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Suppressive effect of some microbial agents on root-knot nematode, *Meloidogyne javanica* infected eggplant

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Abstract

Root-knot nematodes, *Meloidogyne* spp. are a growing concern for vegetable producers, because chemical nematicides are not effective. Search for novel, environmental friendly alternatives to manage *Meloidogyne* populations are needed to solve the problem. The effectiveness of different bacterial and fungal genera against *M. javanica* on eggplant was evaluated under laboratory and greenhouse conditions. Treatments with *Bacillus subtilis*, *B. thuringiensis*, *Pseudomonas fluorescens* and *Serratia marcescens* each alone or in a mixture caused 50.5–90.3% inhibition on *M. javanica* egg-hatch and 2nd stage juveniles (J_2) activity and showed 56.5-86.8% reduction in the number of nematode root galls, egg-masses/root system, number of J_2/250 cc soil and 50.9-73.7% increase in the root and shoot dry weights of eggplant. Treatment with *Arthrobotrys conoides*, *A. oligospora*, *Paecilomyces lilacinus* and *Saccharomyces cerevisiae* caused significant reductions (69.5-89.5%) in the number of nematode root galls, egg-masses/root system and number of J_2/250 cc soil and showed 53.7-60.9% increase in root and shoot dry weights of eggplant. The potential of *P. lilacinus* in colonization *M. javanica* egg masses and eggs formed on eggplant roots ranged from 45.2- 99.2%, compared to the control treatment. This study can help growers to develop new biocontrol agents, to suppress root-knot nematode populations under field conditions.

Keywords: *Arthrobotrys* spp., *Bacillus* spp., Eggplant, *Meloidogyne* spp., *Paecilomyces lilacinus*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Serratia marcescens*
Abbreviations: 2nd stage juveniles _J_2; Nutrient broth medium_ NB; LSD_Least significant difference; Colony forming units_cfu; Corn meal agar _CMA; Randomized complete block design_ RCBD; Water agar_WA.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are sedentary obligate endoparasitic nematodes, which common in Egypt and worldwide and cause severe crop damage especially in light soils that cause major economic damage to crops (Khan et al., 2008). Eggplant (*Solanum melongena* L.) is severely damaged by the root-knot nematode species (Netscher and Sikora, 1990).

Root-knot nematodes can be managed effectively by chemical treatments but many of the nematicides are expensive, pose human and environmental risk or have been withdrawn from the use (Abd-Elgawad, 2008). Due to environmental concerns and increased regulations on use of chemical nematicides, more effective management strategies for root-knot nematodes (*Meloidogyne* spp.) are currently being investigated (Nico et al., 2004). Among the biological control agents that have been assessed are antagonistic bacteria, nematophgous fungi and yeasts (Kiewnick and Sikora, 2005; Muwaffaq, 2013).

The mechanisms of biological control of nematodes by antagonistic bacteria have been used as the subjects of many studies in the past two decades (Crickmore et al. 1998; Yap, 2013). Bacteria have a wide range of suppressive activities on nematodes by a variety of modes, producing toxins, antibiotics and enzymes or interfering with nematode–plant-host recognition (Ali et al., 2002; Khan et al., 2004; Huang et al., 2009). The most thoroughly studied *Bacillus* includes *Bacillus subtilis* and *Bacillus thuringiensis* (Krebs et al., 1998). The enzymes and antibiotics produced by *B. subtilis* have successful as biocontrol activity on root-knot nematodes and protect the host plant through stimulating systemic resistance (Kavitha et al., 2012). *B. thuringiensis* has drawn attention of many researchers' due to its availability to produce one or more parasporal crystal inclusions (Cry or δ-enotoxin). Additionally, a number of studies have also been reported that direct antagonistic effects of other bacteria to pyto-pathogenic nematodes belonged to the genera *Heterodera* and *Meloidogyne* (El-Hadad et al., 2010; Khan et al., 2008).

Nematophagous fungi are natural enemies of nematodes and have developed very sophisticated strategies to either capture vermiform nematodes or parasitize nematode eggs and egg masses (Nordbring, 2004; Mostafa et al., 2012; Perveen and Shahzad, 2013). The nematode trapping fungi *Arthrobotrys* spp. capture nematodes by using special hyphae which forms a three dimensional networks (Anders et al., 1994).

Among different opportunistic soil Hypomycetes, *Paecilomyces lilacinus* was found to be capable of parasitizing both the stages of eggs and larvae of *Meloidogyne* spp. and might become a potential eco-friendly biological control agent in the management *Meloidogyne* spp. in different crops (Devrajan and Rajendran, 2002; Raja and Renganathan, 2012). It has been recognized as a common egg pathogenic fungus of root-knot and cyst nematodes (Rumbos and Kiewnick, 2006).
Saccharomyces cerevisiae is promising plant growth-promoting yeast for different crops. Applicability of *S. cerevisiae* as a biocontrol agent of the root-knot nematode under greenhouse and field conditions resulted in reducing

nematode reproduction ability (Youssef and Soliman, 1997; Muwaffaq, 2013).

The goal of this research was to evaluate; (i) the performance of *Bacillus megaterium*, *B. subtilis*, *B. thuringiensis*, *Pseudomonas fluorescens* and *Serratia marcescens* on egg-hatch and J_2 activity inhibition under laboratory condition; (ii) the potential of the previous bacterial species, *Arthrobotrys conoides* and *A. oligospora*, *P. lilacinus* and *S. cerevisiae* in comparison with the synthetic nematicide, Furadan®10G to manage *M. javanica* infected eggplant cultivar Black Beauty. (iii) The efficacy of *P. lilacinus* in colonization *M. javanica* egg masses and eggs under greenhouse condition.

Results

Laboratory experiment

Effect of B. megaterium, B. subtilis, B. thuringiensis, P. fluorescens, S. marcescens and Furadan®10G on M. javanica egg-hatch and J_2 activity

Treatments with Furadan®10G and bacterial mixture caused the highest inhibition (84.4– 98.8%) of egg-hatch and J_2 activity. Treatments with *B. thuringiensis* and *P. fluorescens* showed 64.9-79.1% inhibition, while *B. subtilis* and *S. marcescens* showed moderate inhibition of 50.5 - 62.0% in egg-hatch and J_2 activity. The lowest inhibition % of egg-hatch and J_2 activity (33.7- 48.8%) were obtained with treatment of *B. megaterium* (Table 1).

Greenhouse experiments

Effect of B. megaterium, B. subtilis, B. thuringiensis, P. fluorescens, S. marcescens and Furadan®10G on eggplant cv. Black Beauty infected with M. javanica

Treatment with Furadan®10G caused the highest reduction (98.0% - 99.1%), followed by treatments with bacterial mixture, each of *B. thuringiensis*, *P. fluorescens* and *S. marcescens* alone and *B. megaterium* and *B. subtilis* which showed (72.0 - 86.8%) and (54.3 - 67.7%) reduction in number of nematode root galls, egg masses/root and number of J_2/250 cc soil compared to check treatments (Table 2). Treatment with bacterial mixture showed significant increase (72.6-73.3%) in dry weight of shoot and root systems, followed by treatments with Furadan®10G and each of *B. thuringiensis*, *P. fluorescens* and *S. marcescens* alone which caused (69.5 - 70.0%) and (58.9-65.8%) increase in dry weight of shoot and root systems, respectively. Treatments with *B. megaterium* and *B. subtilis* showed (44.9 - 55.1%) increase in dry weight of shoot and root systems compared to check treatments (Table 3).

Effect of A. conoides, A. oligospora, P. lilacinus, S. cerevisiae and Furadan®10G on M. javanica on eggplant

The highest reductions (82.4-99.0%) were achieved with Furadan®10G, *P. lilacinus* and *A. oligospora*, followed by *A. conoides* which showed 76.0-78.9% reductions in the number of nematode root galls, egg-masses/root system and numbers of J_2/250 cc soil. Treatment with *S. cerevisiae* showed 69.5-71.7% reduction in the number of nematode root galls, egg-masses/root system and number of J_2/250 cc soil compared to the check treatment (Table 4).

Treatments with Furadan®10G and *A. oligospora* resulted in significant increases (62.0-63.9%) in dry weights of shoot and root systems followed by treatments of *A. conoides*, *P. lilacinus* and *S. cerevisiae* which showed 53.7-60.9% increase (Table 5).

Efficacy of P. lilacinus in colonization M. javanica eggs and egg masses on eggplant

Treatment with ($1X10^8$ cfu/kg soil) of *P. lilacinus* recorded a colonization of 45.2-52.4% on egg masses and eggs after 1^{st} twenty days, which increased to 76.4-82.0% in 2^{nd} twenty days and achieved 98.0-99.2% at the end of the experiment compared to check treatment (Table 6).

Discussion

The present investigation revealed that treatments with the different tested bacterial species; *A. conoides*, *A. oligospora*, *P. lilacinus* and *S. cerevisiae* caused significant reduction in nematode reproduction and enhanced plant growth parameters under laboratory and greenhouse conditions. These results are in harmony with those of (Ismail et al., 2005; Noweer and Hasabo, 2005; Hashem et al., 2008; Ashoub and Amara, 2010).

According to Huang et al. 2009, our results on *Bacillus*, may be due to the nematicidal volatile products produced by this bacterium and characterized to include mainly the benzeneacetaldehyde, 2-nonanone, decanal, 2- undecanone and dimethyl disulphide, which were active against *Meloidogyne* spp. juveniles. Fluorescent pseudomonads can produce a large number of toxic secondary metabolites such as phenazines, indoles, compounds, phenylpyrroles and pterines (Lindberg 1981). Moreover, it is known that *B. thuringiensis* produces chitinolytic enzymes, i.e., chitinases, which is responsible for degrading chitin in cell walls of the nematode eggs and egg masses, so it is named as chitinolytic bacteria (Muzzarelli, 1977).

Arthrobotrys species are trapping fungi which immobilize nematodes using non-adhesive knobs and constricting rings. They ensnare active nematodes using one or more types of mycelial traps (Viaene et al., 2006; Kalele et al., 2010). Mostafa et al (2012) indicated that *A. conoides* and *A. oligospora* were effective in reducing *M. incognita* reproduction.

Different yeast strains are promising biocontrol agents for different crops against root knot nematode infection, which reduced nematode reproduction and increased plant growth parameters (Muwaffaq, 2013). Shawky et al. (2006) reported that *S. uvarum* and *S. ludwigii* proved harmful to *M. javanica* juveniles, egg masses and numbers of galls but the effect magnitude differed from one candidate to another. Youssef and Soliman, 1997 reported that the effect of *S. cerevisiae* on *Meloidogyne* spp. might be due to the activity of *S. cerevisiae* to convert carbohydrates to ethyl alcohol and CO_2, which toxic to nematodes.

P. lilacinus is a soil-inhabiting fungus that is capable of parasitizing nematode eggs, juveniles and females; thus, reduce soil population densities of plant parasitic nematodes (Kiewnick and Sikora, 2005; Ganaie and Khan, 2010). The elective effect of *P. lilacinus* was attributed to the toxic and enzymatic principles such as acetic acid and proteolytic and chitinolytic enzymes released during fungal interaction with nematode which might cause killing/inhibiting the root-knot nematode directly or indirectly promoting plant growth and ultimately enhancing yield (Mahapatra and Sahani, 2007). Strains of this fungus have been formulated for use in controlling nematodes in several countries (Ganaie and Khan, 2010).

Table 1. Effect of *Bacillus megaterium, B. subtilis, B. thuringiensis, Pseudomonas fluorescens, Serratia marcescens,* bacterial mixture and Furadan®10G in both experiments on egg-hatching and J_2 activity of *M. javanica* (MJ).

Treatments	Treated Eggs after 48 h			Treated J_2 after 48 h	
	No. of hatched eggs [w]	Relative [x] hatch %	Hatch [y] Inhibition %	No. of Active J_2 [w]	Activity [z] Inhibition %
Dis. water + MJ alone*	200.0 a	100	0.0	200.0 a	100
NB medium** + MJ	198.5 a	99.3	0.7	197.0 a	1.5
B. megaterium + MJ					
1×10^3 cfu/ml	132.5 b	66.3	33.7	124.5 b	37.8
1×10^6 cfu/ml	119.1 bc	59.6	40.4	102.4 bc	48.8
B. subtilis + MJ					
1×10^3 cfu/ml	99.0 bc	49.5	50.5	81.3 bc	59.4
1×10^6 cfu/ml	85.5 bc	42.8	57.2	77.6 c	61.2
B. thuringiensis + MJ					
1×10^3 cfu/ml	70.1 c	35.1	64.9	53.4 cd	73.3
1×10^6 cfu/ml	60.4 c	30.2	69.8	41.8 d	79.1
P. fluorescens + MJ					
1×10^3 cfu/ml	60.1 c	30.1	69.9	53.6 cd	73.2
1×10^6 cfu/ml	49.3 cd	24.7	75.3	49.7 d	75.2
S. marcescens + MJ					
1×10^3 cfu/ml	92.5 bc	46.3	53.7	96.5 bc	51.8
1×10^6 cfu/ml	85.4 bc	42.7	57.3	76.0 c	62.0
Mixture*** + MJ					
1×10^3 cfu/ml	31.2 d	15.6	84.4	24.0 de	88.0
1×10^6 cfu/ml	23.5 d	11.8	88.2	19.5 e	90.3
Furadan®10 G + MJ					
0.025 mg a.i./ml dis. water	8.4 e	4.2	95.8	5.0 f	97.5
0.05 mg a.i./ml dis. water	5.2 e	2.6	97.4	2.4 f	98.8

*= Check treatment. **= Nutrient broth medium, ***= All used bacterial species together. [w]= 200 MJ eggs and active J_2/treatment. Values of the same column followed by the same letter (s) are not significantly different at $P \leq 0.05$ of LSD. Data are averages of 10 replicates. [x] Relative hatch % = No. of hatched J_2 in each treatment/No. of hatched J_2 in water×100. [y] Hatch inhibition % = 100 – Relative hatch (%). [z] Activity inhibition% = [No. of active J_2 in control - No. of active J_2 in each treatment]/ No. of active J_2 in the control × 100.

Table 2. Effect of *Bacillus megaterium, B. subtilis, B. thuringiensis, Pseudomonas fluorescens, Serratia marcescens* and Furadan®10G on *M. javanica* (MJ) infected eggplant cv. Black Beauty

Treatment	No. of galls/root	Reduction [x] %	No. of egg-masses/root	Reduction [x] %	No. of J_2/250 cc soil	Reduction [x] %
Dis. water + MJ alone*	2896.5 a	----	2881.2 a	---	3642.2 a	---
NB medium**alone+MJ	2889.0 a	0.3	2877.0 a	0.1	3615.5 a	0.7
1X 10^6 cfu/kg soil + MJ:-						
B. megaterium	1041.5 b	64.0	1025.5 b	64.4	1665.0 b	54.3
B. subtilis	953.0 b	67.1	931.2 b	67.7	1584.0 b	56.5
B. thuringiensis	431.4 cd	85.1	425.0 cd	85.2	678.5 de	81.4
P. fluorescens	540.2 c	81.3	523.2 c	81.8	1018.4 c	72.0
S. marcescens	479.4 cd	83.4	468.2 cd	83.7	703.0 d	80.7
Mixture***+MJ	398.1 d	86.3	379.6 d	86.8	618.1 e	83.0
Furadan®10 G [y] + MJ	36.3 e	98.7	25.4 e	99.1	72.4 f	98.0

*= Check treatment. **= Nutrient broth medium. ***= All bacterial species. [x] = Reduction %= [Check-treatment]/check × 100. [y] = 2 mg a.i./kg soil. Data are averages of 5 replicates. Values of the same column followed by the same letter (s) are not significantly different at $P \leq 0.05$ of LSD.

Materials and Methods

Bacterial inoculum preparation

Bacillus megaterium de Bary, *B. subtilis* (Ehrenberg) Cohn, *B. thuringiensis* Berliner, *Pseudomonas fluorescens* Migula and *Serratia marcescens* Bizio single colonies were obtained from the bacterial culture collection of Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. Bacterial cultures were multiplied on nutrient broth (NB) medium (Ramaley and Burden, 1970). Their cultures were mixed in 100 ml of 5% sugar solution. Final concentration of 1×10^6 colony forming units (cfu)/ml distilled water was made for each bacterial species alone or in the complex mixture.

Nematophagous fungi inoculum preparation

Arthrobotrys conoides Drechsler and *A. oligospora* Fresenius hyphae were isolated from the agricultural soil collected from Jazan province, South of Saudi Arabia. Ten grams of soil were sprinkled onto plates contained 1.7% Difco corn meal agar

Table 3. Effect of *Bacillus megaterium*, *B. subtilis*, *B. thuringiensis*, *Pseudomonas fluorescens*, *Serratia marcescens* and Furadan®10G on growth parameters of eggplant cv. Black Beauty infected with M. javanica (MJ).

Treatments	Shoot dry weight (g)	Increase [x] %	Root dry weight (g)	Increase [x] %
Dis. water + MJ alone[*]	4.0 d	-	2.7 d	-
NB medium[**] alone+MJ	4.1 d	2.4	2.8 d	3.6
1X 10 6 cfu/kg soil + MJ:-				
B. megaterium	7.9 c	49.4	4.9 c	44.9
B. subtilis	8.9 c	55.1	5.5 c	50.9
B. thuringiensis	9.9 bc	59.6	6.9 c	60.9
P. fluorescens	10.9 b	63.3	7.9 b	65.8
S. marcescens	9.7 bc	58.8	7.0 c	61.4
Mixture***+MJ	14.6 a	72.6	10.1 a	73.3
Furadan®10 G [y] + MJ	13.1 ab	69.5	9.0 ab	70.0

Legend, as in Table 2. [x] = Increase % = [Treatment-check]/treatment × 100.

Table 4. Effect of *Arthrobotrys conoides*, *A. oligospora*, *Paecilomyces lilacinus*, *Saccharomyces cerevisiae* and Furadan®10G on M. javanica (MJ) infected eggplant cv. Black Beauty.

Treatment	No. of galls/root	Reduction [x] %	No. of egg-masses/root	Reduction [x] %	No. of J_2/250 cc soil	Reduction [x] %
Dis. water + MJ alone[*]	3590.2 a	-	3567.0 a	--	4986.0 a	-
1× 10^8 spores/kg soil +MJ of:-						
A. conoides	863.0 c	76.0	850.4 c	76.2	1054.2 c	78.9
A. oligospora	604.5 d	83.2	593.5 d	83.4	876.0 d	82.4
P. lilacinus	589.2 d	83.6	565.0 d	84.2	523.4 e	89.5
S. cerevisiae	1095.0 b	69.5	1078.4 b	69.8	1412.3 b	71.7
Furadan®10 G [y] +MJ	43.6 e	98.8	35.2 e	99.0	52.6 f	98.9

Legend, as in Table 2.

Table 5. Effect of *Arthrobotrys conoides*, *A. oligospora*, *Paecilomyces lilacinus*, *Saccharomyces cerevisiae* and Furadan®10G on growth parameters of eggplant cv. Black Beauty infected with M. javanica (MJ).

Treatment	Shoot dry weight (g)	Increase [x] %	Root dry weight (g)	Increase [x] %
Dis. water + MJ alone[*]	6.9 c	---	5.2 c	--
1× 10^8 spores/kg soil +MJ of:-				
A. conoides	14.9 b	53.7	12.0 b	56.7
A. oligospora	18.9 a	63.5	14.4 a	63.9
P. lilacinus	15.6 b	55.8	12.9 b	59.7
S. cerevisiae	15.1 b	54.3	13.3 b	60.9
Furadan®10 G [y] +MJ	18.6 a	62.9	13.7 a	62.0

Table 6. Efficacy of *Paecilomyces lilacinus* on colonization *M. javanica* (MJ) eggs and egg masses formed on eggplant cv. Black Beauty.

Treatments	1st 20 days	%	2nd 20 days	%	3rd 20 days	%
Dis. water + MJ alone[*]	0.0 a	-	0.0 a	-	0.0 a	-
1X 108 spors/kg soil of *P. lilacinus*+MJ:-						
No. of colonized Eggs[**]	22.6 b	45.2	41.0 b	82.0	49.6 b	99.2
No. of colonized Egg masses[**]	26.2 b	52.4	38.2 b	76.4	48.4 b	98.0

Legend, as in Table 2. [**]=50 *M. javanica* eggs and egg masses/replicate.

(CMA) and 2% water agar (WA). About 200 free living nematode, Rhabditis spp. was added to the surface of each Petri dish as bait for the nematode-trapping fungi. The plates were incubated at 25 ± 2°C for two weeks and examined every other day. Each nematophagous fungus obtained was maintained on Petri dishes contained CMA (15 g/l) media by using 5 mm fungal disc from 7-day-old cultures and incubated at 25 ± 2°C for 7-10 days. The nematophagous fungi obtained were purified and single spore isolation was made for each fungus on CMA medium. Spore suspension was adjusted to a concentration of 1×10^8 spores/ml distilled water (Barron, 1977). *A. conoides* and *A. oligospora* were identified and classified according to Cooke and Godfrey, 1964 and Van Oorschot, 1985.

P. lilacinus (Thom.) Samson hyphae was obtained from the culture collection of Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt. *P. lilacinus* was grown on autoclaved wheat grains. Spore suspension of *P. lilacinus* was prepared by washing cultures grown on the wheat grains with sterile tap water containing 0.01% Triton X-100. The suspension was passed through a muslin cloth and the spore suspension was adjusted a concentration of 1×10^8 spores/ml distilled water (Rao and Parvk-Rhead, 2001).

Yeast preparation

Commercial dry yeast containing *S. cerevisiae* Franz Meyen ex Emil Christian Hansen, cells (5 g/l) was added to Erlenmeyer flasks containing sterile sucrose solution (50 g/l) and supplemented with streptomycin (0.2 g/l) at pH 4.5, and incubated at 35± 2°C for 24 h. The 24-h-old cultures was inoculated (10%, v/v) to a sterile glucose solution (10 g/l) and incubated for 2 days and then centrifuged at 12,000 rpm for 10 minutes. After centrifugation the pellet was washed and re-suspended in 10 ml of sterile distilled water, for making a suspension adjusted to 1×10^8 spores/ml distilled water (Karajeh, 2013).

Nematode inoculum preparation

The root-knot nematode *M. javanica* (Treub) Chitwood were established from single egg-masses of the adult females previously identified by the morphological characteristics of the female perineal patterns (Taylor and Sasser, 1978) and reared on tomato plants (*Lycopersicon esculentum* Mill) cv. Rutgers in a greenhouse. Nematode eggs were extracted from the infected tomato roots using NaOCl solution as described by Hussey and Barker (1973). Eggs were allowed to hatch for 48 h at 30±2°C in an incubator to obtain 2*nd* stage juveniles (J_2) which used in all tests.

Laboratory experiment

Effect of B. megaterium, B. subtilis, B. thuringiensis, P. fluorescens, S. marcescens and Furadan®10G on M. javanica egg-hatch and J_2 activity

Treatments were done in 24-well tissue culture plates; each well received 2 ml of each treatment. A total of 200 *M. javanica* eggs & J_2 was added in 50 µl of water/well. Two doses of 1×10^3 and 1×10^6 cfu/ml distilled water were used for each bacterial species alone or bacterial mixture. Two concentrations of 0.025 and 0.05 mg a.i./ml distilled water of Furadan®10G were tested to study their effect on egg-hatching and J_2 activity. Each treatment was replicated ten times. *M. javanica* eggs or J_2 were placed in sterile distilled water or sterilized NB medium served as check treatments. Treatments were maintained at 27±2 °C in an incubator. Observations on the effects of different treatments were taken 24 h after adding the nematode eggs or J_2.

Greenhouse experiments

Effects of B. megaterium, B. subtilis, B. thuringiensis, P. fluorescens, S. marcescens and Furadan®10G on M. javanica infected eggplant cv. Black Beauty

Forty five plastic pots were treated with 1×10^6 cfu/kg soil of *B. megaterium*, *B. subtilis*, *B. thuringiensis*, *P. fluorescens* and *S. marcescens* either alone or as a mixture. Furadan®10G was used at the rate of 2 mg a.i./kg soil. Treatments were applied at the same time of *M. javanica* inoculation and 10 days later. Additional two untreated check treatments of ten pots each received *M. javanica* inocula in sterile distilled water or sterilized NB medium.

Effect* of *A. conoides* and *A. oligospora*, *P. lilacinus*, *S. cerevisiae* and Furadan®10G on *M. javanica* on eggplant cv. Black Beauty

Thirty plastic pots were treated with 1×10^8 spores/kg soil of each of *A. oligospora*, *A. conoides*, *P. lilacinus* and *S. cerevisiae*. Furadan®10G was used at the rate of 2 mg a.i./kg soil. Treatments were applied at the same time of *M. javanica* inoculation and 10 days later. Five pots, received only *M. javanica* eggs & J_2 in sterile distilled water were served as a check treatment.

Efficacy of P. lilacinus in parasitism on M. javanica eggs and egg masses formed on eggplant cv. Black Beauty

Twenty plastic pots were treated with (1×10^8 spores/kg soil) of *P. lilacinus*. Twenty egg masses were hand-picked up/root three times intervals after nematode infection. Egg masses were placed on WA plates 2% (w/w), five egg masses/plate. Plates were incubated at room temperature (27 ± 2 °C). Egg masses were observed under stereomicroscope (45x), 4 days later to detect the emerging mycelial from egg masses surfaces as a sign of *P. lilacinus* colonization. Eggs of *M. javanica* were extracted from the infected roots three times. The 1st collection of egg masses and eggs was 20 days after nematode inoculation. The 2nd and 3rd collections were 20 days intervals. A 10 µl of a prepared egg suspension (50 eggs/ml) was pipetted onto a slide, stained with lactophenol cotton blue and examined under a compound microscope (40x) for *P. lilacinus* colonization. Five pots, received *M. javanica* in sterile distilled water were served as a check treatment.

The used plastic pots were 20 cm diameter, filled with 1 kg sandy clay soil (2:1, v:v). Pots were transplanted with three-week-old eggplant seedlings cv. Black Beatty (one seedling/pot) and inoculated with *M. javanica* 4000 eggs & J_2/kg soil. All Pots were maintained at 27 ± 2 °C and irrigated daily. The experiments were terminated 60 days after nematode inoculation. Numbers of nematode root galls, egg-masses/root system, number of J_2/250 cc soil, number of colonized egg masses and eggs with *P. lilacinus* were detected. Dry weights of shoot and root systems were determined.

Statistical analysis

Pots were arranged in randomized complete block design (RCBD) with five replicates/treatment. Data obtained were statistically analyzed according to SAS software program (SAS, 1997). Data of the numbers of nematode root galls, egg-masses and J_2 were transformed to $\sqrt{x+1}$ before statistical analysis. Comparison among means was made via the least significant difference (LSD) ≤ 5% level of probability.

Conclusion

Management of root knot nematode, *M. javanica* infecting eggplant using different antagonistic bacterial or fungal species is an effective and ecologically safer approach as a substitute of chemical nematicides that pollute our environment.

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