

# EVALUATION OF PLANT EXTRACTS AND *PSEUDOMONAS* SPP. FOR CONTROL OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* ON TOMATO

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## ABSTRACT

Abo-Elyousr, Kamal A., Zakaullah Khan, Magd El-Morsi Award, and Montaser Fawzy Abedel-Moneim 2010. Evaluation of plant extracts and *Pseudomonas* spp. for control of root-knot nematode, *Meloidogyne incognita* on tomato. *Nematropica* 40:289-299.

Fresh leaf extracts of *Azadirachta indica* (neem), *Allium sativum* (garlic) and *Tagetes erecta* (African marigold) and bacterial suspensions of *Pseudomonas fluorescens* and *P. aeruginosa* were tested against *Meloidogyne incognita* on tomato under *in vitro*, pots and field conditions. All treatments immobilized juveniles (J2); with the highest effect caused by neem leaves extract and the lowest by *P. aeruginosa* after 24 h and 48 h of exposures. In soil all treatments significantly reduced root galling, nematode population, and enhanced plant growth and yield, with bacterial treatments being the most effective. Nematode populations were reduced greater by nematicide (Vydate 10 G) treatment but the greatest increase in yield (67%) was obtained with *P. fluorescens*; the treatment reduced root galling by 68-70% and 48% in pot and field experiments, respectively. Among the plant species, garlic demonstrated best control reducing root galls by 57% in pots under greenhouse and 33% in field conditions and increasing fruits yield by 47%.

*Key words:* African marigold, biocontrol, garlic, *Meloidogyne incognita*, neem, rhizobacteria.

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## RESUMEN

Abo-Elyousr, Kamal A., Zakaullah Khan, Magd El-Morsi Award and Montaser Fawzy Abedel-Moneim 2010. Evaluación de extractos de plantas y de *Pseudomonas* spp. para el control de *Meloidogyne incognita* en tomate. *Nematropica* 40:289-299.

Se probaron extractos frescos de hojas de *Azadirachta indica* (nim), *Allium sativum* (ajo) y *Tagetes erecta* (clavelón africano) y suspensiones bacteriales de *Pseudomonas fluorescens* y *P. aeruginosa* para el control de *Meloidogyne incognita* en tomate *in vitro*, en macetas y en campo. Todos los tratamientos inmovilizaron los juveniles (J2); observándose el mayor efecto con el extracto de hojas de nim y el menor con el extracto de *P. aeruginosa* después de 24 y 48 h de exposición. En el suelo, todos los tratamientos redujeron significativamente el agallamiento y la densidad de población del nematodo, y aumentaron el crecimiento y rendimiento de la planta, siendo los tratamientos bacteriales los más efectivos. La mayor reducción en las poblaciones del nematodo se observó con el tratamiento con nematicida (Vydate 10 G), pero el mayor aumento en rendimiento (67%) se obtuvo con *P. fluorescens*; cuyo tratamiento redujo el agallamiento en 68-70% y 48% en macetas y campo, respectivamente. Entre los tratamientos vegetales, el ajo demostró el mayor control de agallamiento, con una reducción del 57% en macetas y 33% en campo, y un aumento del rendimiento del 47%.

*Palabras clave:* ajo, *Azadirachta indica*, control biológico, *Meloidogyne incognita*, rizobacteria, *Tagetes erecta*.

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## INTRODUCTION

The root-knot nematodes *Meloidogyne* spp. are serious pathogens of many economic crops and causing approximately 5% of global crop loss (Sasser and Carter, 1985). They are capable of severely damaging a wide range of crops, in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005). Use of chemical nematicides has been one of the primary means of controlling plant-parasitic nematodes for the past five decades. However, detrimental environmental effects associated with chemical control and the recent losses of methyl bromide as a multipurpose soil fumigant have spurred research into nematode control alternatives (Nico *et al.*, 2004). Biocontrol appears to offer an environmentally safe and ecologically feasible option for plant protection with great potential for promoting sustainable agriculture. Bacteria, fungi, protozoans, viruses, nematodes and plants have been reported as antagonists to plant-parasitic nematodes (Stirling, 1991). The beneficial effects of certain types of plant derived materials and microorganisms in soil have been attributed to a decrease in the population densities of plant-parasitic nematodes (Akhtar, 2000).

Plants are an important source of naturally occurring pesticides, and many compounds with nematicidal activity have been found in plants (Gommers, 1981; Chitwood, 2002). In recent years a variety of plants including neem (*Azadirachta indica* A. Juss), garlic (*Allium sativum* L.) and marigolds (*Tagetes erecta* L.), and their by-products have been evaluated for their nematicidal properties and efficacy in the management of plant parasitic nematodes but most of the studies lack field data (Ferris and Zheng, 1999; Zasada *et al.*, 2002; Agbenin *et al.*, 2005; Natarajan *et al.*, 2006;

Bharadwaj and Sharma, 2007; Javed *et al.*, 2007, 2008; Ntalli *et al.*, 2009).

Plant-associated microorganisms have important roles in natural and induced suppressiveness of soil-borne diseases. Several culturable rhizobacteria have been tested for their biocontrol potential against plant parasitic nematodes (Becker *et al.*, 1988; Kloepper *et al.*, 1992; Oka *et al.*, 1993; Hallmann *et al.*, 1997; Siddiqui and Shaukat, 2002, 2003; Khan *et al.*, 2008; Son *et al.*, 2008, 2009). Reductions in nematode damage have been reported by the use of bacteria but most of the studies lack the data on field efficacy, which is very important from practical point of view. Nevertheless, certain root-associated strains of fluorescent *Pseudomonas* spp. have been considered as potential biocontrol agents for root-knot nematodes, they produce and excrete metabolites that are inhibitory to nematodes and induce systemic resistance against root-knot nematodes (Siddiqui and Shaukat, 2000: 2003).

The aims of the present study were to evaluate the efficacy of leaf extracts of neem, garlic and African marigold which grow in Egypt, and to examine the performance of local isolates of *Pseudomonas fluorescens*, and *P. aeruginosa* for control of root-knot nematode (*M. incognita*) on tomato (*Lycopersicon esculentum*) cultivar Super Marmande in greenhouse and field conditions compared with the use of Vydate® 10 G (nematicide).

## MATERIALS AND METHODS

### *Nematode inoculum*

To obtain nematode inoculum for *in vitro* and pot experiments, pure culture of *M. incognita* was raised from single egg mass and maintained on tomato roots in greenhouse. Infected plants were uprooted from soil and the entire root system was

dipped in water and washed gently to remove adhering soil. Egg masses of *M. incognita* were picked with forceps. Egg masses were rinsed with sterile water then placed in 0.5% sodium hypochlorite (NaOCl) solution agitated for 4 minutes and rinsed with sterile water on a 26  $\mu\text{m}$  sieve (Hussey and Barker, 1973). The eggs were incubated for 3-5 days using a modified Baermann funnel method (Southey, 1986) to obtain second stage juveniles (J2). Population density of J2 was determined from 5 replications of one ml aliquots of an inoculum suspension for *in vitro* and pot experiments.

#### *Preparation of plant extracts*

Fresh leaves of garlic and African marigold taken from two months old plants and neem leaves from a 10 year old tree grown in Assuit University farm, Assuit, Egypt were used to prepare water extracts. For that twenty five grams of fresh leaves from each plant were mixed in 250 ml sterilized distilled water (1g/10ml basis) using an electric blender for three minutes. The resultant mixture was left at room temperature for 72 h, and then passed through 15-mm-diam Whatman no. 1 filter paper. Filtrates obtained were used as such without any further dilution in various experiments.

#### *Preparation of bacterial strains*

Bacterial strains used in this study were isolated from tomato rhizosphere and identified on the basis of morphological, physiological and biochemical characteristics (Schaad, 1980; Krieg and Holt, 1984). The isolates were stored under appropriate conditions in facility of Plant Pathology Department, Assiut University, Assiut, Egypt. The 48 h-old-culture in King's medium B (KBM) was centrifuged at 10,000 g for 10 min to separate bacterial

cells from culture media. After centrifugation, supernatants were discarded and pellets (bacterial cells) were washed by centrifugation three times with sterilized distilled water (SDW) and finally suspended in SDW. The optical density (OD) of the suspension was adjusted to 0.45 (A610 nm) with the help of a UV-Visible spectrophotometer (Spectronic® 20, Milton Roy Company USA) equivalent to  $10^8$  CFU/ml. This concentration was used for all experiments.

#### *In vitro assay*

Effects of plant extracts and bacterial suspensions of *P. fluorescens*, and *P. aeruginosa* were evaluated against *M. incognita* J2 under laboratory conditions. For this experiment, 300 freshly hatched *M. incognita* J2 were transferred to 2.5-cm-diam Petri dishes containing 5 ml of each of plant extracts (25 g leaves in 250 ml SDW) or bacterial suspensions ( $10^8$ CFU/ml) separately. Petri dishes were maintained at  $25 \pm 2^\circ\text{C}$  in an incubator (XR20C, Dixell, Pieve d Alpage, Italy). Immobilized J2 were counted under stereoscopic microscope at 60X magnification after 24 h and 48 h of incubation. Each treatment was replicated 5 times and the experiment was repeated once.

#### *Pot experiments*

Six-week-old tomato seedlings of susceptible cultivar Super Marmande were transplanted into 30-cm-diam clay pots containing 2 kg steam-sterilized sandy loam soil (one seedling/pot). Three days later, plants were inoculated with 2000 J2 of *M. incognita* dispensed in 10 ml of water around the root zone with a pipette, and pots were watered slightly. At the same time, 100 ml of each plant extracts (25 g leaves in 250 ml SDW) and 20 ml of each bacterial suspension ( $10^8$ CFU/ml) was

added to potting soil around the plant roots; applications were repeated after 25 days. Pots inoculated with nematodes alone and/or treated with a nematicide, Vydate10 G (0.5 g/pot) were included as controls. Each treatment had ten replicates (pots). Pots were arranged in a randomized complete block design in a greenhouse and watered to field capacity whenever needed; the experiment was repeated once.

Forty and sixty days after nematode inoculation, the plants were uprooted from the pots, and roots were carefully washed free of soil, to avoid dislodging of egg masses. Roots were assessed for root galling on a 0-5 rating scale according to the percentage of galled tissue (0 = 0-10%; 1 = 11-20%; 2 = 21-50%; 3 = 51-80%; 4 = 81-90%; and 5 = 91-100% (Barker, 1985). After gall assessment, fresh roots weights were recorded, and *M. incognita* eggs were extracted from infected roots by shaking the roots of each plant in 1% NaOCl for 4 min and rinsed on a 26 µm sieve with water and collected in a beaker. Roots were then macerated in 100 ml water in a blender for 10 sec and poured on the sieve to obtain nematodes from inside the roots. The total numbers of nematodes per root system was determined in two 10 ml aliquot suspensions using stereoscopic microscope at 60X magnification. Soil from each pot was homogenized and nematodes from 200 g soil subsamples were extracted by centrifugal-flotation technique (Jenkins, 1964). Weights of fresh shoots and roots of 5 plants from each treatment were recorded and plants were dried in an oven at 60 C for 72 h before determination of their dry weight.

#### *Field experiment*

Field experiments were conducted in 2008 and repeated in 2009, in a farm natu-

rally infested with *M. incognita* (ca 1000 J2/250 cc soil) at New Valley Governorate. The soil was sandy loam with 2.5% organic matters. Field experiments for each treatment comprised of 4 rows of 3 meter long, 50-cm wide with 50-cm gap between rows. Six weeks old tomato seedlings were transplanted at 50-cm distance on 1st September, 2008 (7 plants per row). One hundred milliliters of plant leaf extracts and 20-ml of bacterial suspensions with same concentrations as was used in pot experiments were added into soil around rhizosphere of each plant separately. Untreated rows of plants and/or treated with a nematicide, Vydate10 G (0.5 g/plant) were included as controls. Each treatment was applied 3 times, at planting and then at 15 day of intervals. All treatments were replicated four times (4 rows) and were laid out in a completely randomized block design for two months duration. Soil and root samples were collected 30 days after final treatments. Twelve plants and 10 soil samples from each plot were taken for analysis. Two hundred and fifty milliliter soil samples were collected from the rhizosphere of tomato plants up to a depth of 20 cm. Nematode (J2) and egg populations and root galling were analyzed in the same way as described in earlier section. Also, the weight of tomato fruits per plant was recorded and yield was expressed in tons/hectare.

#### *Statistical analysis*

All experiments were performed twice. A two-way analysis of variance (ANOVA) was performed on the data followed by mean separation with Fisher's least significant difference (LSD) procedure to compare means among treatments. Differences are reported at  $P \leq 0.05$ . All statistical analyses were done using Analyse-it software for Microsoft Excel. No significant difference

occurred between the repeated experiments; therefore, data from duplicate tests were pooled for analysis.

## RESULTS

### *In vitro* assay

Treatments with plant extracts and rhizobacteria substantially immobilized *M. incognita* J2 after 24 h and 48 h of exposures (Fig. 1). Plant extracts were more effective in immobilizing J2 than bacterial treatments ( $P \leq 0.05$ ); neem extract immobilized J2 by 76.4% after 24 h of exposure, garlic and marigold extracts immobilized J2 by 67% after 24 h of exposures ( $P \leq 0.05$ ). *Pseudomonas fluorescens* and *P. aeruginosa* reduced J2 mobility by 48 and 50% in 24 h of exposures ( $P \leq 0.05$ ), respectively. Effects of all treatments on J2 mobility continued as exposure time increased, although the differences were not significant after 24 h. All J2 in sterile distilled water were alive by the end of experiment.

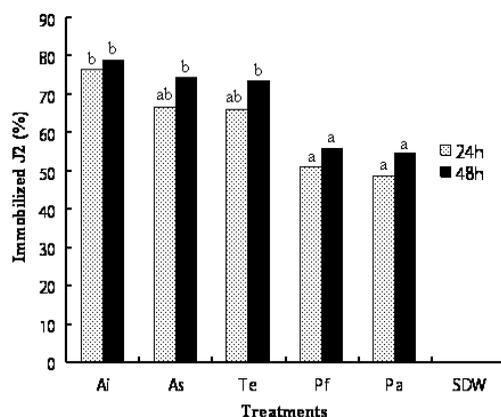


Fig. 1. Effect of plant leaf extracts and bacterial treatments on mobility of *Meloidogyne incognita* juveniles (J2) after 24 h and 48 h of exposures. Ai = *Azadirachta indica* (neem); Te = *Tagetes erecta* (marigold); As = *Allium sativium* (garlic); Pf = *Pseudomonas fluorescens*; Pa = *Pseudomonas aeruginosa*; SDW = sterile distilled water. Means followed by the same letters are not significantly different ( $P \leq 0.05$ ) according to LSD test.

### *Pot experiments*

Treatments with plant extracts, bacterial suspensions and nematicide significantly reduced the disease severity and promoted tomato growth at both observation times, i.e. 40 and 60 days after nematode inoculation ( $P \leq 0.05$ ) (Table 1). However, effects varied among the treatments; maximum reduction of root galling (73.50%) and nematode populations in soil (73%) and roots (52%) occurred in pots treated with Vydate nematicide, followed by *P. fluorescens* and *P. aeruginosa* treatments, which reduced root galling by 57-61% sixty days after inoculation. Among plant extracts, garlic resulted in the best control, while the lowest reduction in root galling, nematode reproduction was found in pots treated with marigold (Table 1). Weights of plants after 60 days were significantly lower in untreated than was treated plants ( $P \leq 0.05$ ) (Table 2). The maximum total plant weights (fresh) were recorded in pots treated with *P. fluorescens* (128g) and *P. aeruginosa* (122g) and the lowest in pots treated with nematicide, Vydate (80.8g).

### *Field experiments*

All treatments caused a significant reduction in root galling and nematode populations, and increased yields compared to untreated control ( $P \leq 0.05$ ) (Table 3). Effects varied among treatments; the highest reduction in root galling and nematodes reproduction was obtained with the nematicide, Vydate but plant growth and yields were not increased by the treatment. Maximum plant growth and fruits yields were obtained from bacteria treated plants ( $P \leq 0.05$ ), both strains reduced root galling by 46-49% and increased fruit yields by 66%. Among the plant species, garlic was the most effective in reducing root galling by 33% and in increasing fruit yields by 47%. Neem and

Table 1. Effect of plant leaf extracts and bacterial preparation on root galling (GI) caused by root-knot nematode (*Meloidogyne incognita*) on tomato roots and on population and egg production in potting soil inoculated with 2000 J2/pot.

Treatments	40 days after J2 inoculation				60 days after J2 inoculation			
	GI	Nematode population		No of eggs	GI	Nematode population		No. of eggs
		in soil	in roots			in soil	in roots	
<i>Azadirachta indica</i> (Neem)	2.4 bc	753 b	58 bc	5694 c	2.4 bc	825 c	65 bc	6028 c
<i>Allium sativum</i> (Garlic)	2.0 cd	621 c	52 cd	4793 d	2.1 bc	805 c	63 bc	4931 d
<i>Tagetes erecta</i> (Marigold)	2.8 b	825 b	65 b	7115 b	2.9 b	1252 b	72 b	7587 b
<i>Pseudomonas fluorescens</i>	1.4 de	432 d	42 de	3973 e	1.9 cd	635 d	49 de	3937 e
<i>Pseudomonas aeruginosa</i>	1.5 de	620 c	45 de	4086 e	2.1 bc	676 d	55 cd	4139 e
Nematicide (Vydate 10 G)	1.0 e	262 e	32 f	1906 f	1.3 d	529 e	44 e	1639 f
Control	4.6 a	1846 a	77 a	11550 a	4.9 a	1952 a	92 a	13890 a

Values in the same column followed by the same letter(s) do not differ at  $P \leq 0.05$ .

Table 2. Effect of treatments of plant leaf extracts and bacterial preparation on growth of tomato plants in potted soil inoculated with *Meloidogyne incognita* (2000 J2/pot).

Treatments	Shoots weight				Root weight			
	FW (g)	Increase (%)	DW (g)	Increase (%)	FW (g)	Increase (%)	DW (g)	Increase (%)
	<i>Azadirachta indica</i> (Neem)	90.0 c	37.8	10.6 bc	89.3	8.4 b	200.0	0.8 b
<i>Allium sativum</i> (Garlic)	78.8 d	20.7	10.5 bc	87.5	6.7 c	139.3	0.7 b	75.0
<i>Tagetes erecta</i> (Marigold)	78.2 d	19.8	9.9 c	76.8	8.1 b	189.2	0.7 b	75.0
<i>Pseudomonas fluorescens</i>	115.9 a	77.5	12.3 a	119.6	12.1 a	332.1	1.5 a	275.0
<i>Pseudomonas aeruginosa</i>	111.0 b	67.0	12.0 ab	114.3	11.2 a	300.0	1.3 a	225.0
Nematicide (Vydate 10 G)	75.2 d	15.2	7.8 d	39.3	5.6 d	81.5	0.6 b	50.0
Control	65.3 e	—	5.6 e	—	2.8 e	—	0.4 c	—

Values in the same column followed by the same letter(s) do not differ at  $P \leq 0.05$ . FW= fresh weight; DW = dry weight.

Marigold leaf extracts were the least effective for increasing fruit yields (33%).

## DISCUSSION

*In vitro* results indicated that leaf extracts of neem, garlic and African mari-

gold, and suspension of *P. fluorescens* and *P. aeruginosa* immobilized J2 of *M. incognita* and that plant leaf extracts were more effective than the bacterial suspension. Ferris and Zheng (1999) have reported that water extracts of several plants including neem bark and seed, and garlic bulb killed

Table 3. Effects of treatments with plant leaf extracts and bacterial preparation on root galling, nematode, egg populations, and fruit yield of tomato plants in a field infested with *Meloidogyne incognita* (1000 J2/250cc soil).

Treatments	Root gall index		J2/250 cc soil		Females in roots		Eggs/root system		Fruits yield	
	GI	CE* (%)	No. of J2	CE (%)	Females /root	CE (%)	No. of eggs	CE (%)	Ton/hectare	CE (%)
<i>Azadirachta indica</i> (Neem)	3.0 bc	30.2	1502 bc	43.4	77 c	51.6	6256 c	59.9	48.5 c	32.5
<i>Allium sativum</i> (Garlic)	2.9 bc	32.6	1420 cd	46.5	75 c	52.8	6100 c	60.9	53.7 b	46.7
<i>Tagetes erecta</i> (Marigold)	3.1 b	27.9	1656 b	37.6	103 b	35.2	8312 b	47.7	48.7 c	33.1
<i>Pseudomonas fluorescens</i>	2.2 cd	48.8	1300 d	51.0	68 c	57.2	5325 c	65.8	61.0 a	66.7
<i>Pseudomonas aeruginosa</i>	2.3 bc	46.5	1322 d	50.2	70 c	56.0	5600 c	64.1	60.7 a	65.8
Nematocide (Vydate 10 G)	1.5 d	65.1	1056 e	60.2	52 d	67.3	3288 d	78.9	53.5 b	46.2
Control	4.3 a	—	2653 a	—	159 a	—	15582 a	—	36.6 d	—

Values in the same column followed by the same letter(s) do not differ at  $P \leq 0.05$ .

\*CE = % change over control.

*M. javanica* J2 and *Pratylenchus vulnus*. They have also determined  $EC_{50}$  and  $EC_{90}$  values (percentage concentrations of plant material, 1g/10 ml basis) for *M. javanica* and *Pratylenchus vulnus*. Agbenin *et al.* (2005) have reported that extracts of neem leaf and garlic bulb completely inhibited hatching of egg masses of *M. incognita* and were lethal to larvae. Kaemptro and myricetin are the chemical components thought to be responsible for nematicidal properties of neem extracts (Qamar *et al.*, 1989). The nematicidal properties of garlic have been reported against root-knot nematodes (Sukul, 1992; Zasada *et al.*, 2002; Agbenin *et al.*, 2005). Korayem and Hasabo (1994) also reported that bulb extract of *Allium sativum* caused 100% immobility to *M. incognita* juveniles after 24 h of exposures. The volatile antimicrobial substance allicin produced in garlic is active against several plant pathogenic bacteria and fungi and suppressed plant diseases (Curtis *et al.*, 2004). The immobility of J2 may be induced by the active component allicin as suggested by Gupta and Sharma (1993).

Marigolds produce a number of potentially bioactive compounds including essential oils; however, it is the thiophenes, particularly  $\alpha$ -terthienyl, that are linked to the nematicidal properties of marigolds (Sivapalan, 1972; Alam *et al.*, 1979).

Bacterial antibiotics and other toxic compound present in metabolites as well as direct interaction might be responsible for the J2 immobility. Production of metabolites by rhizosphere bacteria causes lysis of nematode eggs and affects vitality of J2 of root-knot nematodes (Becker *et al.*, 1988; Westcott and Kluepfel, 1993; El-Sherif *et al.*, 1999; Son *et al.*, 2007). Siddiqui and Shaikat (2003) reported that production of metabolites, including 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN) by *Pseudomonas fluorescens* strains CHA0, inhibit egg hatch and induce mortality in *M. javanica* J2's.

Results from pot and field experiments indicated that addition of treatments with plant extracts, bacterial suspensions or Vydate into soil suppressed root galling and final populations of *M. incognita*, and

except for Vydate promoted plant growth and yield. Hasabo and Noweer (2005) found that soil treatment with aqueous extracts of marigold leaves and neem seeds significantly reduced *M. incognita* J2 in soil and roots of eggplants, J2 population in roots were reduced by 90% and 75% respectively, 4 months after treatments, applied @ 50 ml/plant as soil drench. Zasada *et al.* (2002) reported that aqueous extracts of *Allium sativum* (0.15%) and *Azadirachta indica* (1.6%) reduced root galling by 56% and 75% in potted soil, respectively when applied @ 300 ml extracts/pot, but high concentration of *A. sativum* and *A. indica* extracts were phytotoxic to plants. In present experiments there were no conspicuous adverse or phytotoxic effects of the treatments on tomato plants. Selection of the optimal application dose of plant extracts for management of root-knot nematodes is of primary importance. John and Hebsy (2000) observed that roots of aubergine dipped in neem leaf extract for an hour showed significantly better growth and reduced root galling and number of egg masses. Abbasi *et al.* (2005) observed 67-90% reduction in the number of *P. penetrans* and *M. hapla* in tomato roots grown in soil treated with 1% neem cake. The application of aqueous extracts from various parts of African marigold to rhizosphere significantly suppressed *M. incognita* populations and gall formation on tomato roots, and increased fruit yields (Natarajan *et al.*, 2006). Aqueous extracts of African marigold probably acted by suppressing egg hatch as found by Walia and Gupta (1979) in Petri dish study with aqueous extracts prepared from 30 and 60 days old marigold plants. Similarly, Javed *et al.* (2007) have reported that the protective and curative soil application of neem leaves and neem cake and a neem refined product "aza" significantly reduced the number of egg masses and number of eggs per egg mass

on tomato roots. The reduction in population of *M. incognita* in this investigation may be due to the accumulation of nematocidal components and/or to increase host resistance. The nematode control effects of plant products occur after incorporation into soil and during their decomposition, presumably due to the release of nematotoxic compounds (Stirling, 1991). Plant chemicals probably evolved as a natural defense against diseases and parasites.

In the present study, *P. fluorescens* and *P. aeruginosa* were less effective in *in vitro* assays than the plant extracts, but in pots and field conditions both strains were the most effective in suppressing root galling, nematode reproduction, and promotion of fruit yields. Direct antagonism to pathogens, antibiotic production, competitions with the pathogens for nutrients and induced systemic resistance or inhibition of the nematode's host-recognition process mechanisms in control of plant pathogens by pseudomonads (Gamliel and Katan, 1993). Siddiqui and Shaukat (2002, 2004) demonstrated that *P. fluorescens* induced systemic resistance in tomato roots against *M. javanica*. Growth-promoting effects of tomato plant by bacterial treatment may also result from the production of phytohormones that resulted in elongated stems and expanded root system (Davies, 1987).

Plant extracts and bacterial suspensions applied two and three times at 25 and 15 days intervals provided a reasonable level of nematode control and yield increase. Under the conditions of present experiments it is evident that both bacterial strains were equally effective and gave the best control (46-49% reduction in root galling) effect among all treatments in the soil, garlic and neem have also provided reasonable level of nematode disease control (30-33% reduction in root galling) and yield increase (33%). Thus the rhizobacteria and

plant extracts used have potential in crop management and can be used to reduce the deleterious impact of root-knot nematodes on plants under field conditions.

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