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EFFECT OF SEED TREATMENT WITH CERTAIN BACTERIA AND FUNGI ON THE GROWTH OF MUNGBEAN AND REPRODUCTION OF *MELOIDOGYNE INCOGNITA*

by
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Summary. The effect of *Paecilomyces lilacinus*, *Verticillium chlamydosporium*, *Cylindrocarpon destructans*, *Arthrobotrys oligospora*, *Bacillus subtilis*, *Beijerinkia indica*, *Azotobacter chroococcum* and *Azospirillum lipoferum* on the growth of mungbean, root nodulation and root-knot disease caused by *Meloidogyne incognita* was tested in field microplots. Application of *B. indica* or *A. lipoferum* significantly increased the shoot dry weight of uninoculated plants. The nematode caused significant decreases in plant growth and root nodulation. Application of bacterial and fungal bioagents controlled nematode pathogenesis leading to a decrease of 4.8-26.5, 7.2-30.1 and 11.8-27.6% in the number of galls, egg masses/root system and J2/kg soil, being greatest in the treatments with *A. lipoferum* or *Azotobacter chroococcum*. Plant growth and the biomass of nematode inoculated plants were significantly increased with all the bacteria tested, being greatest with *B. subtilis*. Bacterial and fungal bioagents synergised root nodulation, leading to 21-57.8% increase in the number of functional nodules on nematode infected roots.

Fungi viz., *Paecilomyces lilacinus* (Jatala, 1985), *Verticillium chlamydosporium* (De Leij *et al.*, 1993), *Arthrobotrys oligospora* (Barron, 1975), *Cylindrocarpon destructans* (Tribe, 1979) and *Dactylella oviparasitica* (Stirling and Mankau, 1978) have demonstrated their considerable potential to parasitise eggs and/or juveniles of various plant parasitic nematodes. However, these fungi have failed to produce satisfactory control of plant parasitic nematodes in field conditions.

Plant growth promoting organisms enhance plant growth and productivity by solubilising vital nutrients such as phosphate and nitrogen, and making them available to the plant roots (Gaur, 1990). Some of them also produce toxins such as avermectin, *Streptomyces avermitilis* (Stertton *et al.*, 1987), bulbiformin, *Bacillus subtilis* (Vasudeva *et al.*, 1958) and α-endo-toxin, *B. thuringiensis* (Mankau, 1981), which may antagonise plant parasitic nematodes.

In the present investigation, the effect of true parasites of nematodes and of plant growth promoting organisms were tested as seed treatments on plant growth and root nodulation of mungbean and on root-knot disease in a field microplot experiment.

Materials and methods

Sixty microplots, each 1.5x1.5 m, were prepared in a field plot with loamy soil in which 20 treatments (Table I) were maintained to examine the effect of the nematode parasitic fungi, *Arthrobotrys oligospora* Fresh, *Cylindrocarpon destructans* (Zinssmeister) Scholten, *Verticillium chlamydosporium* Goddard and *Paecilo-*

myces lilacinus (Thoms.) Samson and the plant growth promoting bacteria *Azospirillum lipoferum* Beijerinck, *Azotobacter chroococcum* Beijerinck, *Beijerinkia indica* Banerjee and *Bacillus subtilis* Cohn emend. Prazmowski on the plant growth of mungbean, nodulation and on the root-knot disease caused by *Meloidogyne incognita* (Kofoid *et* White) Chitw. Pure cultures of the microorganisms were obtained from the Institute of Microbial Technology, Chandigarh, India. The culture of *P. lilacinus* was obtained from the Indian Agricultural Research Institute, New Delhi. Sub-culturing and rearing of bacterial bioagents were done on specific media (Subba Rao, 1975). Mass cultures of fungal bioagents i.e. *P. lilacinus. A. oligospora, V. chlamydosporium* and *C. destructans* were prepared on Richard's liquid medium, corn meal agar, potato carrot agar and potato sucrose agar, respectively. To compare efficacy of the microorganisms, a treatment with fenamiphos at 200 ppm (20 g a.i./ha) was also applied.

Before sowing, the nematode suspension was added at nine spots in a microplot. At each of the spots, a suspension containing 1500 J2 was added to the soil and mixed in an area of 10 cm diameter and 15 cm depth. Next day, seeds of mungbean, *Vigna radiata* (L.) Wilczek var. T-44, treated with *Rhizobium* and fungal/bacterial bioagents were sown in each spot. For application, the seeds were first coated with 2% sucrose solution followed by *Rhizobium* and bioagents. For nematicide treatments, the seeds were first dipped in fenamiphos solution in water (200 ppm) for 5 min and then were treated with the *Rhizobium.* Immediately after sowing, 0.75 kg diammonium phosphate and 0.25 kg urea were broadcasted on the 60 microplots. Within a week, the seedlings emerged and were irrigated 15 days after sowing. After irrigation, the seedlings were thinned to one per spot. The plots were irrigated a further six times at fortnightly intervals. Weeds were removed manually at 15 days intervals. Fifteen weeks after sowing, five alternate plants from each microplot were harvested, averaged and considered as one replicate. Length, fresh and dry weight of shoots, number of functional (pink coloured), non-functional (brown coloured) and total nodules, galls and egg masses per root system and soil population of *M. incognita* were determined. The data on plant growth variables and nodules were subjected to a two factor analysis of variance (ANOVA) and Critical Difference (CD) was calculated for each variable at the probability level of 0.05. The data on galls, egg masses and soil population were analysed by a single factor analysis of variance and C.D. was calculated (Dospekhov, 1984). To identify a significant treatment, uninoculated (without nematodes) plants treated with the microorganisms were compared with uninoculated-untreated plants (control). Similarly, inoculated and treated plants were compared with the inoculated-untreated plants (control).

Results and discussion

Application of *A. lipoferum* on the seeds of mungbean led to a significant increase in the length of shoots in comparison with untreated plants (Table I). Biomass production (shoot dry weight) was increased by 21 and 13% due to the application of *B. indica* and *A. lipoferum,* respectively. Inoculation with 1500 J_2 of *M. incognita* caused significant decline in the plant growth variables compared to the control (Table I). Application of bioagents reduced the adverse effect of the nematode and increased plant growth of mungbean. The greatest enhancement in the shoot length of nematode-inoculated plants was associated with the application of *A. chroococcum* (15%), followed by *B. subtilis* (and *P. lilacinus* (12%) and *B. indica* (8%) compared with the inoculated untreated plants. Fresh weight of shoots was significantly increased in the treatments with *A. chroococcum, B. indica, B. subtilis* and *A. lipoferum.* Relatively smaller increases (10-13%) occurred

TABLE I - *Effect of seed treatment with certain fungal and bacterial antagonists on the plant growth of mungbean in the presence and absence of* Meloidogyne incognita.

Antagonist	Shoot length cm	Fresh weight g	Dry weight g
Nematode absent			
Control	103	165	31.5
Arthrobotrys oligospora	101	163	31.0
Verticillium chlamydosporium	104	164	31.0
Cylindrocarpon destructans	103	161	29.8
Paecilomyces lilacinus	102	167	29.5
Azotobacter chroococcum	105	172	33.5
Bacillus subtilis	105	169	33.1
Azospirillum lipoferum	110*	167	35.5*
Beijerinkia indica	109	166	38.0*
Fenamiphos	102	162	30.0
Nematode present			
Control	89*	140*	27.1*
A. oligospora	93	145	28.0
V. chlamydosporium	95	149	29.3*
C. destructans	95	146	28.5
P. lilacinus	99*	143	28.4
A. chroococcum	102*	167*	29.1*
B. subtilis	100*	163*	30.5*
A. lipoferum	96*	163*	29.7*
B. indica	96*	164*	30.1*
Fenamiphos	94	159*	29.4*
C.D. at P=0.05	6.9	13.7	2.2
F-value			
Antagonists (df=9)	NS	5.0*	5.1*
Nematode (df=1)	22.2*	45.9*	4.9*
Interaction (df=9)	4.2*	NS	NS

* Significantly different from the respective controls at P=0.05.

in the dry weight of shoots due to *B. subtilis*, *B. indica* and *A. lipoferum*. Among the fungal bioagents, only *V. chlamydosporium* significantly promoted the dry weight of shoots of mungbean inoculated with *M. incognita*. Nematicide application resulted in a 14% increase in the fresh weight of shoots (Table I). The two factor analysis of variance revealed that overall the nematode significantly reduced plant growth. Individual effects of the antagonists was significant for the dry weight of shoots. Overall application of the microorganisms significantly increased the length and dry weight of shoots (Table I).

Seed treatments with the antagonists led to the promotion of root nodulation in the plants

inoculated with *M. incognita* and in uninoculated plants. The numbers of total and functional nodules per root system were significantly increased due to the application of the bacteria in the presence or absence of root-knot nematode, being greatest with *A. chroococcum* (with nematode) and *B. indica* or *A. lipoferum* (without nematode) compared to the respective controls (Table II). Their application also led to a significant decrease in the number of non-functional nodules. Nematicide application caused a significant inhibition in nodule formation. Rhizobia are quite sensitive to nematicides. Application of fenamiphos can decrease their population in soil

TABLE II - *Effect of seed treatment with certain fungal and bacterial antagonists on the root nodulation of mungbean in the presence and absence of* M. incognita.

Antagonist	Number of nodules		Total
	functional	non-functional	
Nematode absent			
Control	30	6	36
Arthrobotrys oligospora	29	6	35
Verticillium chlamydosporium	29	6	35
Cylindrocarpon destructans	31	5*	36
Paecilomyces lilacinus	28	6	34
Azotobacter chroococcum	34*	5*	39
Bacillus subtilis	33*	5*	38
Azospirillum lipoferum	38*	4*	42*
Beijerinkia indica	38*	5*	43*
Fenamiphos	24*	5*	29*
Nematode present			
Control	18*	7*	25*
A. oligospora	23*	4*	27
V. chlamydosporium	24*	5	29*
C. destructans	23*	4*	27
P. lilacinus	24*	4*	28*
A. chroococcum	30*	4*	34*
B. subtilis	32*	5	37*
A. lipoferum	27*	5	32*
B. indica	30*	3*	33*
Fenamiphos	17*	6*	23
C.D. at P=0.05	2.02	0.97	2.60
F-value			
Antagonists (df=9)	57.9*	NS	93.3*
Nematode (df=1)	46.9*	NS	50.4*
Interaction (df=9)	41.8*	NS	89.2*

* Significantly different from the respective controls at P=0.05.

leading to decrease in root nodulation (Clarkson *et al.*, 1982). Nematode infection decreased by 40 and 31% the numbers of functional and total nodules, respectively (Table II). Non-functional nodules were, however, increased by 17%. Seed treatment with all the fungal and bacterial antagonists resulted in significant increase in the nodulation of nematode inoculated plants compared to the nematode inoculated control. The greatest increase in the number of total (48%) and functional (60%) nodules was recorded with *B. subtilis* (Table II). According to two factor ANOVA, individual and interactive effects of antagonists and nematode were significant for total and functional nodules.

Application of antagonists adversely affected the nematode pathogenesis, leading to considerable decrease in gall formation, egg mass production and soil population (Table III). Application of *A. chroococcum*, *A. lipoferum*, *B. subtilis*, *B. indica* and fenamiphos led to a significant decrease in the number of galls per root system with a decrease of 21-27%. Egg mass production was significantly suppressed with all the antagonists, except *A. oligospora*, *V. chlamydosporium* and *C. destructans*. Maximum decrease in number of egg masses per root system occurred due to *A. lipoferum* (30%), followed by *B. subtilis* and *B. indica* (27%). Application of *P. lilacinus* significantly suppressed egg mass production. Significant decline in the soil population of *M. incognita* was recorded with all the treatments, except *A. oligospora* and *V. chlamydosporium*. The most suppressive treatment in this regard was *A. chroococcum* with a decrease of 28%. Single factor ANOVA revealed significant F-values for the galls, egg masses and soil population at P=0.05 (Table III).

The present study has revealed that soil application of plant growth promoting microorganisms, especially *B. subtilis* or *A. chroococcum*, may decrease root-knot disease, but the microorganisms alone may not fully control the disease in field conditions.

TABLE III - *Effect of seed treatment with certain fungal and bacterial antagonists on soil population, gall formation and egg mass production of* M. incognita *on mungbean.*

Antagonist	Soil population (Pf) (J_2/kg)	Galls	Egg-masses
Control	1851	83	83
Arthrobotrys oligospora	1835	79	77
Verticillium chlamydosporium	1741	76	75
Cylindrocarpon destructans	1566*	78	77
Paecilomyces lilacinus	1611*	78	65*
Azotobacter chroococcum	1341*	61*	61*
Bacillus subtilis	1466*	65*	60*
Azospirillum lipoferum	1475*	61*	58*
Beijerinkia indica	1502*	63*	60*
Fenamiphos	1602*	65*	68*
C.D. at P=0.05	234.7	7.8	9.8
F-value			
Control agents (df=9)	292.38*	86.03*	73.60*

* Significantly different from the respective controls at P=0.05.

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