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Full Length Research Paper

Biocontrol Management of *Rhizoctonia solani* and *Meloidogyne javanica* infecting Watermelon Plants Using *Pseudomonas* spp.

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A survey study was conducted, during watermelon growing seasons 2014 & 2015, to identify root-rot fungi and the root-knot nematodes infecting watermelon plants cultivated in Abu-Arish governorate fields, Jazan province, southwest Saudi Arabia. Soil and root samples revealed the presence of three root-knot nematode species, e.g. *Meloidogyne arenaria*, *M. incognita* and *M. javanica* was the most prevalent species in all collected samples followed by *M. incognita* and *M. arenaria*. Also, three root-rot fungi, e.g. *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated from watermelon root samples and the most prevalent fungus was *R. solani*. The mixed infection with both pathogens; *M. javanica* and *R. solni* was common in most of the surveyed fields. *In vitro* tests using the bioagents, *Pseudomonas aeruginosa*, *P. chlororaphis*, *P. fluorescens* showed significant inhibitions in growth of *R. solani*, egg-hatch and juvenile mortality % of *M. javanica*. Under greenhouse conditions, watermelon plants inoculated with *R. solani* alone showed a root-rot infection of 39.6 % increased up to 69.5% with the presence of *M. javanica* (mixed inoculation). Under a field condition, during the two growing seasons 2014 & 2015, application of the three *Pseudomonas* species resulted in suppressive effects on *R. solani* and *M. javanica* infecting watermelon plants, reduced percent of fungal root-rot infection, nematode population and improved plant growth parameters compared to the check treatment.

Keywords: Root-knot nematode, survey, soil borne pathogens, biological control, antagonistic organisms, frequency of occurrence, *Citrullus lanatus*.

INTRODUCTION

Cucurbits are highly susceptible to a number of root and soil borne diseases causing great losses in yield and quality (Chehri *et al.,* 2010). Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai is a cucurbit fruit, grown throughout the world and consumed as fresh fruit (Bharath *et al.* 2005). Watermelon cultivars suffer considerable yield losses due to infection by root-knot nematodes (*Meloidogyne* spp.) in tropical sand areas (Al-Hazmi *et al*., 1983; Al-Yahya, 2006 and Mokbel, 2014). Root-rot diseases are a serious and persistent disease problem of major crops. Several root-rotting fungi, e.g. *Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani*

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attacked watermelon during different growth stages and resulted in considerable yield losses (Zhou and Everts, 2004). The association of root-knot nematodes, *Meloidogune* spp. with root-rot fungi produced great losses than the presence of either pathogen alone (Parveen *et al*., 1998). In Pakistan, Ghaffar (1995) stated that watermelon plants was found infected with both root-knot nematodes and root-rot fungi. There is a growing concern, both in developed and developing countries, about the use of hazard chemical pesticides for controlling plant disease pathogens because its residues accumulated in the soil particles and soil solution interferes with numerous biological activities (Chet, 1987 and Davis, 2007). Thus, the development of alternate control strategies and long-term integrated approaches is urgently needed in order to replace chemical treatments (Martin, 2003). Management of plant pathogens by using natural enemies is a promising method of control. Soil inoculation with bio-control agents reduced the percentage of infected plants and disease severity (Faheem *et al*., 2010). Antagonistic bacteria belonging to the Gram-negative bacterium; *Pseudomonas* have been widely used against phytopathogens attacking vegetable crops (Bent *et al.,* 2008). Furthermore, the bacterial strains of *P. chlororaphis, P. aeruginosa* and *P. fluorescens* provide sufficient control of root-knot nematodes and root-rot disease on vegetable crops and enhance plant growth (Burr *et al*., 1978; Kaiser *et al*.,1989; Haas and Defago, 2005).

The present study was undertaken to (i) identify and determine frequency of occurrence of root-knot nematodes and root-rotting fungi attacking or associated with watermelon plants samples collected from different fields in Abu-Arish governorate, Jazan region, Saudi Arabia, (ii) determine the effectiveness of *P. aeruginosa*, *P. chlororaphis* and *P. florescence* against *M. javanica* compared with the synthetic nematicide Furadan®10G under laboratory condition, (iii) evaluate the efficiency of the previous bioagents on linear growth of *R. solani* compared with the synthetic fungicide Rizolex-T under laboratory condition, (iv) estimate the influence of mixed inoculation with *M. javanica* and *R. solani* on watermelon plants under greenhouse condition, (v) evaluate the impact of *P. aeruginosa*, *P. chlororaphis*, *P. florescence*, Furadan®10G and Rizolex-T on watermelon plants inoculated with both pathogens under field condition.

MATERIALS AND METHODS

Survey Study:

A survey study was carried out during the watermelon growing seasons of 2014 and 2015 to determine the frequency of occurrence of root-knot nematodes and root-rotting fungi infected or associated with watermelon plants cultivated in different fields in Abu-Arish governorate, Jazan region, Saudi Arabia. A total of 185 rhizosphere watermelon soil and root samples were collected and examined. Adult females of root-knot nematodes were isolated from the infected galled roots and identified to the species level using the perineal pattern technique (Taylor and Sasser, 1978).

Root-rot fungi isolation procedures were carried out according to the method described by Dhingra and Sinclair (1985) and Bridson (1995). The isolated fungi were purified using the hyphal tips technique and then subculture on slant plain agar medium and kept at 4°C. The isolated fungi were identified according to the cultural characteristics described by Barnett and Hunter (1972) and Nelson *et al*., (1982). The frequency of occurrence (FO) was calculated for each root-knot nematode species, isolated root-rotting fungi and for the mixed infection by both pathogens.

Nematode inoculum preparation:

Females and egg-masses of *M. javanica* (Treub.) Chitwood were isolated from the infected watermelon roots. Culture of this nematode species was established from single egg-masses of adult females previously identified by the morphological characteristics of the female perineal patterns (Taylor and Sasser, 1978) and reared on eggplant cv. Long Purple in a greenhouse. The root-knot nematode eggs were extracted from the infected eggplant roots using sodium hypochlorite (NaOCl) solution as described by Hussey and Barker (1973).

Rhizoctonia solani inoculum preparation:

Pure culture of *R. solani* isolated from the infected watermelon roots was prepared. Roots were tap washed free of soil, surface sterilized with 2% sodium hypochlorite solution for 2 min. Isolated fungus was maintained on Czapek's Dox agar medium in Petri plates at (27±5 °C) in order to mass-produce pure culture, then transferred to flasks containing sorghum seeds and incubated at (27±1 °C) for 10 days.

Pseudomonas culture and inoculum preparation:

The three tested bacterial bioagents, *P. aeruginosa* (Schroeter) Migula, *P. chlororaphis* Bergey, and *P. florescence* Migula were obtained from the culture collection of the Biology Department, Jazan University, Saudi Arabia. Each *Pseudomonas* species was cultured on conical flasks containing 500 ml of autoclaved King's 'B' broth medium. The flasks were incubated at 30±1 °C for 5 days, shaken two times a day, then a concentration of 3×10^{6} (cells)/ml distilled water for each bioagent was prepared (King *et al.,* 1954).

***Laboratory experiments*:**

Effect of *Pseudomonas* spp. and Furadan®10G on *M. javanica* egg-hatch and J_2 mortality:

Treatments were done using 24-well tissue culture plates; each well received 2 ml of each treatment. A total of 150 *M. javanica* eggs or J_2 was added in 50 µl of distilled water/well. Two doses of 3×10^3 and 3×10^6 cells cells/ml distilled water were used for each *Pseudomonas* spp. and one dose of 0.25 g Furadan®10G /ml distilled water (2 ml/well) were used to study their effects on egg-hatch and J_2 mortality. Also, 150 eggs or J_2 of *M. javanica* were added in sterile distilled water to serve as a check treatment. Treatments were maintained at 27±2 °C in an incubator. Each treatment was replicated ten times. After 24 h of incubation in different bioagents or nematicide treatments, numbers of hatched eggs or number of alive and dead J_2 were counted and mortality % was calculated.

Effect of *Pseudomonas* spp. and Rizolex-T on *R. solani* growth inhibition:

To determine the antifungal activity of *P. aeruginosa*, *P. chlororaphis* and *P. florescence*, Petri plates (9 cm) filled with Czapek's Dox agar medium were used. The fungicide, Rizolex-T WP 50% [20 % Telcolofos-methyl (0, 2, 6 dichloro-4-methylphenyl 0, 0 dimethyl phosphoro thioate) and 30% thiram] was applied at the rate of 3 g / L (10 ml/plate) against *R. solani*. About 5-day old culture, mycelial disc (5 mm) of *R. solani* was placed at one side of the Petri plate and the respective bioagents and or Rizolex-T were placed on the plate opposite to each other. Plates inoculated only with mycelial discs of *R. solani* were served as a check treatment. Treatments replicated ten times. Plates were incubated at 28±2 °C then inhibition zone were recorded after 5 days of incubation.

***Greenhouse experiment*:**

Effect of *R. solani* inoculation alone or in combination with *M. javanica* on watermelon plants:

The effect of *R. solani* alone or in combination with *M. javanica* on watermelon cv. Balady was studied under greenhouse conditions. Watermelon seeds were sown in 15 cm diam clay pots filled with (3:1, v:v) sterilized sandy clay soil. Seedlings were thinned to two/pot, one week after emergence. Fungal inoculation was applied at 35 g of barley grains infested with *R. solani*/pot. Treated pots were inoculated with 2000 nematode eggs and J_2/pot at the same time of fungal inoculation. Pots, which inoculated with either *R. solani* or *M. javanica* alone were served as check treatments.

Treatments were replicated five times. Pots were arranged in a randomized complete block design. The experiment was terminated 45 days after nematode inoculation. Numbers of root galls and egg-masses/plant, number of J_2/250 cc soil and dry weights of shoot and root systems were determined.

To determine root-rot infection %, roots of infected plants were cut into 1 cm pieces, washed with distilled water, then acidified and stained with 0.5% trypan blue in lactophenol (v/v). Five stained pieces were mounted on a slide in a lactophenol drop and presence of *R. solani* mycelium was estimated (Buysens *et al.,* 1996). The root infection was calculated by measuring the infected area in relation to total length of root piece.

***Microplot experiments*:**

Efficacy of *Pseudomonas* spp., Furadan®10G and Rizolex-T on controlling *R. solani* and *M. javanica* on watermelon cv. Balady:

Two microplot studies were conducted during the two watermelon growing seasons of 2014 & 2015 at Abu-Arish governorate, Jazan province, Saudi Arabia. To determine the efficacy of *P. aeruginosa*, *P. chlororaphis* and *P. florescence*; the nematicide, Furadan®10G and the fungicide, Rizolex-T on watermelon plants cv. Balady cultivated in sandy loam soil contained 3.0% organic matters, which naturally infested with both of *M. javanica* and *R. solani*. The initial population (P_i) of *M. javanica* was estimated by collecting twenty soil samples of 250 cc soil/each, before watermelon cultivation. The P_i was 2500 J_2/kg soil. Prior to watermelon seeds cultivation, the soil was plowed to a depth of 20 to 25 cm and divided into rows of 20 cm height, two meter long and 50-cm wide with 25-cm gap between rows. All rows were irrigated to its full water holding capacity and watermelon seeds were sown in hills (5 hills/row) as five seeds/hill. Ten days later, seedlings were thinned to two seedlings/ hill. A week later, watermelon seedlings were treated with one concentration of 3×10^6 cells/seedling for each *Pseudomonas* species. The nematicide, Furadan®10G was applied at the rate of 2.5 g/seedling and the fungicide, Rizolex-T was applied at the rate of 3 g/seedling.

Separate rows, which naturally infested with both *R. solani* and *M. javanica*, irrigated only with water were served as check treatments. All treatments were replicated five times (5 rows) and were laid out in a randomized complete block design.

At harvest time, numbers of nematode root galls, egg-masses/plant, J_2/250 cc soil, dry weights of shoot and root systems and root-rot infection % were determined.

Statistical analysis:

Data obtained were statistically analyzed using SAS software program (SAS Institute, 1997). Numbers of nematode root galls, egg-masses and J_2/250 cc soil were

Table 1. Number of positive samples and frequency of occurrence % (FO) of root-knot nematode species infected watermelon plants cultivated in Abu-Arish governorate

No. of collected watermelon samples	Number of positive soil samples & FO		
	M. arenaria	*M. javanica*	*M. incognita*
250	33[a] , 13.2[b]	138 , 55.2	39 , 15.6

[a] = number of positive samples containing root-knot nematode species, [b] = FO = (number of positive samples/total number of the collected samples) × 100.

Table 2. Frequency of occurrence of *Meloidogyne* spp. and each of *F. solani*, *M. phaseolina* and *R. solani* infected watermelon plants cultivated in Abu-Arish governorate

Isolated fungus	Naturally infected watermelon samples with			
	Fungus alone	*Meloidogyne* spp. + fungus (mixed infection)		
		M. arenaria	*M. javanica*	*M. incognita*
F. solani	19[a] , 7.6[b]	6 , 2.4	- , -	- , -
M. phaseolina	16 , 6.4	- , -	- , -	8 , 3.2
R. solani	58 , 23.2	5 , 2.0	133 , 53.2	- , -

Number of collected watermelon soil and root samples = 250 sample. [a] = Number of positive soil samples. [b] = Frequency of occurrence % = No. of positive sample/ No. of collected samples ×100.

transformed to before statistical analysis. Moreover, means were compared using revised LSD test at 5% level of probability.

RESULTS

Data presented in Table (1) indicated the presence of the three root-knot nematode species, namely *M. arenaria*, *M. javanica* and *M. incognita* in the watermelon collected samples. The most common root-knot nematode species was *M. javanica,* with FO of 55.2 % followed by *M. arenaria* and *M. incognita* with 13.2 and 15.6 % FO, respectively.

Results of Table (2) showed number of positive samples and FO of root-rotting fungi and root-knot nematode species in the watermelon collected samples. Data indicated the presence of mixed infection with both pathogens in the watermelon collected samples. The most prevalent fungus was *R. solani* with 23.2 FO. However, *M. phaseolina* and *F. solani* showed 6.4 and 7.6 FO%, respectively.

The most watermelon samples were found naturally infected with both *M. javanica* and *R. solani* with 53.2 FO. On the other hand, the mixed infection with either of *M. aernaria* or *M. incognita* showed 2.0- 3.2% FO (Table 2).

The highest egg-hatch or J_2 mortality inhibition % (94.0 – 96.7%) was achieved with Furadan®10G treatment, followed by treatments with (3×10^3 and 3×10^6 cells/ml) of *P. fluorescens* which showed 66.7 – 87.3 % inhibition. Meanwhile, treatments with either 3×10^3 and 3×10^6 cells/ml of *P. aeruginosa* or *P. chlororaphis* inhibited egg-hatch and J_2 mortality % of root-knot nematode by 33.7-53.1 (Table 3).

The effects of *P. aeruginosa*, *P. chlororaphis*, *P. florescence* and Rizolex-T on growth of *R. solani* were presented in Table (4). Data indicated that the highest growth inhibition % of *R. solani* (94.4 %) was achieved with treatment of Rizolex-T followed by treatment with *P. florescence*, which showed 86.7% inhibition. In addition, treatments with either *P. aeruginosa* or *P. chlororaphis* against *R. solani* resulted in 74.4-77.8 % growth inhibition (Table 4).

The highest numbers of root galls, nematode egg-masses and numbers of J_2/250 cc soil on watermelon plants were recorded with *M. javanica* infection (Table 5). However, no significant differences were detected, in

Table 3. Effects of *Pseudomonas* and Furadan®10G on egg-hatching and J_2 mortality % of *M. javanica* after 24 h of exposure, under laboratory condition

Treatment	No. of hatched Eggs	Relative hatching [x] %	Inhibition [y] %	Number of alive J_2 after 24 h & inhibition %	
				Alive	Mortality [z] %
Check (MJ alone)	150.0 a	*100.0*	-	150 a	-
P. aeruginosa					
3×10^3 cells/ml	86.5 bc	*57.7*	*42.3*	85.0 bc	*43.3*
3×10^6 cells/ml	70.4 c	*46.9*	*53.1*	71.0 c	*52.7*
P. chlororaphis					
3×10^3 cells/ml	90.2 b	*60.1*	*39.9*	99.5 b	*33.7*
3×10^6 cells/ml	75.5 bc	*50.3*	*49.7*	79.5 bc	*47.0*
P. florescence					
3×10^3 cells/ml	50.0 d	*33.3*	*66.7*	40.5 d	*73.0*
3×10^6 cells/ml	23.2 e	*15.5*	*84.5*	19.0 e	*87.3*
Furadan®10G					
0.25 g/ml	9.0 f	*6.0*	*94.0*	5.0 f	96.7

Initial population = 150 *M. javanica* eggs/treatment for egg hatching and 150 J_2/ treatment for mortality% experiments. [x] = Relative hatching % = No. of hatched J_2 in each treatment/No. of hatched J_2 in check treatment×100. Data are averages of 10 replicates. Values of each column, followed by the same letter(s), are not significantly different at $P \leq 0.05$ of LSD test. Inhibition [y] %= 100 – Relative hatch%, Mortality %= [No. of dead J_2 in the check -No. of dead J_2 in treatment / No. of dead J_2 in the check treatment]×100.

Table 4. Effect of *Pseudomonas* spp. and Rizolex-T on growth inhibition of *R. solani* under laboratory conditions

Treatment	Zone of Inhibition (cm)	Inhibition %[**]
Check[*]	9.0 a	-
Pseudomonas conc. (3×10^6 cells/ml):		
P. aeruginosa	2.0 b	77.8
P. chlororaphis	2.3 b	74.4
P. florescence	1.2 c	86.7
Rizolex-T (3 mg/ml)	0.5 d	94.4

[*] = Check treatment = Untreated plates. [**] = Inhibition%= growth zone in check plate-growth zone in test plate/growth zone in check plate×100. Data are averages of 10 replicates. Values, within each column, followed by the same letter (s) are not significantly different at $P \leq 0.05$ of LSD test.

nematode parameters with mixed inoculation of *R. solani* and *M. javanica* compared with that of positive control treatment (mixed infection). Data in Table (5) indicated that root-rot infection % of watermelon was increased up to 69.5% in pots inoculated with both pathogens; *M. javanica* and *R. solani* (Table 5).

Results of Table (6) showed the efficacy of *Pseudomonas*, Furadan®10G and Rizolex-T on *M. javanica* and *R. solani* infected watermelon cv. Balady under filed conditions along 2014 and 2015 seasons. In both seasons, data indicated that treatments with Furadan®10G and all *Pseudomonas* species applied treatments reduced root-knot nematode parameters. The highest reduction % of nematode root galls, egg-masses/plant and J_2/250 cc soil was achieved with Furadan®10G with 97.4-99.5% in both seasons followed by that of *P. florescence* treatment, which showed 51.4-57.1 reduction % in the 1st season and raised to be 81.0-85.5% in the 2nd season. Meanwhile, treatments with either *P. aeruginosa* or *P. chlororaphis* reduced number of nematode root galls, egg-masses/plant and J_2/250 cc soil, in the 1st and the 2nd season, by 31.1-41.6 % and 64.1-77.0% , respectively (Table 6).

The greatest reduction (86.5 and 96.4%) in fungal infection was achieved with treatment of Rizolex-T in the 1st and 2nd season, respectively, followed by treatment with *P. florescence* which showed 57.2 reduction % in the 1st season and raised up to 81.5% in the 2nd season. While, treatments with *P. aeruginosa* and *P. chlororaphis* showed reduction in fungal infection of (27.6-39.8%) and (55.8-69.5%) in the 1st and 2nd seasons, respectively. Meanwhile, treatment with Furadan®10G showed fungal infection

Table 5. Effect of infection with *R. solani* (RS) and *M. javanica* (MJ) on number of galls (G), egg masses (EM)/ root system , number of J_2/ 250 cc soil, dry weight of shoot and root systems of watermelon cv., balady under greenhouse condition

Treatment	G	EM	J_2	Dry weight (g)		Root infection* (%)
				Shoot	Root	
Check (MJ alone)	424.0 a	415.8 a	500.4 a	4.5 a	1.1 a	--
Check (RS alone)	-	-	-	4.0 a	0.8 a	39.6 b
RS and MJ	412.4 a	406.8 a	489.0 a	3.6 a	0.7 a	69.5 a

MJ = 2000 nematode eggs & J_2/pot. * = % of root infection with *R. solani*. Data are averages of 5 replicates. Values within a column followed by the same letter are not significantly different at $P \leq 0.05$ of LSD test.

Table 6. Efficacy of *Pseudomonas*, Furadan®10G and Rizolex-T on controlling both *M. javanica* (MJ) and *R. solani* (RS) infected watermelon cv. Balady, under filed condition

Treatment	1st season						Fungal infection (%)	Reduction %
	Nematode parameter and reduction % (R)							
	No. of galls/root	R	No. of egg-masses/ root	R	No. of J_2/250 cc soil	R		
Pseudomonas spp. (3×10^6 cells /seedling):								
P. aeruginosa	568.5 b	*41.6*	563.0 b	*41.8*	884.0 b	*32.0*	60.2 bc	*39.8*
P. chlororaphis	585.0 b	*39.9*	574.2 b	*40.7*	895.5 b	*31.1*	72.4 b	*27.6*
P. florescence	420.5 c	*56.8*	415.0 c	*57.1*	632.0 c	*51.4*	42.8 c	*57.2*
Furadan®10G 2.5 g/seedling	22.4 d	*97.7*	18.0 d	*98.1*	33.5 d	*97.4*	98.4 a	*1.6*
Rizolex-T 3 g/seedling	970.5 a	*0.31*	960.2 a	*0.81*	1290.0 a	*0.77*	13.5 d	*86.5*
MJ & RS*	973.5 a	*0.0*	968.0 a	*0.0*	1300.0 a	*0.0*	100.0 a	*0.0*

ranged from 1.6-3.3% in both 1st and 2nd seasons compared with the check treatment (Table 6).

Data presented in Table (7) showed that treatments with *P. florescence* resulted in significant increases of 63.0-80.6% in dry weights of shoot and root systems in both 1st and 2nd seasons, followed by treatments with *P. aeruginosa* and *P. chlororaphis* which showed 42.5-59.3% increase in both seasons. Meanwhile, slight increase in dry weights of shoot and root systems (2.5-12.9 %) was noticed with Furadan®10G and Rizolex-T treatments range from in both seasons compared with the check treatment (Table 7).

DISCUSSION

The present survey results indicate the presence of three common species of root-knot nematodes; *M. arenaria*, *M. javanica* and *M.incognita* infecting watermelon plants grown in all the surveyed fields in Abu-Arish governorate, Jazan province Saudi Arabia. *M. javanica* was the most common root-knot species with 55.2 FO, followed by *M. arenaria* and *M. incognita* with 13.2 and 15.6 % FO, respectively. These data are in agreement with those of Al-Yahya (2006) and Mokbel (2014), who indicated the presence of root-knot nematodes in vegetables fields in Abu-Arish governorate. They found that *M. javanica* and *M. incognita* were the most prevalent nematode species associated with watermelon soil and root samples.

Also, the present data showed the presence of three root-rotting fungi; *F. solani, M. phaseolina* and *R. solani* associated with watermelon soil and root samples. The most prevalent root-rotting fungus was *R. solani*. These results are in agreement with results of Ghaffar (1995) and Mousa (1994). They found that the most prevalent fungi which could be attacked and associated with watermelon plants were *F. solani* and *R. solani.*

Table 6. *Continued*

Treatment	2nd season						Fungal infection (%)	Reduction %
	Nematode parameter and reduction % (R)							
	No. of galls/root	R	No. of egg-masses/ root	R	No. of J_2/250 cc soil	R		
Pseudomonas spp. (3×10^6 cells /seedling):								
P. aeruginosa	325.0 c	*76.7*	319.2 c	*77.0*	486.5 c	*74.1*	30.5 c	*69.5*
P. chlororaphis	419.5 b	*69.9*	410.5 b	*70.4*	674.0 b	*64.1*	44.2 b	*55.8*
P. florescence	208.0 d	*85.1*	201.5 d	*85.5*	357.0 d	*81.0*	18.5 d	*81.5*
Furadan®10G 2.5 g/seedling	12.0 e	*99.1*	6.5 e	*99.5*	20.5 e	*98.9*	96.7 a	*3.3*
Rizolex-T 3 g/seedling	1338.5 a	*0.47*	1376.0 a	*0.86*	1863.5 a	*0.88*	3.6 e	*96.4*
MJ & RS*	1395.0 a	*0.0*	1388.0 a	*0.0*	1880.0 a	*0.0*	100.0 a	*0.0*

* = Check treatment. The P_i was 2500 J_2/kg soil. Legend as in Table,5.

Table 7. Effects of *Pseudomonas* spp., Furadan®10G and Rizolex-T on growth parameters of watermelon plants infected with *M. javanica* (MJ) and *R. solani* (RS) under filed condition

Treatment	1st season				2nd season			
	Shoot dry weight (g)	Increase (%)	Root dry weight (g)	Increase (%)	Shoot dry weight (g)	Increase (%)	Root dry weight (g)	Increase (%)
Pseudomonas spp. (3×10^6 cells /seedling):								
P. aeruginosa	11.0 ab	*50.7*	8.9 ab	*58.9*	12.9 ab	*59.3*	9.6 b	*54.8*
P. chlororaphis	10.4 b	*42.5*	8.3 ab	*48.2*	11.8 b	*45.7*	9.2 b	*48.4*
P. florescence	11.9 a	*63.0*	9.8 a	*75.0*	13.8 a	*70.4*	11.2 a	*80.6*
Furadan®10G 2.5 g/seedling	8.1 cd	*10.6*	6.3 cd	*12.5*	8.9 c	*9.9*	7.0 c	*12.9*
Rizolex-T 3 g/seedling	8.0 c	*9.6*	6.1 c	*8.9*	8.3 cd	*2.5*	6.9 cd	*11.3*
MJ & RS*	7.3 d	*0.0*	5.6 d	*0.0*	8.1 d	*0.0*	6.2 d	*0.0*

Legend as in Table,6.

Under greenhouse conditions interaction between *M. javanica* and *R. solani* on watermelon plants showed no significant differences neither in nematode reproduction nor in watermelon growth parameters. These results are disagree with results of Mehta *et al*. (1990) who reported that interaction between *M. javanica* and *R. solani* had no effect on French bean growth parameter, but *R. solani* infection affected reproduction of *M. javanica.* Also, AL-Hazmi (1985) and Mehta *et al*. (1990) reported that the combined infection with the nematode and fungus decreased nematode reproduction and this may be due to production of fungal toxins, adverse effect of the fungus on the nematode penetration and/or fungal invasion of giant cells which disrupts nematode feeding.

The present study revealed that some strains of fluorescent *Pseudomonas* showed significant activity against root-rotting fungi and root-knot nematodes under both laboratory and filed conditions. All treatments significantly suppressed both pathogens *R. solani* and *M. javanica* and enhanced plant growth parameters. These results are in agreement with many other workers (Siddiqui and Ehteshamul-Haque (2001) and Jiskani *et al*., 2007). They reported that the bacteria belonging to fluorescent *Pseudomonas*, which colonize roots of a wide range of

crop plants, are reported to be antagonistic to soil borne plant pathogens.

Many investigations revealed that the antagonistic potential of *P. fluorescens* and *P. aeruginosa* against root-rot fungi and root-knot nematodes could be attributed to the production of antibiotics and siderophore as one of the mechanisms involved in antagonism or due to induced systemic resistance (Kloepper *et al*., 1980; Bakker *et al*., 1993; and De Meyer and Hofte*,* 1997). Raajimakers and Weller (1998) reported that the fluorescent *Pseudomonas* species produced the antifungal metabolite 2, 4-diacetylphloroglucinol in the infected plant roots. Meanwhile, Chin-A-Woeng *et al*. (1998) reported that *P. chlororaphis* produces the antifungal metabolite phenazine-1-carboxamide which controls root-rot disease. Park (1990) reported that suppression of root pathogens is also due to competition for food and ability to colonize the roots. Also, the bacterial strain *P. fluorescens* produces plant growth promoting substances, thereby enhancing plant growth and yield (Burr *et al*., 1978; Kaiser *et al*., 1989).

Endo-parasitic nematodes like *Meloidogyne* spp., form specialized feeding cells in the plant tissue and remains embedded in the tissue, whereas parasitic fungi also proliferate inside the host tissues and absorbs the nutrients. Due to protection by surrounding plant tissue, they are difficult to control by soil and rhizosphere microorganisms. Therefore, endophytic microorganisms colonizing plant root tissue may be better able to manage endo-parasitic nematode and fungi due to fact that both occupy the same ecological niche and are close contact (Hallman *et al*., 1997).

CONCLUSION

Our results indicated that application of the antagonistic bacteria belonged to *Pseudomonas* spp. are capable of protecting watermelon from root-knot nematodes and root-rot fungi infection and enhanced plant growth under both greenhouse and field conditions. The application of these bioagents may be an effective and ecologically safer alternative approach as a substitute of chemical pesticides that pollute our environment.

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REFERENCES

AL-Hazmi AS (1985). Interaction of Meloidogyne incognita and Macrophomina phaseolina in a root-rot disease complex of French bean. Phytopathol. Z., 113: 311- 316.

Al-Hazmi AS, Abl-Hayja ZM, Trabulsi IY (1983). Plant parasitic nematodes in Al-Kharj area of Saudi Arabia. Nematol. Medit., 11: 207-212.

Al-Yahya FAA (2006). Plant-parasitic nematodes associated with plant groups in Abu Arish governorate, Jazan province, Southwest, Saudi Arabia. Research Bulletin., No. 140, Food Sci. & Agric. Res. Center, King Saud Univ., 5-17 pp.

Bakker PAHM, Raaijmakers JM, Schippers B (1993). Role of iron in the suppression of bacterial plant pathogens by fluorescent *Pseudomonads*. In: Iron Chelation in Plants and Soil Microorganisms (Eds. L.L. Barton and B.C. Hemming), Academic Press, San Diego, USA., pp: 269-278.

Barnett HL, Hunter BB (1972). Illustrated Genera of Imperfect Fungi. Burgess. Pub. Co., Minnesota ,USA, pp: 241.

Bent E, Loffredo A, McKenry MV, Becker JO, Borneman J (2008). Detection and investigation of soil biological activity against *Meloidogyne incognita*. J. Nematol., 40: 109-118.

Bharath BG, Lokesh S, Rai VR, Prakash HS, Yashovarma B, Shetty HS (2005). Role of foliar spray in the infection biology and management of fungal diseases of watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai).World J. Agricultural Sciences, 1 (2): 105-108.

Bridson EY (1995). The Oxide Manual 7th Ed., Published by Unipath Limited, Wade Koad, Basingstoke Hampshire, RG 248 PW, England.

Burr TJ, Schroth MN, Suslow TV (1978). Increased potato yields by treatment of seed pieces with scientific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathol., 68: 1377-1383.

Buysens S, Heungens K, Poppe J, Hofte M (1996). Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aaeruginosa* 7NSK2. Appl. Environ. Microbiol., 62: 865-871.

Chehri k, Abbasi S, Redd KRN, Salleh B (2010). Occurrence and pathogenicity of various pathogenic fungi on cucurbits from Kermanshah province, Iran. Afr. J. Microbiol. Res., 4: 1215-1223.

Chet I (1987). *Trichoderma* application, mode of action, and potential as a biocontrol agent of soil borne plant pathogenic fungi. Plant disease control, Witey, New York, pp: 137-160.

Chin-A-Woeng TFC, Bloemberg GV, Van der Bij AJ, Van der Drift KMGM, Schripsema J, Kroon B, Scheffer RJ, Keel C, Bakker PAHM, Tichy HV, De Bruijin FJ, Thomas-Oates JE, Lugtenberg BJJ (1998). Biocontrol by phenazine-1-carboxamide producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Molecular Plant-Microbe Interactions, 11:1069–1077.

Davis RF (2007). Effect of *Meloidogyne incognita* on watermelon yield. Nematropica, 37: 287-293.

De Meyer G, Hofte M (1997). Salicylic acid produced by rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induced resistance to leaf infection by *Botrytis cinerea* on bean. Phytopath., 87: 588-593.

Dhingra OD, Sinclair JB (1985). Basic plant pathology methods. CRC Press Inc. Boca Raton, Florida, USA. 353 pp.

Faheem A, Razdan VK, Mohiddin FA, Bhat KA, Banday S (2010). Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. J. Phytology, 2 (10): 38-41.

Ghaffar A (1995). Biological control of root-rot and root-knot disease complex of vegetables. Final Research Report, Dept. Bot. Univ. Karachi, Karachi, pp. 752-770.

Haas D, Defago G (2005). Biological control of soil–borne pathogens by fluorescent *Pseudomonads*. Nat. Rev. Microbiol., 3 (4): 307–319.

Hallman J, Quadt-Hallman A, Mahafee WF, Kloepper JW (1997). Bacterial endophytes in agricultural crops. Can. J. Microbiol., 43: 895-914.

Hussey RS, Barker KR (1973). A comparison of methods of collecting inocula of *Meloidogyne* species, including a new technique. Plant Dis. Rep., 42: 865-872.

Jiskani MM, Pathan MA, Wagan HA, Imran M, Abro H (2007). Studies on the control of tomato damping-off disease caused by *Rhizoctonia solani* Kuhn. Pak. J. Bot., 39: 2749-2754.

Kaiser WJ, Harman RM, Weller DM (1989). Biological control of seed rot and pre-emergence damping-off of chickpea with fluorescent *Pseudomonads*. Soil Biol. Biochem., 21: 269-273.

King EO, Ward MK, Raney DE (1954). Two simple media for the demonstration of pyocyanine and fluorescein. J. Lab. Clin. Med., 44: 301-307.

Kloepper JW, Leong J, Teintze M, Schroth MN (1980). Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature, 286: 885-886.

Martin FN (2003). Development of alternative strategies for management of soil borne pathogens currently controlled with methyl bromide. Annu. Rev. Phytopathol., 41: 325-350.

Mehta N, Gupta DC, Mehta N (1990). Antagonistic behaviour of *Rhizocotonia solani* and *R. bataticola* with *Meloidogyne javanica* on french bean (*Phaseolus vulgaris*). Haryana Agricultural University Journal of Research, 20: 287-290.

Mokbel AA (2014). Survey of plant-parasitic nematodes and their associated host plants cultivated in Jazan Province, Southwest Saudi Arabia. Egypt. J. Exp. Biol. (BOT.), 10 (1): 35 -38.

Mousa ESM (1994). Biological management of soil-borne pathogens and root-knot nematode complexes on soybean. Proceedings of the second Afro-Asian Nematology Symposium held at Menoufiya , Egypt, 18-22 December, pp: 60-66.

Nelson PE, Toussoun TA, Marasas WFO (1982). *Fusarium* spp. An Illustrated Manual of Identification. The Pennesylvania Univ. Press Univ., Park, 216 pp.

Park JI (1990). Root colonization by indigenous and introduced microorganisms. In: The Rhizosphere and Plant Growth (Eds. Keister, D. L. and Gregan, P. R.), Kluwer Academic Publ., Dordrecht, Neetherland, pp: 33-42.

Parveen S, Ehteshamul-Haque S, Ghaffar A (1998). Efficacy of *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* in the control of root-rot and root-knot disease complex of some vegetables. Nematol. Medit., 26: 209-212.

Raaijimaker JM, Weller DM (1998). Natural plant protection by 2,4-diacetylphloroglucinol producing *Pseudomonas* spp. In take all decline soils. Mol. Plant-Microbe Interact. 11: 144-152.

SAS Institute (1997). SAS/STAT User's Guide. Release 6.03 Edition-6th SAS Institute Inc., North Carolina, Cury. Inc., 1028 pp.

Siddiqui, I. A. and Ehteshamul-Haque, S. 2001. Suppression of the root-rot and root-knot disease complex by *Pseudomonas aeruginosa* in tomato: The influence of inoculum density, nematode population, moisture and other plant associated bacteria. Plant and Soil, 237: 81-89.

Taylor AL, Sasser JA (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* spp.). Coop Publ. Dep. Plant Pathology, North Carolina State University and U.S. Agency Int. Dev., Raleigh, NC.111 pp.

Zhou XG, Everts KL (2004). Suppression of *Fusarium* wilt of watermelon by soil amendment with hairy vetch. Plant Disease, 88: 1357-1365.