Journal of Biological Control, 27(4): 305–311, 2013

Research Article

Screening and *in vitro* evaluation of native *Pseudomonas* spp., against nematode pathogens and soil borne fungal pathogens

RAJKUMAR*[1], R. RANGESHWARAN, G. SIVAKUMAR and M. NAGESH

National Bureau of Agriculturally Important Insects (NBAII), Bangalore 560 024

[1]Present Address: Central Plantation Crops Research Institute, Kasargod 671 124, Kerala

**Corresponding E-mail* : rajkumarnbaii@gmail.com

ABSTRACT: The objective of this study was to assess the efficacy of native *Pseudomonas* spp., against root-knot nematode, *Meloidogyne incognita* and other soil borne fungal pathogens such as *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii*. The eggs and second stage juveniles (J2) of *M. incognita* were exposed to each isolates of *Pseudomonas* spp., by diluting the standard culture filtrate to fifty percent and to undiluted culture filtrate (100%). Four isolates of *Pseudomonas* spp. (CRS3, CRS6, and CRS8 and CRS10) significantly induced inhibition of egg hatching and mortality of *M. incognita* juveniles. The per cent mortality was proportional to the concentration of culture filtrate and the duration of exposure period. The highest percentage of inhibition of egg hatching was recorded for CRS3 while mortality of second stage juveniles was found in the case of CRS10 in undiluted culture filtrate. The CRS6 caused 48% inhibition of *Sclerotium rolfsii* while CRS8 caused 58% inhibition of *Fusarium oxysporum* f. sp. *lycopersici*. Since meloidogyne infection can predispose plants to plant pathogens, these isolates show promise for management of nematode and disease complex of vegetable crops.

KEY WORDS: Culture filtrate, egg hatching, *Meloidogyne incognita, Pseudomonas* spp., root-knot nematode and soil borne fungi

(Article chronicle: Received: 26-03-2013; Revised: 11-10-2013 Accepted: 15-11-2013)

INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endo-parasites that cause severe yield loss in vegetables and the damage is much higher in tropical and subtropical countries. Among the four species of root-knot nematodes, *M. incognita* is the key nematode pest of tomato, okra, brinjal and cucurbits. Besides, it also affects a wide range of other economically important crops, accounting 64 per cent of the total population of root-knot nematodes occurring in tropical countries (Sasser, 1979; Carter 1985; Knight *et al*., 1997). The vegetable crops are highly prone to soil borne wilt/ root rot causing fungi and root-knot nematodes, the latter being pre-disposer to a wide range of vegetable crops causing disease complexes with synergistic effect on the common host (Goswami *et al*., 2012). Through extensive surveys of vegetable fields in Karnataka during 2010-2011, heavy damage of tomato, brinjal and okra crops were encountered due to root-knot nematodes in the district of Bangalore. Though the use of nematicides is an effective measure to control root-knot nematodes (Reddy 1986; Kuruganti 2005), repetitive usage leads to percolation of toxicity to ground water and having costly disposable procedures besides declining the load of beneficial organisms in root zone. Continuous use can be harmful to both the user and environment. All this has spurred the researchers to find out alternative eco-friendly management methods (Keinwnick and Sikora, 2006). A number of bacterial species such as *Pseudomonas* spp., *Bacillus thuringiensis* and *Bacillus subtilis* has been used as bio-control agents against *Meloidogyne* spp. (Gokte and Swarup, 1988; Dawar *et al.,* 2010; Ashraf and Khan, 2010). They may serve as promising biological agents towards various root pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani* (Vagelas, 2002; Vagelas *et al*., 2003; Mujeebur Rahman Khan *et al*., 2011). The continuous cultivation of high yielding cultivars and mono-cropping system has witnessed an increase in the number of vegetable fields being infested with root-knot nematodes. To counter the nematode infestation, the growers were found to be indiscriminately using banned chemical pesticides *viz*. MBr, DD, carbofuran, phorate etc. thus disturbing soil fertility and biodiversity. The plant protectionists have been attempting to manage soil borne diseases through biological control alternatives for disease suppression without negative effects on the user, consumer or the environment (Johnsson *et al*., 1998). The present study focuses on investigating the efficacy

of native *Pseudomonas* spp., as bio-control agent against *M. incognita* and other soil borne fungal pathogen.

MATERIALS AND METHODS

Survey, isolation and identification of *Pseudomonas* spp.

A survey was conducted in different localities around Bangalore, Karnataka comprising an area approximately 8500 ha, in order to identify the nematodes infection and to isolate the bacterial antagonists from crops rhizosphere (CRS) of the cucurbits, tomato, brinjal, okra, cabbage, grapes, citrus, groundnut and guava. A total of 42 free soil (500 g) and root (100 g) soil sample were collected from the rhizosphere. Serial dilution technique was used for the isolation of *Pseudomonas* spp. One g of rhizosphere soil was dispensed in 9 ml sterile water, from the 10^{-6} dilution, 50 µl were inoculated over Petri plates containing King's B agar media (autoclaved at 121°C for 15 min). The plates were incubated at room temperature 28±2°C for 24 hrs. The isolated *Pseudomonas* strains were identified by standard protocol (King *et al.,* 1954; Annon, 1957; Buchanan and Gibbons, 1974). A total 12 isolates were collected from soil rhizosphere and evaluated for nematicidal/ anti fungal properties against nematodes and soil borne fungus

Collection of culture filtrate

The *Pseudomonas* isolates were grown in KB medium and incubated at 30°C on a shaker for 48 hrs in dark and centrifuged at 2,800 x g for 10 minutes. Pellets were discarded and the culture filtrate was collected in a beaker for subsequent use.

Nematode inoculum and mass culturing

The inoculum of root-knot nematode *M. incognita* was collected from naturally infested tomato crop in field and single egg mass was used to raise pure culture. The species were identified by studying the morphology of the perineal pattern of ten females (Hartman and Sasser, 1985). Mass culturing of nematodes was done on tomato variety (Arka vikas), in order to get regular supply of the inoculums for the experiments. One month old tomato seedlings were inoculated with small volume of egg suspension approximately consisting of 2000 eggs of *M. incognita.* These pots were watered and kept in glass house at temperature 28-35°C.

Hatching of *M. incognita* bioassay

To determine the effect of cell free culture filtrate on the hatching of eggs and mortality of J2 of *M. incognita*, two separate experiments were carried out to evaluate the nematicidal activity of 12 *Pseudomonas* isolates (10^8 cells ml^{-1}). One ml of each of the culture filtrate of collected isolates at 50 and 100% were poured into Syracuse dishes cavity block and 50 eggs were introduced into each dish and incubated at 28±2°C temperature in completely randomized design, replicated thrice. Equal number of cavity blocks were kept with eggs of *M. incognita* in sterile distilled water as control. The required number of fresh eggs for each treatment was collected from egg mass of *M. incognita* infested roots. The eggs were detached from egg mass and undamaged eggs were collected in a beaker containing fresh sterile water (Hussey and Barker 1973; Ioannis *et al*., 2007). Observation on the number of hatched J2 in three replications (cavity block) for each treatment was determined every 12, 24, 36 and 48 hrs with the aid of streomicroscope (x 6). At the end of the experiment, number of unhatched eggs was calculated and per cent egg hatch calculated. The eggs from culture filtrate were transferred to sterile distilled water and their hatching in sterile water was recorded to ascertain the whether the eggs kept in the culture filtrate had been permanently or temporarily inactivated.

Mortality of second stage juveniles (J2) bioassay

Egg masses of *M. incognita* were collected from an infested root and allowed to hatch in distilled water with aeration (Hussey and Barker 1973). The hatched J2 were collected in a beaker. One ml of each of the culture filtrate of collected isolates at 50 and 100% were poured into Syracuse dishes cavity block and fifty J2 were introduced into each dish and incubated at 28±2°C temperature in completely randomized design, replicated thrice. Equal number of cavity blocks was kept with J2 in sterile distilled water as control.

Observation on the number of dead J2 for every 12, 24, 36 and 48hrs of exposure was recorded with the aid of streomicroscope (x 6). The J2 were considered dead when they did not move when probing with a fine needle (Cayrol *et al.*, 1989). Mean percentage of dead J2 were estimated.

Screening of promising isolates against soil borne fungi

A local strain of the root pathogen, *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii* were collected from National Bureau of Agriculturally Important Insects (NBAII, Bangalore, India and were multiplied on PDA (potato dextrose agar) incubated at 28 ± 2°C (Nash and Snyder, 1962). The bacterium was tested for *in vitro* antagonism towards phytopathogenic fungi by standard co-inoculation technique on PDA agar. The fungal culture was initially obtained by growing on

PDA media to obtain a lawn of culture. Fungal cultures of (6 mm diameter) were inoculated fresh PDA plate. After 24 hrs of fungal growth, single bacterial colonies were streaked around the edge of the plate. Five replications were prepared for each set. The plates were incubated at 28°C and the inhibition zone (if any) was measured after five days. Only those isolates that produced a clear inhibition zone were considered effective (Siddiqui *et al*., 2001). Subsequently, the fungal growth inhibition was assayed by measuring the distance between the fungal and bacterial growth in comparison with control. The inhibition of fungi by bacteria was calculated using the formula:

Inhibition percentage = Control – Treated / Control x 100

Data analysis

Data were subjected to analysis of multi-factorial variance analysed by using SAS (9.3) software.

RESULTS AND DISCUSSION

Identification of bacterial isolates

Collected isolates were characterized and identified according to Bergey's Manual of Determinative Bacteriology. The selected isolates were short, rod shaped, Gram negative, motile and non-spore forming organisms. Isolate tested positive to catalase test and negative to methyl red test. Pyoverdin pigment was distinguished under UV light source grown on King's medium (King *et al.,* 1954). Hence, all these isolates were identified as *Pseudomonas* spp. (Table 1).

Identification of *Meloidogyne incognita* species

Diagnostic features of *M. incognita* were identified, striae were distinct, closely spaced and the dorsal arch was high and squared in females (Hartman and Sasser, 1985).

***In-vitro* assay**

Effect of culture filtrates on eggs and second stage juveniles (J2) of *M. incognita*

All treatments (isolates) significantly reduced ($P \leq 0.05$) the egg hatching and found enhanced mortality of J2 over untreated control from 12 to 48 h exposure. Undiluted culture filtrates (100%) were found superior to the diluted culture filtrate (50%) (Table 2 and 3). After 48 hrs undiluted culture filtrate of CRS3 significantly inhibited hatching of eggs by 28% at while diluted culture filtrate inhibited hatching of eggs by 38.67% over other isolates followed by CRS6 ($P \leq 0.05$). The J2 continued to emerge from eggs transferred from culture filtrate to sterile distilled water in all treatments but their numbers were significantly ($P \leq 0.05$) less in the treatments that were treated with undiluted culture filtrate but diluted down to 50% effectively halted hatching (Table 2). The isolate CRS3, CRS6, CRS8 and CRS10 showed significant ($P \leq 0.05$) mortality of J2 by 13.33–60% at diluted culture filtrate. Isolate CRS10 of undiluted culture filtrate

Table 1. Morphological characterization of *Pseudomonas* spp. isolates (Anonymous, 1957)

Pseudomonas spp. isolates	Gram reaction	Cell shape	Cell size	Pigmentation	Colony traits
CRS1	Gram -ve	Rod	Small	Light green	Smooth round
CRS2	Gram -ve	Rod	Small	Bright green	Smooth regular
CRS3	Gram -ve	Rod	Small	Green	circular
CRS4	Gram -ve	Rod	Small	Yellow green	Smooth round
CRS5	Gram -ve	Rod	Small	Light green	Water droplet like
CRS6	Gram -ve	Rod	Small	Yellow	Smooth and elevated
CRS7	Gram -ve	Rod	Small	Green	Smooth regular and elevated
CRS8	Gram -ve	Rod	Small	Yellow orange	Colonies very small
CRS9	Gram -ve	Rod	Small	Light green	Water droplet like
CRS10	Gram -ve	Rod	Small	Deep green	Smooth and elevated
CRS11	Gram -ve	Rod	Small	Light green	circular
CRS12	Gram -ve	Rod	Small	Bright green	Smooth round

Table 2. Efficacy of native *Pseudomonas* spp., culture filtrate on egg hatching of *Meloidogyne incognita*

Treatments	Concentration of culture filtrate (50%)				Concentration of culture filtrate (100%)			
	12 hrs	24 hrs	36 hrs	48 hrs	12 hrs	24 hrs	36 hrs	48 hrs
CRS1	8.67 (4.97)	34.00 (19.89)	38.00 (22.61)	59.33 (36.82)	5.33 (3.05)	28.67 (16.66)	33.33 (19.48)	59.33 (37.29)
CRS2	14.67 (8.45)	30.67 (17.90)	36.00 (21.14)	59.33 (36.49)	5.33 (3.05)	20.67 (11.94)	28.00 (16.27)	40.00 (23.64)
CRS3	7.33 (4.20)	16.00 (9.21)	28.00 (16.30)	38.67 (22.78)	2.67 (1.52)	13.67 (7.86)	23.33 (13.54)	28.00 (16.29)
CRS4	12.00 (6.90)	34.67 (20.37)	49.33 (29.69)	64.67 (40.33)	8.00 (4.59)	34.67 (20.45)	39.33 (23.17)	54.67 (33.23)
CRS5	10.67 (6.13)	34.67 (20.29)	52.67 (31.82)	64.67 (40.40)	6.00 (3.44)	12.00 (6.90)	40.00 (23.61)	44.67 (26.57)
CRS6	7.33 (4.21)	23.33 (13.51)	26.00 (15.09)	45.33 (27.02)	4.67 (2.67)	24.00 (13.90)	29.33 (17.06)	40.00 (23.60)
CRS7	13.33 (7.67)	41.33 (24.44)	54.67 (33.33)	76.67 (50.56)	8.67 (4.97)	24.00 (13.91)	38.00 (22.36)	49.33 (29.62)
CRS8	7.33 (4.21)	26.00 (15.10)	42.00 (24.91)	62.00 (39.01)	2.00 (1.14)	23.33 (13.53)	40.00 (15.10)	48.67 (23.60)
CRS9	15.33 (8.82)	32.67 (19.08)	50.67 (30.60)	60.00 (37.11)	12.67 (7.28)	29.33 (17.07)	46.00 (27.41)	54.00 (32.70)
CRS10	9.33 (5.36)	22.67 (13.13)	37.33 (21.98)	49.33 (29.58)	10.00 (5.74)	27.33 (15.87)	42.00 (24.87)	57.33 (35.24)
CRS11	13.33 (7.67)	34.00 (19.89)	55.33 (33.68)	71.33 (45.61)	4.00 (2.29)	23.33 (13.51)	41.33 (24.43)	59.33 (36.54)
CRS12	12.00 (6.89)	38.00 (22.37)	58.67 (35.94)	68.67 (43.51)	8.67 (4.97)	30.00 (17.46)	48.67 (29.14)	73.33 (48.11)
Control	20.67 (11.93)	42.67 (25.32)	63.33 (39.44)	86.00 (59.67)	20.67 (11.93)	45.33 (26.97)	64.67 (40.31)	84.00 (57.33)
CD ($P = 0.05$) (Treatments) 2.47	CD (hrs) 1.37		CD (Concentration) 0.97		CD (Treatxhrs) 4.95		CD (Treat x Conc) 3.50	

Figures in the parentheses are Arch-Sine Transformed values

Table 3. Efficacy of native *Pseudomonas* spp., culture filtrate on mortality of second stage juveniles (J2) of *Meliodogyne incognita*

	Concentration of culture filtrate (50%)				Concentration of culture filtrate (100%)			
Treatments	12 hrs	24 hrs	36 hrs	48 hrs	12 hrs	24 hrs	36 hrs	48 hrs
CRS1	4.67 (2.67)	20.67 (11.96)	26.00 (15.08)	32.67 (19.08)	10.00 (5.74)	22.67 (13.10)	22.67 (13.11)	34.67 (20.30)
CRS2	7.33 (4.20)	18.00 (10.38)	34.00 (19.91)	42.00 (24.85)	11.33 (6.51)	22.67 (13.11)	38.00 (22.34)	46.67 (27.92)
CRS3	14.00 (8.05)	28.00 (16.27)	42.67 (25.29)	57.33 (35.01)	22.00 (12.72)	41.33 (24.43)	50.00 (30.02)	76.00 (49.68)
CRS4	9.67 (5.51)	27.33 (15.92)	33.33 (19.48)	39.33 (23.17)	14.67 (8.44)	22.00 (12.71)	34.00 (19.89)	45.33 (26.99)
CRS5	2.67 (1.52)	24.67 (14.29)	30.67 (17.88)	47.33 (28.45)	10.67 (6.12)	24.00 (13.89)	27.33 (15.87)	50.67 (30.47)
CRS6	15.33 (8.82)	32.00 (18.67)	48.67 (29.14)	54.67 (33.37)	25.33 (14.69)	38.00 (22.37)	60.67 (37.40)	77.33 (51.06)
CRS7	6.00 (3.44)	29.33 (17.13)	32.00 (18.68)	40.67 (24.03)	6.67 (3.82)	23.33 (13.50)	34.00 (19.89)	44.00 (26.13)
CRS8	13.33 (7.67)	30.67 (17.88)	42.67 (25.29)	55.33 (33.78)	20.00 (11.54)	42.67 (25.27)	52.00 (31.37)	74.67 (48.74)
CRS9	7.33 (4.20)	21.33 (12.35)	31.33 (18.28)	37.33 (21.94)	10.00 (5.74)	22.67 (13.11)	34.00 (19.89)	59.33 (36.54)
CRS10	14.00 (8.05)	34.00 (19.92)	45.33 (26.97)	60.00 (36.94)	28.00 (16.27)	48.67 (29.14)	56.00 (34.17)	78.67 (52.71)
CRS11	8.67 (4.97)	27.33 (15.89)	33.33 (19.48)	41.33 (39.99)	16.00 (9.21)	26.00 (15.08)	35.33 (20.70)	58.67 (36.17)
CRS12	6.00 (3.44)	20.67 (11.96)	32.00 (18.67)	46.67 (24.44)	12.00 (6.89)	22.67 (13.11)	43.33 (25.76)	47.33 (28.33)
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (Treatments) 1.88	CD (hrs) 1.04	CD (Concentration) 0.74	CD (Treat x hrs) 3.14		CD (Treat x Conc) 2.61		CD (hrs x Conc) 1.48	

Figures in the parentheses are Arch-Sine Transformed values

was most toxic, killing 78.67% J2 compared with other isolates at undiluted culture filtrate (Table 3). However, no other isolate showed any relationship between inhibition of egg hatching and mortality of J2. The isolates CRS8 and CRS10 were effective on inducing mortality of J2 but did not show any effect on inhibition of egg hatching.

Effect of isolates on soil borne fungal pathogens

The isolate CRS6 showed the largest inhibition zone against *Sclerotium rolfsii* 48% and CRS8 isolate exhibited 58% inhibition zone against *Fusarium oxysporum* f. sp. *lycopersici* when compared to other isolates with that of control.

Substantial work has been done regarding the use of bacteria as biological control agents of soil-borne pathogenic fungi. However, less work has been reported on the potential of *Pseudomonas* spp. in managing plant parasitic nematodes in crop plants. In the present study, culture filtrates from native isolates of *Pseudomona*s spp., inhibited hatching and mortality of J2 of *M. incognita* as well as soil borne fungi *in vitro*.

In the preliminary screening of isolates CRS3, CRS 6, CRS8 and CRS10 were selected for further study based on their profound effects on *M. incognita* J2 and egg hatching, *in vitro*. Ovicidal and larvicidal action of the selected antagonist isolates was measured on *M. incognita* (Sharma *et al*., 1998; Mohamed Hashem and Ab-Elyousr 2011). Among the antagonist isolates CRS6 were progressively and consistently exerted their antagonistic effect on eggs and J2 of *M. incognita* in the two concentrations tried (Table 2 & 3). The failure to regain its activity after separating from culture filtrate placed over sterile water demonstrates the numbers of metabolites that had longer systemic activity. These results are in agreement with the findings that showed that *Pseudomonas* spp., are lethal to juveniles of *M. incognita* (Khan *et al.,* 2005; Kiewnick and Sikora 2006). Siddiqui and Shaukat (2003) reported metabolite production, including 2, 4- diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN) by *P. fluorescens* strain CHAO that inhibited egg hatching and induced mortality of root-knot nematodes. *Pseudomonas* spp. has been reportedly acting as biological agent against plant-parasitic nematodes. Production of metabolites by rhizosphere bacteria (Oostendorp & Sikora, 1990) affects the vitality of juveniles of *Meloidogyne* spp. (Becker *et al*., 1988). The effects of rhizobacterial toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as direct killing of nematodes by causing paralysis and convulsive movements (Siddiqui and Mahmood, 1999). These observations are also exemplified in many lines of experimental evidence as indicated by Regina *et al*. (1998) and Hanna *et al*. (1999) who have reported that mortality of *M. incognita* increased with increase in exposure time as well as the concentration. The results prove that in addition to the nematicidal effect the pseudomonas isolates suppressed soil borne fungal pathogens, *Sclerotium rolfsii* (CRS6) and *Fusarium oxysporum* f. sp. *lycopersici* (CRS7) under *in vitro*. These observations were similar to Vagelas, (2002) Li bin *et al*. (2005) reported *Pseudomonas* spp., enhanced plant growth and suppress soil borne pathogens, such as *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia* solani and *Pythium aphanidermatum*. Similarly, Shankra *et al.* (2011), demonstrated that the microorganisms that can grow in the rhizosphere provide the front line defence for roots against nematodes and soil borne pathogens attack and are ideal for use as bio-control agents.

We could conclude that identification of single isolate of *Pseudomonas* spp., that have lethal effect on hatching of eggs and J2 of *M. incognita* as well as suppression of soil borne fungus *via* production of anti-fungal/anti-nematode metabolites and inducing systemic resistance in plants is better option than choosing two different isolates of *Pseudomonas* spp., for targeting nematode and soil borne fungus. The study highlighted the effect of 4 native isolates of *Pseudomonas* spp. as effective alternatives for the management of nematode-fungal disease complex but further field efficacy studies are required.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Director, NBAII, Bangalore for providing necessary infrastructure facilities for the investigation and we wish to thank research fellows for providing technical assistance during the study.

REFERENCES

Anonymous 1957. *Manual of microbiological methods*. Society of American Bacteriologists, McGraw Hill Book Company, Inc., New York, 315 pp.

Ashraf MS, Khan TA. 2010. Integrated approach for the management of *Meloidogyne javanica* on eggplant using oil cakes and bio-control agents. Arch. Phytopathology. *Pl Prot.* **43**: 609–614.

Becker JO, Zavaleta-Mejia, Colbert SF, Schroth MN, Weinhold AR, Hancock JG, Van Gundy SD. 1988.

Effects of rhizobacteria on root-knot nematodes and gall formation. *Phytopathol* **78**: 1466–1469.

Buchanan RE, Gibbons NE. 1974. *Bergey's Manual of Determinative Bacteriology*. Baltimore, Williams & Wilkins, 8th edition, pp. 13–45.

Carter CC. 1985. Literature search: host range of *Meloidogyne hapla*. *Internatl Nematol Network Newslett.* **2**: 16–24.

Cayrol JC, Djian C, Pijarowski I. 1989. Studies on the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue de Nematologie* **12**: 331–336.

Dawar S, Wahab S, Tariq M, Zaki MJ. 2010. Applications of *Bacillus* species in the control of root-rot disease of crop plants. *Arch Phytopathol Pl Prot.* **43** (4): 412–418.

Gokte N, Swarup G. 1988. On the potential of some bacterial biocides against root-knot and cyst nematodes. *Ind J Nematol.* **18**: 152–153.

Goswami BK, Bhattacharya Chaitali, Singh Neetu, Paul MP Reject.2012. An IPM package for the management of soil borne fungal and root-knot nematode diseases on tomato and okra. *Pesticide Res J.* **24**(1): 91–95.

Hanna AL, Raid FW, Tawfik AE. 1999. Efficacy of antagonistic rhizobacteria on the control of root-knot nematode, *Meloidogyne incognita* in tomato plants. *Egyptian J Agri Res.* **77**: 1467–1476.

Hartman KM, Sasser JN. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. pp. 69–77. In: Barker KR, Carter, CC, Sasser JN (ed.). *An Advanced Treatise on Meloidogyne*. *Vol II, methodology,* North Carolina State University Graphics, Raleigh, NC, USA.

Hussey RS, Barker KR. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Pl Dis Rep*. **61**: 328–331.

Ioannis K, Vagelas, Baerbark Pembroke, Simon R, Gowen and Keith G. Davies. 2007. The control of root-knot nematodes (*Meloidogyne* spp.) by *Pseudomonas oryzihabitans* and its immunological detection on tomato roots. *Nematol.* **9** (3): 363–370.

Johnsson L, Hökeberg M, Gerhardson B. 1998. Performance of the *Pseudomonas chlororaphis,* biocontrol agent MA 342 against seed-borne diseases in field experiments. *European J Pl Pathol.* **104**: 701–711.

Khan Z, Park SD, Shin SY, Bae SG, Yeon IK, Seo YJ. 2005. Management of *Meloidogyne incognita* on tomato by root dip treatment in culture filtrate of the blue green alga, *Microcoleus vaginatus*. *Biores Technol.* **96**: 1338–1341.

Kienwnick S, Sikora RA. 2006. Biological control of the root-knot nematode, *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biol Cont.* **38**: 179–187.

King EO, Ward MK, Raney DE. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *J Lab Clin Med.* **11**: 441–449.

Knight KWL, Barber CJ, Page GD. 1997. Plant-parasitic nematodes of New Zealand recorded by host association. *J Nematol.* **29**: 640–656.

Kuruganti K. 2005. Effects of pesticide exposure on developmental task performance in Indian children, *Youth and Environ.* **15** (1): 83–114.

Li bin, Xie Guan-lin, Soad A, Coosemans J. 2005. Suppression of *Meloidogyne javanica* by antagonistic and plant growth promoting rhizobacteria. *J Zhejjang.* 496–501.

Mohamed Hashem, Kamal Abo-Elyousr A. 2011. Management of root-knot, *Meloidogyne incognita* on tomato with combinations of different bio-control organisms. *Crop Prot.* **30**: 1–8.

Mujeebur Rahman Khan, Shaharana Majid, Fayaz A, Mohidin, Nibilah Khan. 2011. A new bioprocess to produce low cost powder formulations of bio-control bacteria and fungi to control fusarial wilt and root-knot nematodes of pulses. *Biol Cont.* **59**: 130–140.

Nash SN, Snyder WC. 1962. Quantitative estimation of plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathol.* **52**: 567–572.

Oostendorp M, Sikora RA. 1990. *In vitro* inter-relationships between rhizosphere bacteria and *Heterodera schachtii. Rev de Nematol.* **13**: 269–274.

Reddy Parvatha P.1986. Chemical control of plant parasitic nematodes infecting citrus and grapevine. *Ind J Hort* **43**: 303–305.

Regina MDGC, DeSouza IS, Belarmino LC. 1998. Nematicidal activity of *Bacillus* spp. strains on juveniles of *Meloidogyne javanica*. *Nemat Brasiliera* **22**: 12–21.

Sasser JN. 1979. Economic importance of *Meliodogyne* in tropical countries, pp. 359–374. In: Lamberti, F., Tylor, C. E. (Eds.) Root-knot nematodes (*Meloidogyne* species) Systematics, Biology & Control. Academics Press. London.

Shankra T, Pavaraj M, Umamaheswari Prabhu D, Baskaran S. 2011. Effect of *Pseudomonas aeruginosa* on the root-knot nematode, *Meloidogyne incognita* infecting tomato, *Lycopersicum esculentum*. *A J Ent.* **4** (3): 114–117.

Sharma SB, Rupela OP, Ansari MA, Gopalkrishnan S. 1998. Suppression of *Meloidogyne javanica* egg hatch by on isolate of *Pseudomonas striata*. In: III International Symposium of Afro-Asian Society of Nematologists, 16-19 April 1998, Coimbatore, India.

Siddiqui ZA, Ehteshamul-Haque S, Shukat SS. 2001. Use of rhizobacteria in the control of root rot- root-knot disease complex of mungbean. *J Phytopathol.* **149**: 337–346.

Siddiqui I, Shukat S. 2003. Suppression of root diseases by *Pseudomonas fluorescenes* CHAO in tomato: Importance of bacterial secondary metabolities 2, 4-diacetylphloroglucinol. *Soil Biol Biochem.* **35**: 1 615 – 1623.

Siddiqui ZA, Mahmood I. 1999. Role of bacteria in the management of plant parasitic nematodes: a review. *Bio-resource Tech.* **69**: 167–179.

Vagelas IK. 2002. Efficacy of *Pseudomonas oryzihabitans* as bio-control agent of root pathogens. Ph.D. Thesis, Department of Agriculture, University of Reading, UK.

Vagelas IK, Gravanis FT, Gowen SR. 2003. Control of *Fusarium oxysporum* and *Meloidogyne* spp. with *Pseudomonas oryzihabitans*. In: Proceedings of the BCPC International Congress – Crop Science and Technology. Vol. 1. Glasgow, UK., 419–424.