass into valuable products, such as nanocellulose (NC). An in silico approach using substrate docking and molecular dynamic (MD) simulation was used to predict the order in which fungal enzymes, endocellulase and exocellulase, degrade the multilayers of cellulosic polymers, namely lignin, hemicellulose, and cellulose in oil palm leaves (Bahaman et al. 2020). Blind docking and multiple sequence alignment revealed that molecular recognition by *T. harzianum* BglT12 occurred through interactions between the -1,3-glucan, -1,3/1,4-glucan, and chitin components of *M. phaseolina*, with corresponding binding energies of -7.4, -7.6, -7.5, and -7.8 kcal/mol (Mohammad et al. 2022). Secondary metabolites are produced, as well as antibiotics such as viridine, gliotoxin, gliovirine, peptaibols, trichodermin, suzukaciline, and alameticine, which can inhibit egg hatching and immobilize J2 (Candelero et al. 2015; Feyisa et al. 2015), as well as nematode cuticle hydrolyzing enzymes such as chitinases, glucanases, peroxidases, and chitobiose (Jiménez et al. 2013; Bhattacharjee and Dey 2014; Shafeeq et al. 2021). The ability of *Trichoderma* spp. to control plant-parasitic nematodes is mainly determined by the fungal species, its origin, its interactions with its plant host, and its adaptation to the environment. *T. harzianum* has been reported to suppress *M. incognita*, *M. javanica*, and *M. arenaria* populations while increasing yields in vegetables and other crops (Sharon et al. 2001; Shafeeq et al. 2021). The objective of this study was to determine the efficiency of *P. penetrans* and *T. harzianum* in suppressing root-knot nematode infection in tomato plants.

MATERIALS AND METHODS

Bacteria

Pasteuria penetrans was used as a parasitic on root-knot nematodes. It was isolated from the agricultural fields in Al-Zaafaraniya area, Southeast of Baghdad.

Fungus

Isolates of the *T. harzianum* grown on corn cob cracked + wheat bran (3: 1 w/w) medium.

Root-knot nematode *Meloidogyne javanica*

In this study, a pure culture of *M. javanica* nematode was collected from infected tomato roots.

Plant

Pearson cultivar of tomato plants (*Lycopersicon esculentum*) was used for the experiment. This cultivar was found to be sensitive to root-knot nematodes.

Effect of different levels of bacterial spores on second-stage juveniles for control of *Meloidogyne javanica* on tomato plants

Four different levels of *P. penetrans* spores (1-3, 4-8, 9-14, 15-25 spores/J2 are a range of spore concentration) were used in second-stage juveniles of nematodes. A 5 mL of aqueous suspension of spores (1.4×107 spores) was added to 20 mL of water containing 2000 second-stage juveniles of *M. javanica* for each treatment in a glass container (Saleh et al. 1999). A sample of J2 was examined every five minutes to count the number of spores attached to J2. Two thousand second-stage juveniles loaded with different levels of spores for each treatment were added to four-week-old tomato plants grown in 2 kg plastic pots of soil containing a sterile mixture of soil and peat moss at a ratio of 2:1 in the greenhouse. Each treatment was repeated four times and performed in a randomized complete block design. Control was also maintained for the comparison. The data were recorded after five weeks of adding the inoculum by calculating the gall index (estimated according to the scale 1 = 0, 2 = 1 - 25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100% of roots galled) and the number of eggs, J2 and females were estimated using a nematode count slide. The infection of J2 and females with bacteria was also calculated.

Effect of *Pasteuria penetrans* on the biological control of *Meloidogyne javanica* on tomato plants

Three days were selected to add *P. penetrans* and J2 of *M. javanica* in tomato plant (at the time of transplanting 3 and 7 days before transplanting), 1 and 2g bacterial inoculum was added (one gram containing 1.4×106 spores) to lima bean root powder containing bacterial spores and a sterile mixture of soil and peat moss was added in a 2: 1 ratio for a 2 kg capacity pot. The nematode inoculum was added at 2000 J2/pot, according to the following treatments: (i) *M. javanica* only, (ii) *P. penetrans* (1 g) + *M. javanica*, (iii) *P. penetrans* (2 g) + *M. javanica.*

Tomato seedlings were planted at the age of 4 weeks in pots. According to a complete random block design, each treatment was repeated five times, and the pots were placed in a greenhouse, and after five weeks of planting, the results were taken by calculating the root gall index and the number of eggs using a nematode count slide.

Effect of *Trichoderma harzianum* on the biological control of *Meloidogyne javanica* on tomato plants

Three days were selected to add *T. harzianum* (at the time of transplanting, 3 and 7 days before transplanting), whereas 2gm,4gm per pot (one gram contains 1.6×107 spores) fungal inoculum was added for each plastic pot of 2kg soil. A sterile mixture of soil and peat moss in a ratio of

2:1 and the nematode inoculum was added at 2000 eggs per pot according to the following treatments: (i) *M. javanica*, (ii) *T. harzianum* (2 g) + *M. javanica*, (iii) *T. harzianum* (4 g) + *M. javanica*.

Tomato seedlings were planted at the age of 4 weeks in pots. Each treatment was repeated five times according to a complete random block design, the pots were placed in the greenhouse. The results were taken by calculating the root gall index, and the number of eggs was estimated using a nematode count slide.

Data analysis

The data were statistically analyzed, and the averages were compared using the Duncan test method at a probability level of 5%.

RESULTS AND DISCUSSION

Effect of different levels of bacterial spores on second-stage juveniles for the control of *Meloidogyne javanica* on tomato plants

The results showed that all levels of *P. penetrans* inoculum achieved good control against *M. javanica* nematodes, which led to a decrease in root gall index values as well as in the number of eggs/g root as compared to the control treatment (Table 1). The result exhibited that levels of 9-14 and 15-25 spore/J2 achieved the most significant reduction in root gall index and the number of eggs/g root, i.e., 2.4, 1.8, 1726, 563, respectively, compared to the other treatments and the control treatment. The parasitic nature of bacteria lies in their ability to inhibit the activity and movement of the J2 of root-knot nematodes (Akyazi and Dickson 2014; Ciancio 2018). This reduces the ability of J2 to penetrate the roots (Cho et al. 2013; Kokalis-Burelle 2015). Even if it manages to penetrate the root and develop inside it and reach the full female stage, it loses its ability to lay eggs, and even huge numbers of bacterial spores are formed inside it (Stirling 1984). Previous studies indicated that the average number of bacterial spores per female of root-knot nematodes ranged between 2.1×106 and 5.5×106 spores/mL (Mankau 1981). The results from Table (1) showed that the level of 15-25 spore/J2 showed the highest significant reduction in the number of eggs/g root (563 eggs), the number of females in the root (8 females) and the number of J2 (13 J2) in 250 g of soil compared with other levels. It also achieved the highest percentage of infected females in the roots and the highest percentage of J2 in the soil, reaching 78.2 and 56.6%, respectively. This is consistent with previous studies that the higher the number of spores attached to the J2, the better the control of nematodes. Saleh et al. (1999) showed that levels 21-40 and more than 40 spores/J2 of *M. javanica* control on tomatoes had the best significant reduction in the number of eggs and J2 in the roots.

Table 1. The effect of different levels of *Pasteuria penetrans* spores on *Meloidogyne javanica* reproduction and damage potential on tomato plants

Spores/ J2	Gall index	No. eggs/g root	Nematode final population		% of Bacteria-infected nematode	
			Female/ g root	J2/250 g soil	Females in root	J2 in soil
1-3	3.8 b*	8527 b	61 b	367 b	22.5 d	11.3 c
4-8	3.2 c	4214 c	43 c	153 c	41.2 c	27.1 ab
9-14	2.4 d	1726 d	27 d	59 d	63.4 b	35.3 a
15-25	1.8 e	563 e	8 e	13 e	78.2 a	56.1 d
M. javanica	4.6 a	264o5 a	94 a	1983 a	0.0 e	0.0 d

Note: *According to the Duncan multiple range test, means in a column separated by the same letters are not significantly different (P: 0.05)

Table 2. Effect of application time of *Pasteuria penetrans* on the reproduction of *Meloidogyne javanica* on tomato plants

Treatments	Application time					
	At the time of transplanting		3 days before transplanting		7 days before transplanting	
	Gall index	No. eggs/g root	Gall index	No. eggs/g root	Gall index	No. eggs/g root
M. javanica only	4.8 a*	12158 a	4.8 a	13773 a	4.6 a	10206 a
P. penetrans(1g) + *M. javanica*	3.2 b	641 b	2.8 b	402 b	2.2 b	195 b
P. penetrans (2g) + *M. javanica*	2.6 c	467 c	2.0 c	161 c	1.4 c	78 c

Note: *According to the Duncan multiple range test, means in a column separated by the same letters are not significantly different (P: 0.05)

Table 3. Effect of application time of *Trichoderma harzianum* on the reproduction of *Meloidogyne javanica* on tomato plants

Treatments	Application time					
	At the time of transplanting		3days before transplanting		7days before transplanting	
	Gall index	No. eggs/g root	Gall index	No. eggs/g root	Gall index	No. eggs/g root
M. javanica only	4.8 a*	12748 a	4.8 a	10606 a	4.6 a	11317 a
T. harzianum (2g) + *M. javanica*	3.8 b	8189 b	3.4 b	5731 b	2.8 b	1352 b
T. harzianum (4g) + *M.javanica*	3.6 c	4949 c	2.8 c	2127 c	2.0 c	885 c

Note: *According to the Duncan multiple range test, means in a column separated by the same letters are not significantly different (P: 0.05)

Effect of *Pasteuria penetrans* on the biological control of *Meloidogyne javanica* on tomato plants

The results of this study showed that the addition of *P. penetrans* spores at levels of 1 g and 2 g/pot and for all the days showed high efficiency in combating *M. javanica* (Table 2). It has been observed that the addition of 2 gm of bacterial inoculum achieved the best result in reducing the root gall index and the number of eggs/gm root as compared to the treatment of 1 gm of bacterial inoculum for all three days. It was also noted that the addition of 2 g of bacterial inoculum at 3 and 7 days before transplanting showed the best result in reducing root gall index and the number of eggs/g root, which were 2.0, 161 eggs and 1.4, 78 eggs compared with the treatment of 1 g of bacterial inoculum and the control treatment (Table 2). In a previous study, a natural biological control was found in the soils of vineyards due to the presence of type of bacteria, *P. penetrans* (Stirling 1984). These bacteria have characteristics that make them suitable for use as a biocontrol in the field of application because these bacteria are tolerant of high temperatures, chemical pesticides and drought (Cho et al. 2013; Akyazi and Dickson 2014; Ciancio 2018).

Effect of *Trichoderma harzianum* on the biological control of *Meloidogyne javanica* on tomato plants

The results showed a significant effect in reducing root gall index and the number of eggs compared with the control treatment *of M. javanica* alone (Table 3). The best day to reduce infection with root-knot nematodes was 7 days before transplanting at a level of 4 gm of fungal inoculum for each plant, as it showed a significant reduction in the root gall index (2.0) and the number of eggs (885 eggs) compared to the treatment of the level of 2 gm of the fungal inoculum and *M. javanica* treatment, which were 2.8, 1352 eggs and 4.6, 11317 eggs, respectively. This is consistent with previous studies that indicated that the best protection against root-knot nematodes was when *T. harzianum* was added to the soil 7 days before transplanting (Stephan et al. 2002). *Trichoderma* spp. reduces the incidence of root-knot nematodes due to the secretion of some inhibitory substances such as viridine, trichodermine and some enzymes such as chitinase enzyme that degrades the outer wall of eggs (Bhattacharjee and Dey 2014; Candelero et al. 2015; Ghazanfar et al. 2018).

In conclusion, the result of the present study revealed that *P. penetrans* and *T. harzianum* on tomato plants showed the best efficiency in the biological control of *M. javanica*. It was also observed that 15-25 spores/J2 were more efficient in reducing the number of eggs and females in the root. The best day for the application of *T. harzianum* to reduce infection of root-knot nematodes was 7 days before transplanting at a level of 4 g. Further studies should be conducted for the persistence of *P. penetrans* and *Trichoderma* spp. antagonistic activity against *M. javanica*.

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