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EVALUATION OF BIOCONTROL POTENTIAL OF *PSEUDOMONAS FLUORESCENS* AGAINST ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA*) INFECTING CHILI

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ABSTRACT

Plant parasitic nematodes are considered worst enemies of mankind because of devastation they cause to crops. They are distributed all over the world in different kinds of habitats and cause severe losses to economically important crops. Up to 40% nematode infection has been observed in chilli. Present study was aimed at checking the antagonistic activity and developing systemic resistance against nematodes. The influence of rhizobacteria *Pseudomonas fluorescence* on mortality of nematodes was studied in-vitro as well as the seed treatment of rhizobacteria on growth parameters of chilli. The results obtained were highly significant that revealed *P. fluorescence* that induced systemic resistance against *Meloidogyne javanica.* Seedling of chilli were treated with *P. fluorescence* to see the efficacy on disease development and different growth parameters. The results achieved were highly significant. This showed that *Pseudomonas fluorescence* enhanced the growth of chilli by controlling deleterious microorganisms and increased plant height /plant, fresh and dry weight of shoot and root and decreased number of galls/plant due to the reason that *P. fluorescence* was found protease producer. It was concluded from the studies that rhizobacteria *P. fluorescence* is a potential biocontrol agent in chilli plants.

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INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are one of the most economically important pests causing severe damages to a wide variety of crops particularly to chilies (Kayani et al., 2017, 2018; Mukhtar et al., 2017; Tariq-Khan et al., 2016). Root-knot nematodes and root infecting fungi have also been found implicated in disease complexes aggravating yield losses (Aslam et al., 2017a; Aslam et al., 2017b; Fateh and Mukhtar, 2017). On the other hand, entomopathogenic nematodes can reduce incidence and severity of root-knot nematodes (Rahoo et al., 2018a; Rahoo et al., 2017; Rahoo et al., 2018b). Various techniques including crop rotation, planting of resistant cultivars and nematicide application have been used for the management of this nematode but crop yield losses persist (Hussain et al., 2016; Kayani and Mukhtar, 2018; Khan et al., 2017; Mukhtar, 2018; Mukhtar et al., 2017). Nematicide use is limited for safety and economic reasons, resistance can be affected by shifts in nematode races, and crop rotation is often effective but may be restricted by the need to select certain crops, by the increase of other nematode populations, and by the ability of the eggs to remain

dormant for years (Meyer et al., 1998; Young, 2001). In view of the above-mentioned drawbacks of a single management procedure, there is a growing interest in finding alternative control approaches for use in integrated pest management programs. Existing management procedures could be enhanced by the development of biocontrol strategies. Plant-associated micro-organisms have been extensively examined for their roles in natural and induced suppressiveness of soil-borne diseases. Among the many groups of such organisms are root-associated bacteria, which generally represent a subset of soil bacteria. Rhizobacteria are a subset of total rhizosphere bacteria which have the capacity, upon reintroduction to seeds or vegetative plant parts, to colonize the developing root system in the presence of competing soil microflora (Kloepper et al., 1999).

Root knot nematode infection was found in five vegetables including chilies, okra, brinjal, tomato cucurbits and that these were found to be infested with other phytonematodes (Anwar et al., 1991). Certain root-associated strains of fluorescent *Pseudomonas* spp. produce and excrete metabolites that are inhibitory to soil-borne plant pathogens (Dowling and O'Gara, 1994; Thomashow and Weller, 1996). Among these metabolites, 2, 4-diacetylphloroglucinol (2, 4-DAPG) has received particular attention because of its production by a wide range of Pseudomonads used for the biological control of root diseases (Dowling and O'Gara, 1994; Thomashow and Weller, 1996). 2,4-DAPG is a phenolic compound with broad spectrum antifungal, antibacterial, antihelminthic, nematicidal and phytotoxic activity (Dowling and O'Gara, 1994). Siddiqui and Shaukat (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). Becker (1988), Westcott Iii (1993), El-Sherif et al. (1994) and Son et al. (2009) illustrated that bacterial antibiotics and other toxic compounds present in metabolites as well as direct interaction might be responsible for the juvenile (J2) immobility. Production of metabolites by rhizosphere bacteria causes lysis of nematode eggs and affects vitality of J2 of root-knot nematodes. Gamliel (1993) studied that *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 induce systemic resistance or inhibition of the nematode's host-recognition process mechanisms in control of plant pathogens by Pseudomonads.

Rhizobacteria support plant growth by generating growth regulators, smooth the progresses of nutrient uptakes, speed up mineralization, diminish plant trauma, excite nodulation, offer nitrogen fixation, support mycorrhizal fungi, restrain plant diseases, and purpose as nematicides and insecticides. Many of the PGPR are fluorescent Pseudomonads (*Pseudomonas fluorescens*), but other bacteria (*Bacillus* sp., *Azotobacter* sp., *Acetobacter* sp., *Azospirillum* sp.) are identified a number of these organisms as well have been invented into bio-fertilizers and are easily available. Keeping in view the above-mentioned facts, the present study was planned to evaluate the biocontrol potential of *Pseudomonas fluorescens* against root knot nematodes infecting chili.

MATERIALS AND METHODS

Isolation of *Pseudomonas fluorescens:* Rhizospheric soil samples were collected from the chili fields and serial dilution technique was used to isolate bacterial population from each 1 gram of soil sample on Nutrient agar. Purification of bacterial isolates was done by picking single colony and sub-cultured on selective media King's B (KB) agar medium (King et al., 1954) to isolate *P. fluorescens*. Gram test, KOH test and florescent under UV light were done to screen the bacterial colonies.

Preparation of nematode suspension: Culture of root-knot nematode was taken from the Department of Plant pathology, Nematology Lab. The galled roots from stock cultures were washed without dislodging the egg masses. The hand-picked egg masses from cultured pot plants were transferred carefully to a wire gauze sieve containing two layers of facial tissue paper trimmed down to edge of wire gauze and kept in a Petri dish holding sufficient sterile water to remain in contact with the bottom of Petri dish and incubated at 25±20°C. After 24 h, the larvae hatching from the egg masses passed through the tissue paper and population counts were made under stereo-microscope with the help of Fenwick's multi chamber on one milliliter counting slide. Based on the requirement, the suspension was diluted with sterile water.

Preparation of cell free filtrates of *P. fluorescens* isolates: A loopful of *P. fluorescens* isolates was inoculated into the conical flasks having King's B broth separately and

incubated for 24 h at room temperature. The bacterial cells were harvested using centrifuge at 12,000 rpm for 20 minutes and the supernatant was collected which was finally passed through Millipore filter of 0.22 µm. The filtrates thus obtained for all these strains were designated as standard filtrates (Niknam and Dhawan, 2002).

***In vitro* efficacy of *P. fluorescens* isolates against *M. javanica*:** *In vitro* effect of cell free filtrates on mortality of second-stage juveniles of *M. javanica* was done by following the method described by Li Bin et al. (2005). All the treatments including distilled water as control were replicated three times. One milliliter each of the bacterial cell free filtrates was poured into separate Syracuse dish. *M. javanica* was introduced into each dish at 100 nematodes in 1 ml of sterile water and incubated at 28 ± 2°C. The inactive nematodes were transferred separately into sterile distilled water and kept overnight to check whether mortality was permanent or temporary. Observations were recorded on the mortality of nematodes after 24 and 48 h exposure period and per cent mortality was calculated.

Evaluation of effective *P. fluorescens* strains in pot culture method: A pot culture experiment was conducted to study the influence of three selected isolates of *Pseudomonas* (Ps-1, Ps-3 and Ps-9) on seed germination, growth and nutrient uptake of chili plants under net house conditions. Seeds of test plant were surface sterilized with 2.4 per cent sodium hypochlorite (NaOCl) solution for 2-3 min and were soaked in 10 ml of the bacterial suspension (10^6 cfu/ml) for 24 h. A set of sterile blank nutrient broth served as control. The seeds were then blot dried and sown in pots containing sterilized soil with five replications. Hoagland's nutrient solution was added to earthen pots at weekly intervals. The germination per cent was estimated at 10 days after sowing (DAS). Without disturbing the roots of chili and seedlings were de-potted at 30 and 60 DAS and observations on root length, shoot length, fresh weight of plant and dry weight of biomass were taken after drying samples to a constant weight in an oven and vigor index was calculated.

Statistical analysis: The data were found normally distributed and did not require transformation. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The means were compared by Fisher's Protected Least Significant Difference Test at 5%.

RESULTS AND DISCUSSION

From the rhizospheric of chilli plants a total of ten bacterial strains were isolated having gram negative results and were found florescent under UV light when grown on King's B media. These gram negative, florescent bacterial isolates were used for further studies.

In vitro* evaluation of *P. fluorescens* isolates against *M. javanica: All the ten strains tested produced compounds that resulted in the death of *M. javanica* ranging from 3 to 57%. As compared to control, the maximum mortality was caused by Ps-9 strain of *P. fluorescence* which gave the juvenile mortality of 57% after 48 h. On the other hand, the strains Ps-5 and Ps-7 caused lowest mortality of 3% after 24 h and 12% and 16% respectively after 48h. The bacterial strains Ps-3 caused mortality of *M. javanica* by 39%. Other strains of bacteria caused the mortality about 6-23% (Table 1).

Table 1. Evaluation of rhizobacterial isolates on mortality of *M. javanica* after 24 and 48h.

Strain	% Mortality	
	After 24 h	After 48 h
Ps-1	39	43
Ps-2	12	23
Ps-3	24	37
Ps-4	6	19
Ps-5	3	12
Ps-6	14	21
Ps-7	3	16
Ps-8	24	19
Ps-9	48	57
Ps-10	15	23
Control	6	10

All the ten strains tested against nematodes showed mortality against *M. javanica* from 3-57%. Ps-9 caused maximum mortality of juveniles after 48 h which was 57% as compared to control. According to Nasima et al. (2002) nematode mortality up to 17-96% was recorded after 48 h when seaweed at the concentration of 2 mg/ml was used against *M. Javanica*. The reason should be that microorganisms antagonistic to nematodes secrete different metabolites which could be responsible for nematode mortality. In the present study proteases were detected and might be responsible for mortality of *M. javanica.*

Efficacy of *P. fluorescens* strains in pot culture method: Effect of *P. fluorescence* on the growth parameters of chilli and development of root-knot nematode disease was measured in pot experiment in net house (Table 2). Comparison of treatment means showed that height was the maximum with Ps-9 treatment which showed 9.8 cm as compared to control (7.88 cm) and was the lowest with positive control treatment (Nematodes alone) that was 5.06 cm. All the three rhizobacterial isolates were effective but Ps-9 showed maximum results as compared with positive and negative control. Similarly, in case of fresh and dry shoot weight, Ps-9 showed highest results (0.86 g and 0.52g respectively) as compared with positive and negative control (Table 2).

Table 2. Effect of rhizobacterial application on plant growth promotion and nematode population on chilli.

Treatments	Height (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Juveniles/ 250 g soil	Egg masses/ root
Ps-1+Nematode	9.86 a	0.76 a	0.47 a	0.39 d	0.31 d	112 c	9 d
Ps-3+Nematode	8.81 c	0.66 c	0.37 c	0.51 b	0.40 b	87 b	7 c
Ps-9+Nematode	10.11 b	0.86 b	0.52 b	0.63 e	0.51 c	54 c	4 d
Positive control	5.06 d	0.26 e	0.13 e	0.27 a	0.20 a	482 a	20 a
Control	7.88 c	0.46 c	0.27 d	0.31 e	0.29 e	0.0 d	0.0 d

In case of fresh and dry root weight, again all the three rhizobacterial isolates showed effective results but Ps-9 showed the maximum values (0.63g and 0.51g respectively) as compared with other treatments. In case of nematode population, maximum nematode population/250 g soil was seen in positive control that was 482 and number of egg masses were 20/root while minimum number of juveniles/250 g soil was 54 in case of Ps-9 and also minimum egg masses/root was observed in case of Ps-9 that was 4 (Table 2).

Effect of *P. fluorescence* on growth parameters of chilli and on the development of root knot nematode was observed in growth chamber. Results of different parameters of growth revealed that application of biocontrol agents enhanced plant growth characteristics such as plant height, fresh weight and dry weight of shoot and significantly reduced nematode population in soil as well as in root. Moreover, they reduced number of egg masses per root system. Treatment with Ps-9 showed greatest increase in plant height likely due to indole acetic acid producer. Phosphate solubilization and indole acetic acid production by *Pseudomonas* spp. has also been reported by Debora et al. (2007).

Plant growth promoting rhizobacteria can reduce the disease by inhibiting the pathogens competition for Fe (III), resistance induced in plants, inhibiting of pathogen by volatile products, and massive colonization in roots and plant growth stimulation (Kloepper et al., 1999; Siddiqui and Shaukat, 2002, 2004; Thomashow and Weller, 1996). In a study *Pseudomonas* spp. caused massive root colonization than the other species of plant growth promoting rhizobacteria. It is due to the reason that *Pseudomonas* spp. caused greater increase in the growth of plant and high reduction in the multiplication of nematode than the other species of bacteria used in study. Some other scientists have also reported that *Pseudomonas* spp. caused a greater increase in plant growth and inhibit the invasion of pathogen to plants (Aalten et al., 1998; Bora et al., 2004). *P. polymyxa* and *P. alcaligenes* are also known to increase plant growth and reduce disease severity (Gamliel, 1993; Kloepper et al., 1992). It is, therefore, concluded from the present study that indigenous strains of *Pseudomonas fluorescens* can be effectively used for the management of root-knot nematodes.

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