Effect of *Paecilomyces lilacinus* and plant growth promoting rhizobacteria on *Meloidogyne incognita* inoculated black gram, *Vigna mungo* plants

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ABSTRACT

An experiment was carried out to study the interaction of root knot nematode, *Meloidogyne incognita, Paecilomyces lilacinus* and plant growth promoting rhizobacteria, *Bradyrhizobium* on the growth of black gram, *Vigna mungo*. In the present investigation it was revealed that *Meloidogyne incognita* resulted in significant decrease in the growth of black gram, root-nodule development, nitrogen contents of root and shoot, and nitrogenase activity at all inoculum levels. Treatment of *Bradyrhizobium* and *Paecilomyces lilacinus* resulted in significantly lesser damage to plant growth of blackgram than the plants treated with bacteria at the time of inoculation with root-knot nematode, *M. incognita* fallowed by plants where bacteria and fungus was applied 10 days after nematode inoculation. .Treatment of *Bradyrhizobium* significantly increased the nitrogen content of root and shoot in all the treatments. Nitrogenase activities in nematode infected plants was higher in plants treated with *P. lilacinus* and *Bradyrhizobium* before and at the time of nematode inoculation in comparison to plants which were treated by *P. lilacinus* and *Bradyrhizobium* 10 days after nematode inoculation.

Key words: Black gram, *Meloidogyne incognita,* nitrogen content, *Paecilomyces lilacinus*, plant growth, rhizobacteria,

INTRODUCTION

Knowledge of biological nitrogen fixation at genetic, biochemical, and physiological levels has expanded rapidly during past few decades. Plant growth promoting rhizobacteria are bacteria that colonize plant roots, and in doing so, they promote plant growth and /or reduce disease or insect. PGPR are free-living bacteria and some of them invade the tissues of living plants and cause unapparent and symptomatic infections (Sturz and Nowak, 2000) when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens (Kloepper *et al*., 1980). Nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is therefore important in plant growth and the production of food and feed. (Dakora and Keya, 1997; Banerjee *et al.*, 2006). Even though numerous obstacles that limit maximum nitrogen fixation still remain unsolved, one of the biological factors affecting nodule formation and its functioning is the presence of phytonematodes in the rhizosphere (Taha,1993) as *Meloidogyne* spp. adversely affect nodulation and N_2 fixation in pulses (Taha,1993; Bhat, *et. al.*, 2009). The application of beneficial rhizosphere bacteria fungus *Paecilomyces lilacinus* protects plant roots from pathogens and increases plant growth stimulation and leaf yield (Manjula and Podile, 2001; Wright *et al*., 2003 Muthulakshmi *et al.*, 2010). Plant growth–promoting rhizobacteria (PGPR) strains have induced systemic resistance (ISR) in plants against multiple pathogens (Ramamoorthy *et al.*, 2001, Siddiqui and Showkat, 2002a, 2002b). Fungi like *Paecilomyces lilacinus*, has been reported as potential biocontrol agents of plant parasitic nematodes especially root knot nematodes (Bhat, *et al*., 2009). The use of fungal parasite of nematode eggs and endophytic bacteria in reducing nematode populations is a promising form of crop protection

against nematode species (Atkins *et al.,* 2005; Vetrivelkalai, 2010). Some combinations of biocontrol agents have resulted in three fold increase in yield (Deepa *et al*., 2011)

Among the pulses, black gram and green gram are important ones. According to Union Government's Third Advanced Crop Estimates, black gram production was estimated at 1.8 million tons and green gram at 1.4 million tons during 2010-11. Blackgram, *Vigna mungo* (L.) Hepper is an important and widely cultivated pulse crop contributing substantially to the annual production of pulses. But during the last few decades the production and yield of blackgram declined and expected target could not be achieved. The root-knot nematode, *Meloidogyne incognita* (Kafoid and White) Chitwood, an important nematode pest of blackgram thus is one of the major constraints in increasing the production of the blackgram. In order to increase the yield of crops the integrated pest management strategy is applied which includes a broad array of microbial pesticides, botanicals, biochemicals derived from micro-organisms and the genetic incorporation of DNA into agricultural commodities that protects them against pest damage (Gupta and Dikshit 2010).Therefore biotechnological approaches in the management of plant pests, diseases and weeds are presently employed for sustainable agriculture. (Wahab, 2009) The objectives of this study were, therefore, to investigate the effect of time of application of biocontrol agents (BCAs) namely *Paecilomyces lilacinus* and plant growth promoting rhizobacteria *Bradyrhizobium* on plant growth, nodulation and nitrogen fixation in black gram.

MATERIALS AND METHODS

The test fungus *Paecilomyces lilacinus,* used in the experiment, was obtained from Indian Type Culture Collection Centre, Plant Pathology Division, IARI, New Delhi. The fungus was cultured on PDA for 15 days at 27 ± 2^0C then inoculated to Richards Medium (Riker and Riker, 1936) for en-masse propagation .The mycelia (100 gm) were blended in distilled water (100 mL.) in warring blender to make mycelial suspension for soil application (10 mL of suspension containing 1gm mycelia). The fungus was applied into the rhizosphere zone by making three or four holes around the plant. Cultures of *Bradyrhizibium* (black gram strain) was obtained from Indian Agriculture Research Institute, New Delhi. I gm. of the bacterial strain was mixed with 10 mL of distilled water and was thoroughly mixed by stirring. A 1 mL of the bacterial suspension was added to rhizosphere zone of black gram seedling at an appropriate time. *Meloidogyne incognita* inoculum consist of second-stage juveniles (J_2) obtained by hatching egg masses collected from root galls of heavily infested egg plant (*Solanum melongena* L.), which were grown in pots on green house benches. To obtain second-stage juveniles (J_2) of *M. incognita*, five handpicked egg masses (pre-treated with 1.0% NaOCl solution) obtained from root galls of eggplants were allowed to hatch in Petri plates containing sterilized distilled water and incubated at 27 ± 2°C. After 24 hrs to 48 hrs J_2 suspension was collected in a beaker and second-stage juveniles (J_2) were counted in 1 mL counting dish under stereoscopic microscope. The nematode was identified using host differential test (Taylor and Sasser, 1978). Seeds of Black gram obtained from Indian Institute of Pulses Research, Kanpur, were surface sterilized (1.0% NaOCl) and sown (5 seeds /pot) in 15 cm diameter earthen pots containing 1 kg mixture of sandy loam soil, coarse sand and manure (3:3:1). One healthy seedling /pot was retained after germination

After 10 days of growth, plant roots were inoculated by adding required inocula through four soil depressions made around each plant. Each plant was inoculated with required number of *M. incognita* (0, 500, 1000 and 2000) juveniles (J_2) and 0 and 10 mL *Bradyrhizobium* suspension (1g/10mL) and fungus *Paecilomyces lilacinus* (1g/10mL) individually, simultaneously and /or sequentially with an interval of 10 days. All treatments were replicated five times. The plants were lightly watered after inoculation and thereafter whenever required. The pots were arranged on green house benches in randomized block design. The experiment was terminated 60 days after inoculation and plant growth parameters such as length and dry weight of shoot and root as well as nodule number (primary and secondary $root^{-1}$ system) from all treatments were

determined (Southey, 1986; Oostenbrink, 1966) The rate of nitrogenase activity and total nitrogen content in shoot and root of every treatment was determined as per Sadasivam and Manikam (1992). The data were analysed statistically for least significant difference calculated at P=0.05 level (Panse and Sukhatme, 1989). All the treatments were replicated five times.

RESULTS

The overall response of *Bradyrhizobium* and *P. lilacinus* on roots was beneficial for plant growth of black gram. In absence of nematode, growth of bacterized plants significantly (P=0.05) increased in comparison to un-bacterized plants. *M. incognita* inoculation resulted in significant (P=0.05) decrease in length and weight of roots and shoots of blackgram at all inoculum levels. The reduction in growth was inversely proportional to inoculum level. However, in the presence *Bradyrhizobium* and *P. lilacinus*, the damage to plant growth was significantly less , except in treatment where bacterial and fungal applications followed nematode inoculation at the same inoculum levels. Inoculation of nematode 10 days prior to bacterial and fungal application was more effective in causing damage to plant growth than those plants that were inoculated with nematode and treated with bacteria and with fungus simultaneously or to plants where nematode followed bacterial inoculation and fungal application. Application of bacteria and *P. lilacinus* prior to nematode inoculation caused significantly (P=0.05) lesser damage compared to plants where fungus and bacteria was applied simultaneously with nematode or after nematode inoculation. Nematode development and gall formation diminished the number of nodules on primary and secondary root system significantly (P=0.05) in all the treatments (Table 1).

The nematode multiplication in terms of reproduction factor (R) and gall number was significantly (P=0.05) higher in unbacterized plants and in absence of *P. lilacinus* than on bacterized and *P. lilacinus* treated plants.

Table 1. Effect of *Bradyrhizobium* and *Paecilomyces lilacinus* on plant growth, nodulation and biochemical parameters of *Meloidogyne incognita* inoculated blackgram plants

Treatment	T*	Plant growth				Nodulation		Nitrogen content (mg)		Nitrogenase activity PM2H4 hr/g
		Length		Dry weight		Nodule number				
		Shoot	Root	Shoot	Root	Primary root	Secondary root	Root	Shoot	Dry weight of nodule
None	T_1	31.8	16.5	5.2	4.2	00.0	00.0	0.20	0.25	00
M.incognita(Mi)	T_2	22.5	13.7	3.2	2.6	00.0	00.0	0.16	0.21	00
M.incognita	T_3	20.7	12.7	2.9	2.3	00.0	00.0	0.15	0.20	00
M.incognita	T_4	19.6	11.2	2.4	1.8	00.0	00.0	0.14	0.18	00
Rh +Mi 500+*Pl****	T_5	27.1	16.1	3.9	3.2	17.3	145.5	0.18	0.24	134
Rh + *Mi* 1000+*Pl*	T_6	24.6	14.8	3.6	2.9	16.1	131.5	0.17	0.23	95
Rh + Mi 2000+*Pl*	T_7	23.4	13.4	3.3	2.7	14.9	114.6	0.16	0.26	47
Rh→Pl → Mi 500	T_8	30.6	17.5	4.5	3.8	19.2	166.3	0.21	0.25	169
*Rh→Pl →Mi*1000	T_9	27.9	16.1	4.2	3.4	18.0	148.0	0.18	0.24	125
*R h→Pl→Mi*2000	T_{10}	26.7	15.5	4.0	3.3	16.1	129.1	0.18	0.23	75
Mi 500 *→Rh→Pl*	T_{11}	23.9	14.8	3.5	2.8	13.9	105.3	0.16	0.23	98
Mi 1000→Rh→*Pl*	T_{12}	22.2	13.9	3.1	2.5	12.7	91.3	0.15	0.22	78
Mi 2000→*Rh→Pl*	T_{13}	21.1	12.5	2.9	2.3	11.3	76.1	0.14	0.21	39
C.D.(P≤ 0.05)		3.5	1.6	0.5	0.4	0.8	6.7	0.015	0.01	4.1

*T_1 = Control T_2 = 500 J_2 alone T_3 =1000 J_2 alone T_4 =2000 J_2 alone T_5 ,T_6 and T_7= Simultaneously, T_8,$T_9$$T_{10}$$T_{11}$$T_{13}$ = Sequential
Data are the mean of three replicates;Sequential inoculation (after an interval of 10 days);***Pl= *Paecilomyces lilacinus*

Table 2. Effect of *Bradyrhizobium* sp. and *Paecilomyces lilacinus* on multiplication of *Meloidogyne incognita* on blackgram

Pathogens	Treatment*	Galls Plant^{-1}	Females Plant^{-1}	R. factor* root + soil
M.incognita(Mi)	T1	175.1	60.1	28.5
M .incognita	T2	229.5	70.9	21.3
M .incognita	T3	300.0	100.4	15.2
*Rh +Pl****+*Mi* 500	T4****	155.5	41.0	26.7
*Rh+Pl+Mi*1000	T5	211.7	55.0	19.1
Rh +Pl+Mi 2000	T6	275.3	70.4	13.0
Rh+Pl → Mi 500	T7	117.8	30.2	21.5
Rh+*Pl* →Mi1000	T8	174.9	40.1	17.3
R*h+Pl →Mi*2000	T9	220.1	50.2	11.4
Mi 500 → *Rh+Pl*	T10	167.4	42.3	26.3
Mi 1000→*Rh+Pl*	T11	218.7	63.1	12.1
Mi 2000→*Rh+Pl*	T12	288.7	80.0	14.3
C.D.(P≤ 0.05)		8.4	5.9	1.0

*T_1 = Control T_1 = 500 J_2 alone T_2 =1000 J_2 alone T_3 =2000 J_2 alone T_4, T_5 &T_6= Simultaneously, T_7,$T_8$$T_9$$T_{10}$ = Sequential; Data are the mean of three replicates; **Sequential inoculation (after an interval of 10 days); ****Pl= Paecilomyces lilacinus;* ***** Simultaneously

Application of *Bradyrhizobium* and *Paecilomyces liacinus* prior to or simultaneously with nematode resulted in comparatively decreased rate of nematode multiplication (R) and gall development on the roots than in treatments where bacteria and fungus application followed by nematode inoculation. All the inoculum levels showed significant (P=0.05) difference in reproduction factor and number of galls formed on the root system, similar observation was found in simultaneous or sequential inoculation of all the treatments (Table 2). Nitrogen content of root and shoot reduced significantly (P=0.05) in all nematode inoculated plants at all inoculum levels. The nitrogen content of root and shoot significantly (P=0.05) increased in all the treatments where bacteria was applied along with the nematode inoculation and prior to the nematode inoculation. A significant (P=0.05) decrease in nitrogenase activity of nodules was observed in nematode inoculated plants, however reduction in nitrogenise activity was directly proportional to increase in nematode population. Nitrogenase activity was its peak in the nodules of plants treated with *Bradyrhizobium* alone. In nematode inoculated plants, nitrogenase activity was higher, in plants treated with *Bradyrhizobium* and *P. lilacinus* along with nematode inoculation and when applied prior to nematode inoculations, however nitrogenase activity was non-significant (P=0.05) different in treatments where bacteria was applied 10 days after nematode inoculation (Table 1).

DISCUSSION

The black gram plants remained stunted and showed poor growth response in absence of *Bradyrhizobium and* fungus *P. lilacinus*, however both these biocontrol agents resulted in increased plant growth and lesser damage to nematode inoculated plants there by indicates that the incorporation of *Bradyrhizobium* and *P. lilacinus* is beneficial for plant growth by increasing mineral uptake of plants and escaping the damage from pathogens (Lin *et al.*, 1983; Kiewnick and Sikora, 2006; Siddiqui, 2006). *Bradyrhizobium* provided increased nitrogen needed for better growth of plant, because it activates expression of noduation (nod) genes in rhizobia (Lhuissier *et al*., 2001). It can be justified by increased nitrogen contents in shoot and root in bacterial treated plants. The principal effect of *M. incognita* was reduction in plant growth an which was improved and the adverse effects of nematode were reduced by *Bradyrhizobium* and *P. lilacinus* as reported earlier (Siddiqui and Husian, 1992; Fazal, 1993; Siddiqui and Ehteshamul-Haque, 2001; Bhat *et al*., 2009). Improvement in plant growth may be due to increased rate of nitrogen fixation and decrease in reproduction rate by inhibiting hatching of eggs

during secondary infection. *Bradyrhizobium* and *P. lilacinus* resulted in least damage to plants, whereas, the damage was of intermediate order in simultaneous application. These findings are in conformity with Kokalis - Burelle *et al.,* 2002; Hashem and Abo-Elyousr, 2010; Tian *et al.* 2007. It seems that earlier establishment of bacteria and *P. lilacinus* protected the plant, in contrary to it, earlier establishment of nematode carried out certain mandatory physiological and biochemical damage, for its own favour inside the host tissue which could not be repaired to greater extent by treating plants with plant growth promoting rhizobacteria and biological control agents after nematode got established. Number of nodules on primary and secondary roots in all treatments was decreased by nematode inoculations as length of roots was shortened by them, occasionally and very few nodules are formed on galls (Hussey and Barker, 1976; Raut, 1980; Spanik, 2000). Ali *et al*. (1981) reported mature females, juveniles and egg masses on nodules on root system of cow pea plants. The decreased number of nodules may be due to short size of the root system. These findings are in conformity with Taha and Raski, 1969; Verdego *et al.* 1988 and Bhat *et al*., 2009. Consequently, it has been suggested that multiple inoculation strategies, in which different microorganisms with different mechanisms of action, are used could enhance biocontrol activity (Siddiqui and Akhtar, 2008; Mendoza and Sikora, 2009). Increased nodulation in treatments with prior application of *Bradyrhizobium* and *P. lilacinus* and decreased nodule formation in simultaneous or delayed applications proves that development of nodules also depends on release of bacteria from infection threads also development of bacterial and host mitotic activity, all of which are affected by phytochrome concentrations and translocations of nutrients. All these systems are disrupted by sedentary endo-parasite, *M. incognita* and thus may hamper nodule development and its formation as well as root growth. *M. incognita* altered the proper functioning of root system and in turn abnormal development of nodules in inoculated plants, thus nitrogenase activity in deformed nodules as well as nitrogen uptake of abnormal root was reduced, which ultimately led to stunted shoot (Ambreen *et al*. 2012). The dinitrogen is reduced to ammonia by the enzyme nitrogenase and the reduced nitrogenase activity forms the basis for reduced fixations of dinitrogen (Smith, 1949; Chahal and Rewari, 1977; Kimenju, 1999; Coyne and Oyekanmi, 2007). Many of the changes in nodulation and associated variation can be interpreted as responses compensating for an unsatisfied demand for nitrogen in plant. It is also reported that rhizobia form intimate symbiotic relationships with legumes by responding chemo - tactically to flavonoid molecules released as signals by the legume host. These plant compounds induce the expression of nodulation (*nod*) genes in rhizobia, which in turn produce lipo-chito-oligosaccharide (LCO) signals that trigger mitotic cell division in roots, and leading to nodule formation (Lhuissier *et al*., 2001 and Damiani *et al.,* 2012). Hence, it may be concluded as microbial inoculants can be used as components in integrated approaches for managing diseases and changing microbial population dynamics in the rhizosphere as well as the suitable combinations of biocontrol agents can further increase the plant growth and resistance to pathogens.

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