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Interaction Between *Meloidogyne incognita*, *Pseudomonas fluorescens* and *Bacillus subtilis* and its Effect on Plant Growth of Black Gram (*Vigna mungo* L.)

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ABSTRACT

Meloidogyne incognita infection produced adverse effects on the growth of black gram (*Vigna mungo* L.). Inoculation of the plants with the second-stage juveniles of *M. incognita*, prior to bacterial inoculation, resulted in reduction of plant growth, when compared with the plants in which bacterial application was followed by nematode inoculation. Plant length, fresh and dry weights, nodules weight, leghaemoglobin content and the number of nodules per plant were found decreased in nematode infected than in infected plants. Application of bacteria *Pseudomonas fluorescens* and *Bacillus subtilis* increased the growth parameters and the number of nodules. Highest number of galls per plant were recorded on the plants infected with the nematode and not treated with bacteria. Gall number was found decreased on the plants inoculated with the nematode and treated with the bacteria than the plants not treated with bacteria. *Meloidogyne incognita* on infecting black gram (*Vigna mungo* L.) in absence of bacteria caused the formation of a number of galls on the roots, decreased plant length, plant weight, number of nodules per plant and amount of leghaemoglobin. Incorporation of bacteria into the soil after 10 days of nematode inoculation resulting in an increase in all the growth parameters considered and decreased root-knot number.

Key words: *Vigna mungo, Meloidogyne incognita, Pseudomonas fluorescens, Bacillus subtilis*

INTRODUCTION

Pulses occupy an indispensable position in the dietary habit of vast majority of the people of India s well as abroad. These are not only the protein source but these also contribute in restoration of soil fertility (Korlovich and Repyev, 1995).

Black gram is an excellent source of easily digestible protein having low flatulence property. It is a short duration and widely cultivated leguminous pulse crop contributing substantially to the annual production of pulses. The production and yield of black gram is declined if the crop field is infested with the nematodes (Ali, 1994). The root-knot nematodes (*Meloidogyne* sp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops including black gram (Sikora and Greco, 1993). The infection starts with root penetration of second-stage juveniles hatched in soil from eggs encapsulated in egg masses laid by the females residing in the infected roots (Barker *et al*., 1985). Available literature shows that *Meloidogyne* sp. adversely affect nodulation and nitrogen fixation in pulse crops (Haung, 1987; Taha, 1993).

Bacteria are the most abundant microorganisms of which about 2 to 5% are rhizobacteria which exert a beneficial effects on plant growth and therefore, are termed as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper and chroth, 1978). Early studies on PGPR were more focused on biological control of plant diseases than on growth promotion as a result of inoculants of fluorescent pseudomonads and *Bacillus subtilis*, antagonistic to soil borne plant pathogens (Kloepper *et al.*, 1989). The PGPR-nematode interactions have been extensively studied with the aim to manage plant-parasitic nematodes.

The objectives of this study were to investigate the effect of sequential inoculation of *M. incognita*, *P. fluorescens* and *B. subtilis* on plant growth, nodulation, fresh and dry biomass in black gram cv. Pant U-30.

MATERIALS AND METHODS

Seeds of Pant U-30 black gram were sown in 15 cm diameter earthen pots filled with autoclaved soil and mixed compost (3:1). Prior to sowing, the seeds were disinfected by immersion on 1% of sodium hypochlorite (NaOCl). After germination, the seedlings were thinned to one per pot.

Culturing of nematodes: To get a large number of second-stage juveniles of *M. incognita* pure culture was maintained on tomato plants grown in field. The required number of freshly hatched second-stage juveniles were obtained from as per requirement.

Extraction of nematodes: For isolation of second-stage juveniles (J_2), tomato plants infected with *M. incognita* were collected and washed properly in running tap water. Egg masses were carefully removed from the roots with the help of forceps and placed over a double layer of tissue paper spread on 3 cm diameter sieve. The sieves were placed on petri dishes containing sufficient water so that their lower part remained partially submerged in water. To avoid evaporation of water the petri dishes were kept covered with their lids. After 24 h onwards second- stage juveniles were collected in the form of suspension and stored for later use.

Culturing of bacteria: *P. fluorescens* and *B. subtilis* were cultured on King's B and nutrient broth media, respectively, containing measured amount of nutrients for their growth and kept at 33°C for best growth for 48 h. Bacterial colonies of *Pseudomonas* sp. fluoresced under UV light at 366 nm were purified on King's B agar medium (King *et al.*, 1954) and identified according to Krieg and Holt (1984). The bacterial population reached $>10^9$ cells mL^{-1} within 7-10 days.

Inoculation: The 10 days old seedlings were inoculated by adding required amount of inocula through four narrow holes made around each plant. Each plant was inoculated with *M. incognita* (1000 J) and bacterial culture as per treatment (10 and 20 mL) sequentially with the interval of 10 days:

T1 = *M. incognita* prior to *Pseudomonas fluorescens* (10 mL)
T2 = *M. incognita* prior to *Pseudomonas fluorescens* (20 mL)
T 3 = *M. incognita* prior to *Bacillus subtilis* (10 mL)
T 4 = *M. incognita* prior to *Bacillus subtilis* (20 mL)
T 5 = *M. incognita* prior to *Pseudomonas fluorescens* (10 mL), *Bacillus subtilis* (10 mL)
T 6 = *M. incognita* prior to *Pseudomonas fluorescens* (20 mL), *Bacillus subtilis* (20 mL)

P. Fluorescens and *B. Subtilis* were also used as per treatment and their detail is follows:

F 1 = *Pseudomonas fluorescens* (10 mL) prior to *M. incognita*
F 2 = *Pseudomonas fluorescens* (20 mL) prior to *M. incognita*
F 3 = *Bacillus subtilis* (10 mL) prior to *M. incognita*
F 4 = *Bacillus subtilis* (20 mL) prior to *M. incognita*
F 5 = *Pseudomonas fluorescens* (10 mL), *Bacillus subtilis* (10 mL) prior to *M. incognita*
F 6 = *Pseudomonas fluorescens* (20 mL), *Bacillus subtilis* (20 mL) prior to *M. incognita*

All treatments were replicated thrice. The plants were lightly watered after inoculation and thereafter, whenever required. The pots were arranged in a randomized block design. The experiment was terminated 60 days after inoculation and different parameters were determined (Southey, 1986; Oostenbrink, 1966).

RESULT

The data on the parameters of black gram var. PU-30 bacteria and *Meloidogyne incognita* have been presented in Table 1-4. The data recorded revealed that the shoot length increased significantly on increasing the dose of bacterium inoculums as is evident from F1 to F6 (Table 3). when compared with control. The shortest length of the shoot was recorded (19.4 cm) in T3 treatment (Table 1). However, maximum length (30.7 cm) of the shoot was recorded in the treatment F6 (Table 3) but the values were significantly lower than the control (33.4 cm). Reduction in shoot length in black gram due to *Meloidogyne incognita* infection has earlier been reported by Singh (1972), Gupta *et al.* (1987), Mohanty *et al.* (1989), Kalita and Phukan (1993) and Haider *et al.* (2003).

The root length of the plants infected with the nematode was found to be increased significantly on increasing the amount of bacterial inoculum (Table 1 and 3). Maximum root length (14.8 cm) was recorded in F6 plants (Table 3) which was significantly less than the control (Table 1). Maximum and significant reduction in the root length (5.8 cm) occurred in the plants of the treatment T3 (Table 1).

Significant variations were observed in shoot and root weights in both the experiments. Highest and significant reductions in the fresh and the dry shoot weights (7.85 and 1.74 g) were noticed

Table 1: Sequential effect of *M. incognita*, *Pseudomonas fluorescens* and *Bacillus subtilis* on *Vigna mungo* L.

	Parameters							
Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (mg)	Root dry weight (mg)	No. of galls per plant	No. of nodules per plant
Control	33.4	16.7	15.75	10.70	9.53	8.73	-	36.66
T1*	20.8	6.7	8.57	2.63	2.74	0.92	96.00	14.33
T2*	25.6	9.6	10.67	4.76	3.74	2.67	91.33	20.33
T3*	19.4	5.8	7.85	1.70	1.74	0.68	97.33	10.33
T4*	22.6	7.5	9.76	3.74	2.85	1.66	92.66	16.33
T5*	27.65	10.7	11.74	5.62	4.82	3.76	77.00	25.66
T6*	29.50	12.6	12.76	7.56	5.56	5.58	73.00	27.33
S.E.	±1.06	±0.79	±0.58	±0.64	±0.58	±0.61	±7.14	±1.84
LSD (0.05)	02.30	1.72	1.26	1.39	1.26	1.32	15.50	4.00

*Sequential inoculation = after an interval of 10 days

Table 2: Sequential effect of *M. incognita*, *P. fluorescens* and *B. subtilis* on Leghaemoglobin, nitrogen content and dry weight of nodules in *Vigna mungo* L.

	Parameters		
Treatments	Leghaemoglobin content (mg g^{-1}) nodule	Nitrogen content in leaves (mg)	Dry weight of nodules per plant (mg)
Control	4.70	0.33	24.62
T1	0.43	0.09	9.65
T2	0.83	0.16	13.79
T3	0.23	0.12	6.91
T4	0.53	0.15	10.86
T5	1.33	0.23	17.60
T6	1.53	0.23	18.58
SE	±0.32	±0.01	±1.2
LSD (0.05)	0.69	0.02	2.61

Table 3: Sequential effect of *Pseudomonas fluorescens*, *Bacillus subtilis* and *M. incognita* on *Vigna mungo* L.

	Parameters							
Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (mg)	Root dry weight (mg)	No. of galls per plant	No. of nodules per plant
Control	33.4	16.7	15.75	10.70	9.53	8.73	-	36.66
F1*	22.5	8.7	10.94	4.84	3.70	2.84	87.33	24.00
F2*	27.6	11.6	12.76	6.75	5.67	4.71	73.33	30.33
F3*	21.8	7.6	9.43	3.68	2.74	1.63	81.66	20.66
F4*	24.5	9.6	11.77	5.64	4.63	3.76	79.00	26.00
F5*	28.6	12.6	13.86	7.66	6.68	5.80	70.33	32.33
F6*	30.7	14.8	14.84	9.63	8.74	6.80	65.66	35.33
S.E.	±0.90	±0.69	±0.45	±0.53	±0.55	±0.53	±6.18	±1.23
LSD (0.05)	1.96	1.50	0.98	1.15	1.19	1.15	13.4	2.66

*Sequential inoculation = after an interval of 10 days

Table 4: Sequential effect of *P. fluorescens*, *B. subtilis* and *M. incognita* on Leghaemoglobin, nitrogen content and dry weight of nodules in *Vigna mungo* L.

	Parameters		
Treatments	Leghaemoglobin content (mg g^{-1}) nodule	Nitrogen content in leaves (mg)	Dry weight of nodules per plant (mg)
Control	4.7	0.33	24.62
F1	1.9	0.17	16.44
F2	3.3	0.17	20.50
F3	0.33	0.17	13.85
F4	2.8	0.18	14.40
F5	3.7	0.23	21.90
F6	4.1	0.23	23.87
SE	±0.30	±0.01	±0.92
LSD (0.05)	0.65	0.21	2.00

in T3, on comparing with the control (Table 1). Increase in the fresh and the dry shoot weights (14.84 and 8.74 g) (Table 3) was maximum in F6 treatment which was, however, lower than the ontrol (15.75 and 9.53). The values of the fresh and the dry weights of the roots (1.70 and 0.68 mg) were maximum when *M. incognita* was inoculated into the soil prior to bacterial inoculation as is evident from T3 treatment. Maximum increase in the fresh and the dry weights of the roots (9.63

and 6.80 g) was recorded in F6 treatment where bacterial inoculum was introduced prior to ematode inoculation (Table 3). The number of galls were highest in T1, where the plants were inoculated with *M. incognita* prior to bacterial inoculation (Table 1). Lowest number of galls was observed in the treatment F6 where higher doses of both the bacteria were applied before nematode inoculation.

The highest number of effective nodules per plant (35.33) was recorded in F6 (Table 3), in which the bacteria were inoculated prior to nematode inoculation, the values were at par with the control (36.66). However, minimum number of root nodules was recorded in T3 treatment (Table 1). When the plants were inoculated with the nematode, *Meloidogyne incognita*, significant reductions in nodule weights were observed. Maximum dry weights of the nodules (23.87 mg) was recorded in F6 treatment where bacterial inoculum was introduced prior to nematode inoculation (Table 4). Leghaemoglobin content of *Vigna mungo* L. nodules was significantly differed at different inoculation levels (Table 2 and 4). F6 treatment showed a significantly maximum leghaemoglobin content compared to rest of the treatments but less than control (4.8). Rhizosphere inoculation of bacteria significantly increased the nitrogen content in leaves less than control. The lowest nitrogen content were recorded in T1 treatment (0.09) (Table 2).

DISCUSSION

Plant growth promoting bacteria play a significant role in the growth and the development of all the leguminous crops. The presence of *Pseudomonas fluorescens* and *Bacillus subtilis* in the roots of black gram usually produces beneficial effects on the plant growth. Occurrence of rhizobacteria in the soil plays a protective role against the nematodes. From the following experiment it is evident that the damage caused to the plant was significantly lower, except in the treatment where bacterial applications followed nematode inoculation. Presence of the nematodes in the soil and their interaction with the plants, in absence of the PGPR, are more damaging for the plants. The results of the experiment revealed that inoculation of the plants with the nematodes prior to bacterial inoculation caused reduction in plant growth as well as nodulation. From the experiment it was found that development of the nodule was suppressed as a result of prior nematode inoculation. The similar findings were reported by Bopaiah *et al.* (1976).

Bacterial application prior to nematode inoculation resulted in an increase in plant growth and reduced the extent of damage to the nematode inoculated plants. From the following study it was inferred that *Pseudomonas* and *Bacillus* both produced beneficial effects on the plant growth even in the presence of the nematode. Similar trend was also reported by Siddiqui and Husain (1992). Bacterial application prior to nematode inoculation resulted in immediate establishment of the bacteria inside the root tissues; on the contrary, earlier establishment of nematode carried out mandatory changes and caused damage to the plant (Varshney, 1982). Increase in amount of bacterial inoculum resulted in increase in plant growth as well as in increase in nodulation. The usual site of nodule formation is the root tissue but nodules were occasionally found on the galls as was reported by Hussey and Barker (1976). Suppressed nodulation is related to the smaller size of the root system that confirms the findings of Taha and Raski (1969). Growth parameters, as shoot and root lengths, fresh and dry weights of the shoot and the root showed more growth in prior pplication of bacteria (Table 1) as compared to nematode (Table 2). The values of these parameters were found to be increased in both the treatments on increasing the dose of bacteria.

Increase in plant growth bacterial inoculated plants, even in the presence of the nematode was mainly due to enhancement of mineralization process, specifically nitrogen uptake and assimilation (Griffiths, 1994). These bacteria have been characterized for production of hydrolytic enzymes,

HCN, phenol oxidation and anti fungal activity (Insunza *et al*., 2002). Nematode, when infects a plant, interferes in plant and bacteria relationship. Colonization of the roots by pathogenic and beneficial organisms is influenced by the nematode parasitism; but a natural antagonism between nematode and organism, specially microorganisms suppresses the deleterious effect of nematode (Kerry, 2000). Root knot nematodes and rhizobacteria occupy similar niches in the soil and roots suggesting the possibility for genetic exchange (Bird *et al*., 2003). *Pseudomonas fluorescens* produces IAA which is helpful to promote physiological effects on plants. Rhizosphere bacteria promote plant growth by improving the availability of nutrients, suppressing the growth of plant pathogens or by production of hormones such as auxins (Jangu and Sindhu, 2011). *Pseudomonas* sp. possessed considerable insecticide tolerance and IAA, siderophores (salicylic acid and 2, 3-dihydroxy benzoic acid), exo-polysaccharides, HCN and ammonia producing traits (Ahemad and Khan, 2011). PGPR may induce plant growth by the production of stimulatory volatiles and phytohormones, lowering of the ethylene level in plant, improvement of the plant nutrient status and stimulation of disease resistance mechanisms (Beauchamp, 1993). *Pseudomonas fluorescens* and *Bacillus subtilis* increased growth of root-knot nematode infected plants of black gram. Higher doses of bacterial were proved more beneficial than lower doses. Application of bacteria prior to nematode inoculation was found more promising. Bacterial inoculated plants without nematodes had conspicuously large and pink coloured nodules where as nodules on nematode infested plants were brownish in colour. The number of nodules per plants and their weight were significantly reduced by nematode infection (Table 1 and 2) which was proved by Hussaini and Seshadari (1975), Sharma and Sethi (1976) and Chahal *et al*. (1985). Root-knot nematode juveniles directly interfere with the establishment of bacteria and the secretion of hydrolytic enzymes or growth regulators produced by nematodes may play a determinative role in nodule development (Barker *et al*., 1972; Ali *et al*., 1981). Leghaemoglobin content of nodules was significantly reduced by nematode infection as compared to control and increased on increasing the bacterial dose. Invading nematodes are considered to disturb the functioning of nodules by altering host nutrition (Doney *et al*., 1970). As leghaemoglobin regulates the supply of oxygen and bacteroids contain nitrogenase enzyme required for the reduction of atmospheric nitrogen to ammonia, then a decrease in these due to nematode infection would lead to a decrease in fixation of nitrogen.

From the results of experiment, it may be concluded that *M. incognita* affects symbiotic nitrogen fixation not only by reducing the number of nodules but also by disturbing the functioning of nodules due to decrease in the photosynthate supply and leghaemoglobin content of nodules.

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