



# Host Status of Commonly Planted Ornamentals to *Meloidogyne incognita* and Management through Endophytic Bacteria

Ghulam Muhae-ud-Din<sup>1,4</sup>, Anam Moosa<sup>1</sup>, Umer Farooq Ghummen<sup>1</sup>, Muhammad Jabran<sup>1</sup>, Amjad Abbas<sup>1</sup>, Muhammad Naveed<sup>2</sup>, Abdul Jabbar<sup>1</sup> and Muhammad Amjad Ali<sup>1,3,\*</sup>

<sup>1</sup>Department of Plant Pathology, University of Agriculture, Faisalabad

<sup>2</sup>Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad

<sup>3</sup>Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad

<sup>4</sup>Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

## ABSTRACT

*Meloidogyne incognita* is the most dangerous plant parasitic nematode species that infects a huge number of crop plants. It also infects the ornamental plants resulting in a serious growth-limiting factor in ornamentals. In this study, the response of ten ornamental plants to *M. incognita* was assessed in pot experiments. All the ornamental plant species showed varying degree of infection of *M. incognita*. *Rhapis excels* and *Ophiopogon japonicus* were moderately resistant, *Cordyline fruticosa*, *Hibiscus syriacus* and *Carrisa macrocarpa* were moderately susceptible, *Murraya paniculata* and *Ficus benjamina* were susceptible, *F. elastica*, *Artemisia stelleriana* and *Duranta repens* were highly susceptible to *M. incognita* infection according to a well established 0-4 galling scale. The highest galling index and reproductivity of nematodes, i.e., total number of egg masses, juveniles and females, were observed in highly susceptible plants. The values of canopy length, dry root weight, fresh root weight, root length, dry shoot weight and shoot height was variable for all plants and the maximum canopy length was recorded in *C. macrocarpa*, while maximum fresh root weight, dry root weight, root length and shoot weight were recorded in *O. japonicus* and *R. excelsa*, respectively. The susceptible plants were subjected to the application of endophytic bacteria *Pantoea agglomerans* strain MN34 and *Pseudomonas putida* strain MN12 in a pot experiment. Both endophytes significantly decreased the galling index and promoted the growth of the plants root length, shoot weight and canopy length; however, the highest root weight was observed in the control treatment. *P. agglomerans* showed more reduction in galling index and increase in plant growth than *P. putida*. The results concluded that *M. incognita* has a wide host range in ornamental plants and they could be managed by using antagonistic, growth promoting endophytic bacteria.

## Article Information

Received 12 November 2017

Revised 06 February 2018

Accepted 06 March 2018

Available online 05 June 2018

## Authors' Contribution

MAA conceived and supervised the study. UFG and AJ planned and conducted study. GM and MJ helped in data collection and analysis. AM and AA reviewed and interpreted results. MN provided the bacterial strains and critically reviewed and interpreted results.

## Key words

Ornamental plants, Biocontrol, *M. incognita*, *P. agglomerans*, *P. putida*.

## INTRODUCTION

Ornamental plants hold high aesthetic value and make the environment better and clean. The climate of Pakistan is conducive to cultivate almost all important ornamental plants. Floriculture has emerged as an attractive business due to social, religious and medicinal benefits of flowers (Raghava and Dadlani, 1999). Plant parasitic nematodes are one of the major limitations to the growth and proliferation of ornamental plants. Root-knot nematodes (RKNs), *Meloidogyne* spp. (Chitwood, 1949), are a serious yield limiting threat to various crops, in particular, under tropical and sub-tropical climates (Koenning *et al.*, 2004).

RKNs are economically important group of sedentary plant parasitic nematodes that cause high yield loss of cultivated crops worldwide, predominantly in developing countries (Sasser, 1979; Sasser and Carter, 1985; Netscher and Sikora, 1990; Ali *et al.*, 2017a). Seasonal ornamental plants are highly susceptible to the attack of root-knot nematodes (Sasser, 1989). The amount of damage by nematodes primarily depends upon the population density, species, host type and environmental factors (Mitkowski and Abawi, 2003). However, the impact of plant parasitic nematode on ornamental plants and other crops remained obscure due to their tiny size, soil-borne nature, enigmatic life cycle and delusive signs of feeding on the plants. Among various plant parasitic nematodes affecting the ornamental plants, root knot nematodes and stunt nematodes (*Tylenchorhynchus* spp. and *Xiphinema* spp.) are the most deleterious pests (Anwar and van Gundy, 1989). These nematodes attack the root system of

\* Corresponding author: [amjad.ali@uaf.edu.pk](mailto:amjad.ali@uaf.edu.pk), [amjad.ali2001@gmail.com](mailto:amjad.ali2001@gmail.com)  
0030-9923/2018/0004-1393 \$ 9.00/0  
Copyright 2018 Zoological Society of Pakistan

plants and hinder the uptake of water, minerals and other essential nutrients from the soil (Walia and Bajaj, 2003; Ali *et al.*, 2015, 2017b).

High yield loss due to plant parasitic nematodes has provoked the search for most reliable and effective management strategies. To date, application of nematicides is the best way to control nematodes. However, nematicides have inflicted a damaging effect on beneficial soil microbes, human health and surroundings in addition to plant growth and metabolic systems (Duncan, 1991; Sharma, 1977). It emphasizes the need to find reliable and eco-safe alternative management options. The use of growth promoting antagonistic bacteria is an environment friendly approach to control plant parasitic nematodes (Chen and Dickson, 1998; reviewed by Ali *et al.*, 2017a). Numerous studies have previously reported the potential of endophytic bacteria to suppress plant parasitic nematodes (Weller, 1988; Emmert and Handelsman, 1999; Munif *et al.*, 2000; Vetrivelkai *et al.*, 2010). Hallmann *et al.* (1998) indicated the positive colonization of *Pseudomonas fluorescens* 89B-61 and *Enterobacter asburiae* JM22 on *M. incognita* infected roots of cotton (*Gossypium hirsutum* L.) and cucumber (*Cucumis sativus* L.) roots. *Pantoea agglomerans* MK-29, *Pseudomonas putida* MT-19, *Cedeca davisae* MK-30 and *Enterobacter* spp. MK-42 significantly reduced the infection of *M. incognita* on tomato (*Lycopersicon esculentum* Mill.) (Munif *et al.*, 2001). The number of infective juveniles of *M. incognita* and root galling was significantly reduced in treated plants. The study indicates the possible role of systemically induced resistance in the biocontrol of *M. incognita*. Isolates from *Pseudomonas* and *Streptomyces* showed biocontrol activity against *M. incognita* (Krechel *et al.*, 2002). Bacterial antagonism is mainly due to production of antibiotics and toxins, competition and the production of cell wall degrading enzymes (Chet *et al.*, 1990). In addition to bacteria, nematophagous fungi have largely been used to control *Meloidogyne* spp. (Hussain *et al.*, 2017a, b). Endophytic bacteria have beneficial effect on plants such as plant growth promotion, supply of nutrients, less exposure to environmental stress and antagonistic control of plant pathogens (Hallmann *et al.*, 1997; Downing and Thomson, 2000; Ashikari *et al.*, 2001).

In the current report, we have studied the host status of selected ornamentals to *M. incognita*. *M. incognita* has a diverse host range in fruits, vegetables, cereal crops and ornamentals. Moreover, we have further investigated the potential of endophytic bacteria *P. Agglomerans* strain MN34 and *Pseudomonas putida* strain MN12 (Naveed, 2013) to analyze the infestation of *M. incognita* in the roots of ornamental plants. *P. agglomerans* has been reported to be an effective antagonistic agent due to competitive

colonization of plants and production of antimicrobial compounds (Stockwell *et al.*, 2002; Poppe *et al.*, 2003). *P. putida* is a ubiquitous endophyte that produces a variety of antimicrobial secondary metabolites against numerous plant pathogens. Guo *et al.* (2016) recently reported the inhibition of *M. incognita* by *P. putida*. Hence, the project was designed to check the antagonistic effect of these endophytes on *M. incognita* infection in ornamentals.

## MATERIALS AND METHODS

### Experimental site and ornamental plants

The experimental site was Research area of Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. Ten species of ornamental plants were selected for this study (Table I). These ten plant species are the most commonly planted in the gardens and for the ornamental purposes throughout the country. The plant species were taken from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan.

**Table I.- Galling index and reaction of ornamental plants to *Meloidogyne incognita* infection.**

Plant species	Common name	Gall index <sup>b</sup>	Reaction <sup>b</sup>
<i>Ficus elastica</i>	Ficus Rubber	39.8 a <sup>a</sup>	HS
<i>Artemisia stelleriana</i>	Silver Spoon	34.4 b	HS
<i>Duranta repens</i>	Golden Duranta	33.2 b	HS
<i>Ficus benjamina</i>	Ficus Starlight	17.8 d	S
<i>Murraya paniculata</i>	Marwa Chinese	24.8 c	S
<i>Cordyline fruticosa</i>	Red Dracaena	7.8 e	MS
<i>Hibiscus syriacus</i>	Hibiscus	5.2 ef	MS
<i>Carrisa macrocarpa</i>	Stuff Karonda	9.4 e	MS
<i>Ophiopogon japonicus</i>	Dwarf lilyturf	1.8 f	MR
<i>Rhapis excels</i>	Lady Finger Palm	1.4 f	MR

<sup>a</sup>Mean values with same letters are not significantly different from each other analyzed by Fischer Protected LSD at  $P \leq 0.05$ , Mean values average of 5 replicates. <sup>b</sup>0 gall = resistant (R); 1-2 galls = moderately resistant (MR); 3-10 galls = moderately susceptible (MS); 11-30 galls = susceptible (S); 31 galls and above = highly susceptible (HS).

### Culture preparation of endophytic bacteria

Selected endophytic bacterial strains MN34 (*P. agglomerans*) and MN12 (*P. putida*) were previously isolated from the rhizosphere of maize (*Zea mays* L.) and were evaluated for improving growth and yield of maize (Naveed, 2013). The culture was prepared in 10% of Tryptic Soy Broth (TSB) in 1 L of sterilized water and incubated for 72 h at 28°C (Naveed, 2013). The culture was grown to an optical density (OD) of 0.5 at 600 nm through dilution in double distilled water to maintain uniform cell density ( $10^7$ - $10^8$  CFU mL<sup>-1</sup>) using a UV-visible spectrophotometer

T60 (PG Instruments Limited, Leicestershire, UK).

#### *Extraction and mass culturing of M. incognita*

*M. incognita* (J2) infective juveniles were isolated from infected tomato plants and their rhizosphere by modified Whitehead and Hemming Tray method (Whitehead and Hemming, 1965). Infected tomato samples were collected from Department of Plant Pathology, University of Agriculture, Faisalabad and Ayub Agriculture Research Institute, Faisalabad, Pakistan. Mature females were identified as *M. incognita* through perineal pattern (Hartman and Sasser, 1985). Tomato cv. Money Maker 3-week-old seedlings were taken from Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. The seedlings were transplanted into earthen pots filled with sterilized sandy loam soil. After one week of transplanting, each plant was inoculated with 1000 freshly hatched 2<sup>nd</sup> stage juveniles (J2s) of *M. incognita*. The pots were kept in greenhouse at 22-25°C and irrigated daily. Eight to nine-week-old infected tomato seedlings were washed gently with clean water to remove the debris. The roots were shredded into 2-3 cm pieces and shaken vigorously for 3-4 min in a plastic jar with a tightly fitting lid, containing 200 mL of 0.5% sodiumoxychloride (NaOCl) to dissolve the gelatinous matrix and to release *M. incognita* eggs. Care was taken that the eggs should not be exposed to more than 1% concentration of NaOCl for no longer than few seconds. The eggs were rinsed with clean water for a few minutes on clean mesh of 150 µm and 25 µm pore size to release freshly hatched juveniles. To obtain J2s, the egg masses were incubated for 3-4 d at 28°C. Infective juveniles (J2s) were collected after every 24 h on clean mesh of 150 µm and 25 µm pore size for a week. During the collection period the infective juveniles were stored in an aqueous suspension at 5°C to further inoculate ornamental plants.

#### *Counting and standardization of M. incognita inoculum*

The inoculum density of *M. incognita* was calculated. The extracted suspension was poured in a measuring cylinder and mixed thoroughly. The number of J2s was estimated in 3×1 mL aliquots in a counting dish under a dissecting microscope at X3.5 magnification. The mean of three replications was multiplied with total volume to assess the total population density. It was difficult to count the nematodes in 1 mL suspension due to high density of nematodes; 0.25-0.5 mL nematode suspension was diluted with 1-2 mL distilled water for population estimate.

#### *Pot experiments*

##### *Host range assessment of ornamental plants*

Two-month-old ornamental plants, washed clean of

soil, were transferred to earthen pots containing sterilized soil. The plants were inoculated after 1 week of transplant by applying 1000 egg/pot of *M. incognita* at four equidistant holes of 4 cm depth made around the root zone of the plant. For each ornamental plant one treatment was infected and the other treatment was uninfected control to compare the response of the plants against *M. incognita* infection. The experiment was a completely randomized arrangement of treatments. The plants were kept in a greenhouse and irrigated with clean water once or twice a week depending upon the temperature. The assessment of nematode development on plants was carried out after 8 weeks for 5 replicates of each treatment. The criteria used for the assessment of infection was the gall index scale of Taylor and Sasser (1978) which states that; 0 = 0 gall, resistant; 1 = 1-2 galls, moderately resistant; 2 = 3-10 galls, moderately susceptible; 3 = 11-30 galls, susceptible; and 4 = 31 galls and above, highly susceptible. To assess root galling, number of galls/root system was counted and rated on gall index scale. The roots were stained in 0.1% acid fuchsin solution (acid fuchsin dissolved in a 1:1:1 mixture of glycerol, lactic acid and distilled water) for 1-3 min prior to counting number of egg masses/root system. The egg masses were stained bright red and the excess stain was removed by dipping the roots in a beaker filled with tap water and a dip in clearing solution (50:50, glycerol and distilled water, acidified with few drops of acetic acid). The roots were chopped into small pieces, transferred into clearing solution in a glass tube and macerated using a silver son laboratory homogenizer at maximum speed for 30 s to count the number of nematodes. To count the number of mature females the shredded roots were placed in two folds of tissue paper to prevent quick drying. The roots were stained for 15-20 min in phloxine B (15 mg L<sup>-1</sup>) (Hartman and Sasser, 1985). The shredded roots were placed in a plastic dish (15 cm in diameter) and the total number of egg masses was counted by using dissecting microscope. Similarly, to estimate the number of J2s, infected roots were kept in a funnel containing sterilized water for 5 d. The nematodes moved into the water at the lower side of the funnel. Few aliquots of water were collected in petri plates and the number of J2s was counted under a stereomicroscope. Growth parameters, i.e., dry root weight (g), fresh root weight (g), dry shoot weight (g), root length (cm), shoot height (cm) and canopy length (cm) were recorded for all ornamental plants in different treatments.

##### *Co-inoculation of selected ornamental species with endophytic bacteria and nematodes*

Four susceptible ornamental plants, i.e., *M. paniculata*, *D. repens*, *A. stelleriana* and *F. elastic* obtained from resistance assessment of ornamental plants in the first

pot experiment were selected for this experiment. Two-month-old ornamental plants were cultivated 1 plant per pot in earthen pots containing sterilized sandy loam soil. *P. agglomerans* and *P. putida* cultures 25 mL each containing  $10^7$  CFU mL<sup>-1</sup> concentration was applied to the soil by making four equidistant holes of 4 cm in depth around the collar region of the plants. After 15 d of bacterial inoculation *M. incognita* inoculum at the rate of 1000 eggs pot<sup>-1</sup> was distributed in the holes around the root zone of the plants. Plants were irrigated with clean water once or twice a week depending upon the temperature. The experiment was a factorial arrangement of treatments in a completely randomized block design including with 5 replications, the positive and negative controls, 1) treated plants (plants treated with *P. agglomerans* or *P. putida* and infected with *M. incognita*) or 2) control plants (plants infected with *M. incognita* only). The plants were harvested after 60 d. The galling index was calculated following the gall index scale of Taylor and Sasser (1978). To calculate number of galls, the plants were gently taken out of the pots. To calculate number of egg masses, roots were washed with clean water and stained in 0.1% acid fuchsin solution (acid fuchsin dissolved in a 1:1:1 mixture of glycerol, lactic acid and distilled water) for 1-3 min. Growth parameters canopy length (cm), plant height (cm), root length (cm) and root weight were also recorded.

#### Statistical analysis

Data from both trials was subjected to statistical analysis using the statistical software Statistix (Ver. 8.1) (available at <https://www.statistix.com/>). The mean values of the treatments were analyzed with Fischer protected LSD and Tukey HSD tests at 5% ( $P \leq 0.05$ ). The significance of data for resistance assessment of ornamental plants and growth parameters was tested by Fischer Protected LSD test at 5% ( $P \leq 0.05$ ). Significant and non-significant interactions from the screening of endophytic bacteria on ornamental plant species were used to explain the results and the significance of treatment groups was tested by Tukey's HSD test at 5% ( $P \leq 0.05$ ).

## RESULTS

Host status of the 10 ornamental plant species assessed through pot trial indicated that all plants were host to *M. incognita* with varying degree of infection (Table I). Maximum galling was observed in *F. elastica* followed by *A. stelleriana*, and *D. repens*, while least was observed in *R. excelsa* at  $P \leq 0.05$ . The reaction of two ornamentals *R. excelsa* and *O. japonicus* was moderately resistant (MR), *C. fruticosa*, *H. syriacus* and *C. macrocarpa* were moderately susceptible (MS), *M. paniculata* and *F. benjamina* were

susceptible (S), *F. elastica*, *A. stelleriana* and *D. repens* showed highly susceptible (HS) reaction to *M. incognita* infection (Table I). Nematode infection on various plant species was also assessed through total number of females, total egg masses and total juveniles. The reproduction parameters of *M. incognita*, i.e., total number of egg masses, females and juveniles, were highest in highly susceptible ornamental plants, i.e., *F. elastica*, *A. stelleriana* and *D. repens* (Table II). Moderately resistant plants *R. excelsa* and *O. japonicus* showed great reduction in reproduction of *M. incognita* ( $P \leq 0.05$ ). The plant species with high degree of infection revealed higher reproduction potential; however, the plant species with low infection showed less reproduction potential of *M. incognita*. The galling index and reproductive parameters of *M. incognita* showed significant differences in all ornamentals  $P \leq 0.05$ . The effect of *M. incognita* infection on growth parameters of all ornamental plant species showed significant variation in the growth of various plant species. The canopy length (cm), dry root weight (g), fresh root weight (g), root length (cm), shoot height (cm) and dry shoot weight (g) were variable for all the ornamentals studied (Table III). All ornamental plant species were different from each other in growth habit. However, superior growth rate was recorded in *C. macrocarpa*, *O. japonicus* and *R. excelsa* respectively, while least growth was observed in *M. paniculata*. All ornamental plant species showed significantly different growth parameters at  $P \leq 0.05$ . The plant growth parameters were independent of the degree of resistance or susceptibility.

**Table II.- Reproduction of *Meloidogyne incognita* on different ornamental plants in pot experiment.**

Plant species	Total females	Total egg masses	Total juveniles
<i>Ficus elastica</i>	119.4 a <sup>a</sup>	159.2 a	2895 a
<i>Artemisia stelleriana</i>	103.2 a	137.6 b	2580 ab
<i>Duranta repens</i>	103.2 a	137.8 b	2490 b
<i>Ficus benjamina</i>	53.4 b	71.2 d	1335 d
<i>Murraya paniculata</i>	74.4 b	99.2 c	1860 c
<i>Cordyline fruticosa</i>	23.4 cd	31.2 e	540 e
<i>Hibiscus syriacus</i>	15.6 cd	20.8 ef	390 ef
<i>Carrisa macrocarpa</i>	28.2 c	37.6 e	705 e
<i>Ophiopogon japonicus</i>	5.2 d	7.2 f	135 f
<i>Rhapis excelsa</i>	4.2 d	5.6f	105 f
LSD ( $P \leq 0.05$ )	13.57	18.10	331.96

<sup>a</sup>Mean values with same letters are not significantly different from each other analyzed by Fischer Protected LSD at  $P \leq 0.05$ , Mean values average of 5 replicates.

**Table III.- Effect of *Meloidogyne incognita* on growth parameters of ornamental plants.**

Plant species	Canopy (cm)	Dry root weight (g)	Fresh root weight (g)	Root length (cm)	Dry shoot weight (g)	Shoot height (cm)
<i>Ficus elastic</i>	20.2 e <sup>a</sup>	13.2 cd	21.0 e	22.6 de	47.1 c	19.4 bc
<i>Artemisia stelleriana</i>	34.4 b	11.2 df	20.4 e	25.8 cd	25.4 e	19.6 bc
<i>Duranta repens</i>	26.2 cd	10.2 ef	19.9 ef	18.0 ef	22.6 ef	21.0 ab
<i>Ficus benamina</i>	26.0 cd	12.5 cd	25.4 d	21.2 df	17.0 fg	21.2 ab
<i>Murraya paniculata</i>	21.0 e	7.4 g	14.0 f	12.2 g	12.4 g	24.2 a
<i>Cordyline fruticosa</i>	27.4 c	11.1 df	22.5 de	29.2 c	17.9 fg	16.8 c
<i>Hibiscus syriacus</i>	22.8 de	14.8 c	33.4 c	46.0 b	59.0 b	18.0 bc
<i>Carrisma macrocarpa</i>	61.4 a	9.0 fg	16.6 f	17.0 fg	28.8 e	23.8 a
<i>Ophiopagan japonicas</i>	20.0 e	40.8 a	63.0 a	52.0 a	35.8 d	7.20 d
<i>Rhapis excels</i>	30.0 c	23.2 b	44.4 b	19.2 ef	78.4 a	16.2 c
LSD ( $P \leq 0.05$ )	4.12	2.45	3.42	4.99	6.74	3.93

<sup>a</sup>Mean values with same letters are not significantly different from each other analyzed by Fischer protected LSD at  $P \leq 0.05$ , Mean values average of 5 replicates.

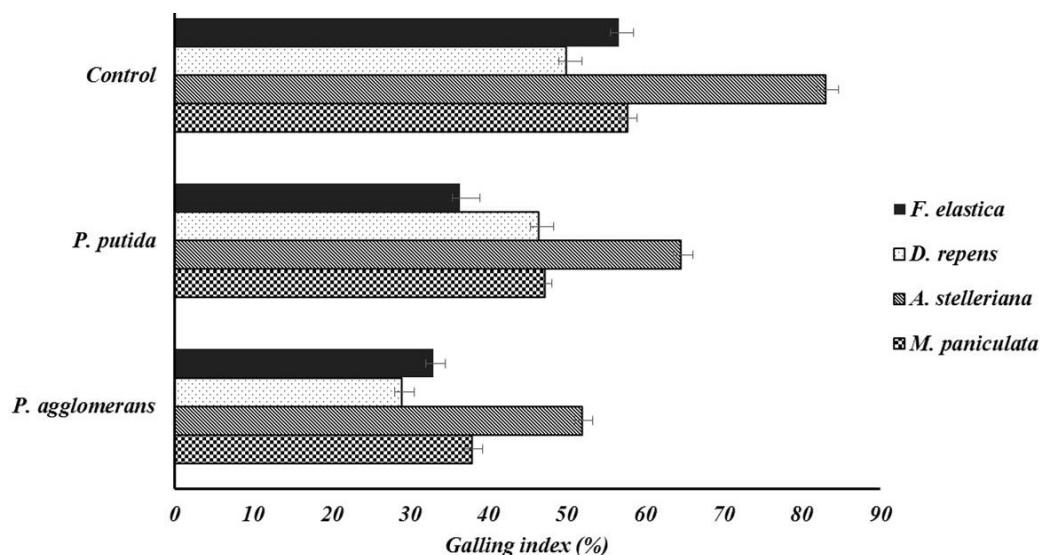


Fig. 1. Effect of endophytic bacteria *P. agglomerans* and *P. putida* on root gall index of *M. incognita* in susceptible ornamental plants *M. paniculata*, *D. repens*, *A. stelleriana* and *F. elastica*. Mean values analyzed by Tukey's HSD test at  $P \leq 0.05$ . Mean values average of 5 replicates.

The effect of endophytic bacteria *P. agglomerans* and *P. putida* on susceptible ornamental plants *M. paniculata*, *D. repens*, *A. stelleriana* and *F. elastic* was assessed in a pot experiment. Plant species (P) and endophytic bacteria (B) alone and their interaction P×B indicated significant effect on galling index at  $P \leq 0.05$ . Growth parameters canopy length (cm), root weight (g), shoot weight (g) and root length of the four ornamental plants were also significantly affected by type of plant (P) and endophytic bacterium (B) alone. The interaction P×B affected the canopy length (cm) and root weight (g) of the plants, while, the root length (cm) and shoot weight (g) were not affected at  $P \leq 0.05$ . Both *P. agglomerans* and *P. putida* were effective

to reduce the infection of *M. incognita*. However, higher reduction in galling index was observed by *P. agglomerans* in *M. paniculata*, *D. repens*, *A. stelleriana* and *F. elastic* (Fig. 1). *P. agglomerans* and *P. putida* had a growth promoting effect on the plants. The canopy length (cm), shoot weight (g) and root length (cm) was highly enhanced in *P. agglomerans* and *P. putida* treated plants compared to untreated control. *P. agglomerans* showed higher growth rate of the plants compared to control at  $P \leq 0.05$  (Fig. 2). The root length was maximum in *P. agglomerans* and *P. putida* treated plants, while the root weight was maximum in the control plants with least length.

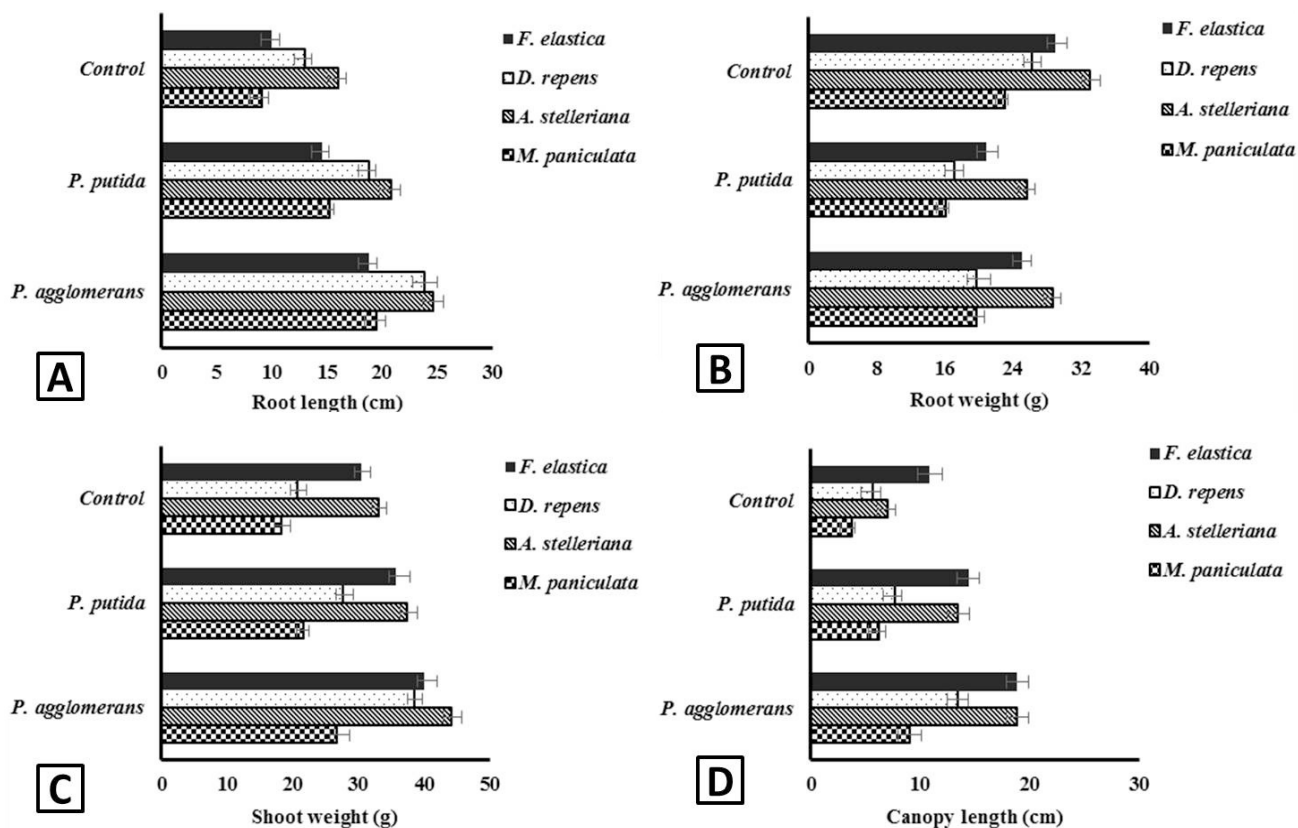


Fig. 2. Effect of endophytic bacteria *P. agglomerans* and *P. putida* on growth parameters (A) root length (cm), (B) root weight (g), (C) shoot weight (g) and (D) canopy (cm) total root length (cm) of susceptible ornamental plants *M. paniculata*, *D. repens*, *A. stelleriana* and *F. elastica* infected by *M. incognita*. Mean values analyzed by Tukey's HSD test at  $P \leq 0.05$ . Mean values average of 5 replicates.

## DISCUSSION

The first approach of this study was to study the host status of ornamental plant species commonly planted in Pakistan to *M. incognita*. The observations of the study clearly indicate the potential of *M. incognita* to infect the ornamental host plants included in this study and further confirmed the diverse host range of *M. incognita*. Ten ornamental plants were screened for their host status to *M. incognita*. It was evident from the observation that all plants were favorable for the infection of *M. incognita* and showed varying degree of infection. Susceptible plants had a high gall index of *M. incognita*. Previously, various reports have stated the infectivity of *M. incognita* on ornamental plants and confirmed the host status of many ornamental species. Siddiqui *et al.* (2014) recorded five ornamental hosts, *i.e.*, *Papaver somniferum*, *Chrysanthemum morifolium*, *Dianthus caryophyllus*, *Calendula officinalis* and *Centaurea montana* of *M. incognita*. Shazad *et al.* (2011) also reported the susceptibility of two perennial

ornamentals *Alternanthera dentata* cv. Brazilian-Red-Hot and *Iresine herbstii* cv. Brilliantissima to *M. incognita*. Similarly, Salawu and Darabidan (2010) reported that *Hibiscus*, *Duranta* and *Cordyline* spp. were susceptible to *M. incognita* in conformity with the present finding. *M. incognita* and other *Meloidogyne* spp. were isolated and identified from plants of *Duranta*, *Ficus*, *Ophiopogon* and *Hibiscus* genera (Brito *et al.*, 2010). *D. repens* and *Carrisa macrocarpa* were reported to be infected by *M. javanica* (El-Sherbiny, 2011). The galling index and reproductivity of nematodes was higher with increasing susceptibility of the plants. The results are in accordance with Siddiqui *et al.* (2014), who reported that the galling index and reproductive parameters were higher in susceptible plant species compared to the resistant ones. Shazad *et al.* (2011) also observed high galling and reproductivity of *M. incognita* in susceptible ornamental plants. Increasing susceptibility of ornamental plants in the present study has resulted in high reproductivity and galling index of nematodes, hence, aggravating the infection. The results

have indicated that susceptibility of ornamentals was directly proportional to infectivity of *M. incognita*.

The management approach used in this study was to test the effect of endophytic bacteria as biocontrol agents to manage *M. incognita* and growth promotion of the plants. The identification of bacterial endophytes with broad spectrum and effective control of *M. incognita* infection in ornamental plants is highly required for biocontrol of this problem. Endophytes live inside the plants without causing any deleterious effect to plant health, but some endophytes provide direct or indirect benefits to their host plants (Hardoim *et al.*, 2008; Shoresh *et al.*, 2010). Endophytes *P. agglomerans* strain MN34 and *P. putida* strain MN12 tested in the pot experiment indicated suppressive effect on *M. incognita* and promoted growth of treated plants. Antibiosis or production of toxic compounds is more likely to be the principle mechanism involved in the control of *M. incognita*. For instance, hydrogen cyanide (HCN) is produced by several bacterial strains like *Pseudomonas* spp. (Naveed, 2013). Endophytic bacteria produce a variety of diffusible compounds (Weller, 2007; Naveed, 2013). HCN is a volatile compound produced by *Pseudomonas* spp. (Paul and Sharma, 2006). They also compete with other microorganisms for food and space, hence, suppressing the growth of other organisms, *i.e.*, root knot nematodes, in this study.

*P. agglomerans* and *P. putida* showed growth promoting effect on plants. Both endophytes significantly increased root length (cm), shoot weight (g) and canopy (cm) of the ornamental plants and decreased the galling index, number of females, number of egg masses and number of juveniles of *M. incognita*. The root weight was maximum 23, 33, 26.2 and 29 g, while root length was least 9, 6, 13 and 10 cm in the control plants of *M. paniculata*, *A. stelleriana*, *D. repens* and *F. elastica*, respectively. It may be attributed to the fact that *M. incognita* produced the highest number of galls on the control plants that resulted in increased weight of the plants. The result of screening of antagonistic bacteria is in line with the earlier findings (Akhtar, 1993; Siddiqui and Shaukat, 2003, 2004; Xin *et al.*, 2009; Khan *et al.*, 2012, 2015; Rogers *et al.*, 2012; Knoth *et al.*, 2013) stating the growth promoting effect of endophytes and reduction in galling index of plant parasitic nematodes. These results are also supported by Racke and Sikora (1992), who reported that the application of endophytic bacteria increased the canopy length, fresh shoot weight, dry shoot weight, shoot height and root length of the ornamental plants. This increase in growth might be attributed by high production of auxin (by these bacterial strains) that is already reported that MN34 strain is hyper-producer of auxin (Naveed, 2013). Benson and Barker (1982) described that ornamental plants that are affected by

the plant parasitic nematodes showed stunted growth as compared to un-affected plants. Similarly, several reports have stated that bacterial endophytes enhance plant growth by absorbing more nutrients from the soil and suppress the growth of plant parasitic nematodes (Sturz *et al.*, 2000; Feng *et al.*, 2006). Moens *et al.* (2009) described that the endophytic bacteria inhibit the entry of phytopathogenic nematodes into the root zone of plants. Hence, it is evident from previous reports and present findings that endophytic bacteria increase plant growth and decrease the infection of *M. incognita* in the plants.

## CONCLUSIONS

The findings of the present study confirms the host status of ten commonly planted ornamentals to *M. incognita* with varying degree of infection. It indicates that *M. incognita* have a diverse host range in ornamentals. It is also concluded that the application of bacterial endophytes *P. agglomerans* and *P. putida* provides a less damaging, reliable option for controlling plant-parasitic nematodes and increases the plant growth without any detrimental effects on the environment.

## ACKNOWLEDGEMENTS

The authors are thankful to Higher Education Commission of Pakistan for financial support through grant number HEC/HRD/SRG/2014/828.

### Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

## REFERENCES

- Akhtar, M. and Alam, M.M., 1993. Control of plant-parasitic nematodes by Nimin - an urea coating agent and some plant oils. *Z. Pflanzenkr. Pflanzenschutz.*, **100**: 337-372.
- Ali, M.A., Abbas, A., Azeem, F., Javed, N. and Bohlmann, H., 2015. Plant-nematode interactions: from genomics to metabolomics. *Int. J. Agric. Biol.*, **17**: 1071-1082. <https://doi.org/10.17957/IJAB/15.0037>
- Ali, M.A., Azeem, F., Abbas, A., Joiya, F.A., Li, H. and Dababat, A.A., 2017a. Transgenic strategies for enhancement of nematode resistance in plants. *Front. Pl. Sci.*, **8**: 750. <https://doi.org/10.3389/fpls.2017.00750>
- Ali, M.A., Azeem, F., Li, H. and Bohlmann, H., 2017b. Smart parasitic worms use multifaceted strategies

- to parasitize plants. *Front. Pl. Sci.*, **8**: 1699. <https://doi.org/10.3389/fpls.2017.01699>
- Anwar, S. and van Gundy, S., 1989. Influence of four nematodes on root and shoot growth parameters in grape. *J. Nematol.*, **21**: 276.
- Ashikari, T.B., Joseph, C.M., Yang, G., Phillips, D.A. and Nelson, L.M., 2001. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling diseases of rice. *Can. J. Microbiol.*, **47**: 916-924. <https://doi.org/10.1139/w01-097>
- Benson, D.M. and Barker, K.R., 1982. Susceptibility of Japanese boxwood, dwarf gardenia, Compacta (Japanese)holly, Spiny Greek and Blue Rugjunipers, and nandina to four nematodes species. *Pl. Dis.*, **66**: 1176-1179. <https://doi.org/10.1094/PD-66-1176>
- Brito, J.A., Kaur, R., Cetintas, R., Stanley, J.D., Mendes, M.L., Powers, T.O. and Dickson, D.W., 2010. *Meloidogyne* spp. infecting ornamental plants in Florida. *Nematropica*, **40**: 87.
- Chen, Z. and Dickson, D., 1998. Review of *Pasteuriapenetrans*: Biology, ecology, and biological control potential. *J. Nematol.*, **30**: 313-340.
- Chet, I., Ordentlich, A., Shapira, R. and Oppenheim, A., 1990. Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria. *Pl. Soil.*, **129**: 85-92. <https://doi.org/10.1007/BF00011694>
- Chitwood, B.G., 1949. Root knot nematode. Part I. A revision of genus *Meloidogyne goeldi* (1887). *Proc. Helminthol. Soc. Wash.*, **16**: 90-104.
- Downing, K.J., and Thomson, J.A., 2000. Introduction of the *Serratia marcescens chiA* gene into an endophytic *Pseudomonas fluorescens* for the biocontrol of phytopathogenic fungi. *Can. J. Microbiol.*, **46**: 363-369. <https://doi.org/10.1139/w99-147>
- Duncan, L.W., 1991. Current options for nematode management. *Annu. Rev. Phytopathol.*, **29**: 467-490. <https://doi.org/10.1146/annurev.py.29.090191.002345>
- El-Sherbiny, A.A., 2011. Phytoparasitic nematodes associated with ornamental shrubs, trees and palms in Saudi Arabia, including new host records. *Pak. J. Nematol.*, **29**: 28-45.
- Emmert, E.A. and Handelsman, J., 1999. Biocontrol of plant disease: A (Gram-) positive perspective. *FEMS Microbiol. Lett.*, **171**: 1-9. <https://doi.org/10.1111/j.1574-6968.1999.tb13405.x>
- Feng, Y., Shen, D. and Song, W., 2006. Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. *J. appl. Microbiol.*, **100**: 938-945. <https://doi.org/10.1111/j.1365-2672.2006.02843.x>
- Guo, J., Jing, X., Peng, W.L., Nie, Q., Zhai, Y., Shao, Z., Zheng, L., Cai, M., Li, G., Zuo, H. and Zhang, Z., 2016. Comparative genomic and functional analyses: Unearthing the diversity and specificity of nematocidal factors in *Pseudomonas putida* strain 1A00316. *Scient. Rep.*, **6**: 29211. <https://doi.org/10.1038/srep29211>
- Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper, J.W., 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.*, **43**: 895-914. <https://doi.org/10.1139/m97-131>
- Hallmann, J., Quadt-Hallmann, A., Rodriguez-Kabana, R. and Kloepper, J.W., 1998. Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber. *Soil Biol. Biochem.*, **30**: 925-937. [https://doi.org/10.1016/S0038-0717\(97\)00183-1](https://doi.org/10.1016/S0038-0717(97)00183-1)
- Hardoim, P.R. van Overbeek, L.S. and van Elsas, J.D., 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.*, **16**: 463-471. <https://doi.org/10.1016/j.tim.2008.07.008>
- Hartman, K.M. and Sasser, J.N., 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: *An advanced treatise on Meloidogyne, Vol. II, Methodology* (eds. K.R. Barker, C.C. Carter and J.N. Sasser). North Carolina State University Graphics, Raleigh, pp. 69-77.
- Hussain, M., Zouhar, M. and Rysanek, P., 2017a. Population dynamics of a nematophagous fungus *Lecanicillium muscarium*, and root knot nematode, *Meloidogyne incognita* to assess the disease pressure and its management. *Pakistan J. Zool.*, **49**: 207-214.
- Hussain, M., Zouhar, M. and Rysanek, P., 2017b. Comparison between biological and chemical management of root knot nematode, *Meloidogyne hapla*. *Pakistan J. Zool.*, **49**: 215-220.
- Khan, Z., Guelich, G., Phan, H., Redman, R. and Doty, S., 2012. Bacterial and yeast endophytes from poplar and willow promote growth in crop plants and grasses. *ISRN Agron.*, **2012**: 890280. <https://doi.org/10.5402/2012/890280>
- Khan, Z., Kandel, S.L., Ramos, D.N., Ettl, G.J., Kim, S.H. and Doty, S.L., 2015. Increased biomass of nursery-grown Douglas-fir seedlings upon inoculation with diazotrophic endophytic consortia. *Forests*, **6**: 3582-3593. <https://doi.org/10.3390/f6103582>



- Knoth, J. L., Kim, S.H., Ettl, G.J. and Doty, S.L., 2013. Effects of cross host species inoculation of nitrogen-fixing endophytes on growth and leaf physiology of maize. *Glob. Change Biol. Bioener.*, **5**: 408-418. <https://doi.org/10.1111/gcbb.12006>
- Koenning, S.R., Wrather, J.A., Kirkpatrick, T.L., Walker, N.R., Starr, J.L. and Mueller, J.D., 2004. Plant-parasitic nematodes attacking cotton in the United States: Old and emerging production challenges. *Pl. Dis.*, **88**: 100-113. <https://doi.org/10.1094/PDIS.2004.88.2.100>
- Krechel, A., Faupel, A., Hallmann, J., Ulrich, A. and Berg, G., 2002. Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. *Can. J. Microbiol.*, **48**: 772-786. <https://doi.org/10.1139/w02-071>
- Mitkowski, N.A. and Abawi, G.S., 2003. Reproductive fitness on lettuce of populations of *Meloidogyne hapla* from New York State vegetable fields. *Nematology*, **5**: 77-83. <https://doi.org/10.1163/156854102765216713>
- Moens, M., Perry, R.N. and Starr, J.L., 2009. *Meloidogyne* species—a diverse group of novel and important plant parasites. *Root-Knot Nematol.*, **1**: 482-483. <https://doi.org/10.1079/9781845934927.0001>
- Munif, A., Hallmann, J. and Sikora, R.A., 2000. Evaluation of the biocontrol activity of endophytic bacteria from tomato against *Meloidogyne incognita*. *Mededel. Facul. Landbouwk. Toegepaste Biol. Wetensch. Univ. Gent.*, **65**: 471-480.
- Munif, A., Hallmann, J. and Sikora, R.A., 2001. Induced systemic resistance of selected endophytic bacteria against *Meloidogyne incognita* on tomato. *Mededel. Facult. Landbouwk. Toegepaste Biol. Wetensch. Univ. Gent.*, **66**: 663-669.
- Naveed, M., 2013. *Maize endophytes—diversity, functionality and application potential*. Ph.D. thesis, Austrian Institute of Technology, BOKU, Tulln, Austria.
- Netscher, C. and Sikora, R.A., 1990. Nematode parasite on vegetables. In: *Plant parasitic nematode in subtropical and tropical agriculture* (eds. M. Luc, J. Bridge and R.A. Sikora). CAB International, Wallingford, UK. pp. 237-284.
- Paul, D. and Sarma, Y.R., 2006. Antagonistic effects of metabolites of *Pseudomonas fluorescens* strains on the different growth phases of *Phytophthora capsici*, foot rot pathogen of black pepper (*Piper nigrum* L.). *Arch. Phytopathol. Pl. Prot.*, **39**: 113-118. <https://doi.org/10.1080/03235400500301182>
- Poppe, L., Vanhoutte, S. and Höfte, M., 2003. Modes of action of *Pantoea agglomerans* CPA-2, an antagonist of postharvest pathogens on fruits. *Eur. J. Pl. Pathol.*, **109**: 963-973. <https://doi.org/10.1023/B:EJPP.0000003747.41051.9f>
- Racke, J. and Sikora, R.A., 1992. Isolation, formulation and antagonistic activity of rhizobacteria toward the potato cyst nematode *Globodera pallida*. *Soil Biol. Biochem.*, **24**: 521-526. [https://doi.org/10.1016/0038-0717\(92\)90075-9](https://doi.org/10.1016/0038-0717(92)90075-9)
- Raghava, S.P.S. and Dadlani, N.K., 1999. Strategy, needs, review. In: *The Hindu survey of Indian agriculture* (ed. N. Ravi). Kasturi and Sons Ltd., Chennai, India, pp. 147-149.
- Rogers, A., McDonald, K., Muehlbauer, M.F., Hoffman, A., Koenig, K., Newman, L. and Lelie, D., 2012. Inoculation of hybrid poplar with the endophytic bacterium *Enterobacter* sp. 638 increases biomass but does not impact leaf level physiology. *Glob. Change Biol. Bioener.*, **4**: 364-370. <https://doi.org/10.1111/j.1757-1707.2011.01119.x>
- Salawu, E. and Darabidan, I., 2010. Screening of selected ornamental plants to *Meloidogyne incognita* in Nigeria. *Pak. J. Nematol.*, **28**: 353-358.
- Sasser, J. and Carter, C., 1985. Overview of the international *Meloidogyne* project. 1975-1984. In: *An advanced treatise on Meloidogyne*, Vol. 1, *Biology and control* (eds. J.N. Sasser and C.C. Carter). North Carolina State University Graphics, Raleigh, North Carolina, USA, pp. 19-25.
- Sasser, J.N., 1979. Pathogenicity, host range and variability in *Meloidogyne* spp. In: *Root-Knot Nematode (Meloidogyne spp.): Systematic, biology and control* (eds. F. Lamberti and C.E. Taylor). Academic Press, London, pp. 257-268.
- Sasser, J.N., 1989. *Plant-parasitic nematodes, the farmer's hidden enemies*. North Carolina State University Graphics, Raleigh, NC, pp. 15.
- Sharma, R., 1977. Nematodes of the cocoa region of Bahia, Brazil: VI. Nematodes associated with tropical fruit trees. *Publ.-Soc. Brasil. Nematol.*, **2**: 109-125.
- Shazad, S., Anwar, S.A., McKenry, M.V., Sahi, S.T., Abid, N. and Ghaffor, B., 2011. *Meloidogyne incognita* infecting two perennial ornamentals. *Pakistan J. Zool.*, **43**: 337-342.
- Shores, M., Harman, G.E. and Mastouri, F., 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol.*, **48**: 21-43. <https://doi.org/10.1146/annurev-phyto-073009-114450>
- Siddiqui, I.A. and Shaukat, S.S., 2003. Suppression

- of root knot disease by *Pseudomonas fluorescens* CHA0 in tomato: Importance of bacterial secondary metabolite, 2, 4-diacetylphologlucinol. *Soil Biol. Biochem.*, **35**: 1615-1623. <https://doi.org/10.1016/j.soilbio.2003.08.006>
- Siddiqui, I.A. and Shaukat, S.S., 2004. Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, *Meloidogyne javanica* is independent of salicylic acid production. *J. Phytopathol.*, **152**: 48-54. <https://doi.org/10.1046/j.1439-0434.2003.00800.x>
- Siddiqui, Y., Ali, A. and Naidu, Y., 2014. Histopathological changes induced by *Meloidogyne incognita* in some ornamental plants. *Crop Prot.*, **65**: 216-220. <https://doi.org/10.1016/j.cropro.2014.08.001>
- Stockwell, V.O., Johnson, K.B., Sugar, D. and Loper, J.E., 2002. Antibiosis contributes to biological control of fire blight by *Pantoea agglomerans* strain Eh252 in orchards. *Phytopathology*, **92**: 1202-1209. <https://doi.org/10.1094/PHYTO.2002.92.11.1202>
- Sturz, A. V., Christie, H.R. and Nowak, J., 2000. Bacterial endophytes: Potential role in developing sustainable systems of crop production. *Crit. Rev. Pl. Sci.*, **19**: 1-30. <https://doi.org/10.1080/07352680091139169>
- Taylor, A.L. and Sasser, J.N., 1978. *Biology, identification and control of root-knot nematodes*. North Carolina State University Graphics, pp. 1-111.
- Vetivelkalai, P., Sivakumar, M. and Jonathan, E.I., 2010. Biocontrol potential of endophytic bacteria on *Meloidogyne incognita* and its effect on plant growth in bhendi. *J. Biopest.*, **3**: 452-457.
- Walia, R.K. and Bajaj, H.K., 2003. *Textbook on introductory plant nematology*. Indian Council of Agricultural Research, KAB, New Delhi, India, pp. 96.
- Weller, D.M., 1988. Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.*, **26**: 379-407. <https://doi.org/10.1146/annurev.py.26.090188.002115>
- Weller, D.M., 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology*, **97**: 250-256. <https://doi.org/10.1094/PHYTO-97-2-0250>
- Whitehead, A. and Hemming, J., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annls. appl. Biol.*, **55**: 25-38.
- Xin, G., Zhang, G., Kang, J.W., Staley, J.T. and Doty, S.L., 2009. A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. *Biol. Fertil. Soils*, **45**: 669-674. <https://doi.org/10.1007/s00374-009-0377-8>