EFFECT OF CERTAIN RHIZOBACTERIA AND ANTAGONISTIC FUNGI ON ROOT-NODULATION AND ROOT-KNOT NEMATODE DISEASE OF GREEN GRAM

M.R. Khan, K. Kounsar and A. Hamid

Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202 002, India

Summary. Effect of soil application of rhizobacteria (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus subtilis* and *Beijerinkia indica*), antagonistic fungi (*Arthrobotrys oligospora*, *Cylindrocarpon destructans*, *Verticillium chlamydosporium* and *Paecilomyces lilacinus*) and fenamiphos on root nodulation, plant growth, biomass production and gall formation and reproduction of *Meloidogyne incognita* were investigated on green gram in field trails. Application of rhizobacteria resulted in a significant increase in the numbers of functional nodules (pink coloured) per root system of nematode inoculated or uninoculated plants; numbers of nonfunctional nodules (brown coloured and degenerated) were decreased. Application of the rhizobacteria significantly increased dry weight of shoots of uninoculated plants. Growth of nematode inoculated plants was enhanced due to application of rhizobacteria and *P. lilacinus*. Treatment with *B. subtilis* or *B. indica* reduced galling by 33-34% and increased the dry weight of shoots by 22-24%, respectively. Corresponding values for the nematicide (fenamiphos) were 43 and 14%, respectively. Egg mass production and soil populations of *M. incognita* were more adversely affected. Maximum decrease in the soil population, 84%, occurred due to *A. lipoferum*.

Green gram, *Vigna radiata* L. Wilczek is an important grain pulse crop in India, and an excellent source of high quality protein. *Meloidogyne* species cause important diseases to green gram (Sikora and Greco, 1990) and are responsible for approximately 28% or more yield loss (Bridge, 1981). Four species (*M. incognita*, *M. javanica*, *M. hapla* and M. *arenaria*) attack green gram. *M. incognita* and *M. javanica* are most important because green gram is extensively cultivated in tropical and sub-tropical countries where both species are prevalent (Sasser, 1979).

Recently, there has been growing interest in the use of rhizobacteria as plant growth promoting organisms (PGPOs) in place of inorganic fertilizers. PGPOs such as *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Beijerinkia indica*, *Bacillus subtilis*, *Pseudomonas stutzeri* etc. solubilise nitrogen and/or phosphorus in the soil and supply them to plant roots in much greater amount than in their absence (Gaur, 1990; Rao, 1990). These organisms can also be used in nematode disease management; some of them produce toxins such as bulbifomin by *B. subtilis* (Vasudeva *et al.*, 1958). Suppressive effects of *M. incognita* on tomato by *B. subtilis*, *P. stutzeri* (Khan and Tarannum, 1999), *P. fluorescens* and *B. polymyxa* (Khan and Akram, 2000) have been observed. Fungi like *Arthrobotrys*, *Dactylaria*, *Monacrosporium* (Mankau, 1980), *Paecilomyces lilacinus* and *Verticillium chlamydosporium* (Stirling, 1991) may suppress nematode populations through predation and/or parasitism.

In the present investigation, relative efficacy of parasites of nematodes (*A. oligospora*, *P. lilacinus*, *V. chlamydosporium* and *C. destructans*) and PGPOs (*A. chroococcum*, *A. lipoferum*, *B. subtilis* and *B. indica*) through soil application was evaluated against a root-knot disease caused by *M. incognita* on green gram in a field trials for two consecutive years. Effects of the microorganisms were also examined on root nodulation, plant growth and on biomass production.

MATERIALS AND METHODS

Sixty microplots, each 1.5 x 1.5 m dimension were prepared in a field of 33 x 5 meters. Twenty treatments were conducted to examine the effect of the microorganisms on plant growth of green gram, nodulation and on the root-knot disease caused by *M. incognita*. Treatment with the nematicide fenamiphos at the rate of 4 kg ai/ha was applied for comparison of results. The nematicide was applied to the soil through drenches after nematode inoculation. The twenty treatments are listed in Table II. Each treatment was replicated three times and randomly distributed in the field.

Cultures of *M. incognita* (Kofoid *et* White) Chitw. were prepared from egg masses, incubated on tissue paper on a wire guaze (coarse sieve) placed in a Baermann funnel in a B.O.D. incubator at 25 °C for 6-8 days. The nematode suspension was diluted to 1500 second stage juveniles (J_2) in 100 ml of water which was injected 15 cm deep and 10 cm dia. in eighteen locations in microplots (6 injection sites/row, 3 rows/plot). The inoculation was made one day prior to sowing seed of green gram.

Pure cultures of rhizobacteria (*Azotobacter chroococcum* Beijeririck, *Azospirillum lipoferum* Beijerinck, *Bacillus subtilis* Cohn *emend.* Prazmowski and *Beijerinkia in-*

Table I. Effect of soil application of rhizobacteria and antagonistic fungi on soil population, gall formation and egg mass production of *Meloidogyne incognita* on green gram.

Microorganisms	Nematode (Pi)	Soil Population (Pf)	Galls	Eggmasses (root system)
Control	1500	1851	83	83
Arthrobotryis oligospora	1500	1819	78	76[a]
Verticillium chlamydosporium	1500	1206[a]	74[a]	74[a]
Cylindrocarpon destructans	1500	1396[a]	78	76[a]
Paecilomyices lilacinus	1500	1589[a]	71[a]	56[a]
Azotobacter chroococcum	1500	824[a]	62[a]	60[a]
Bacillus subtilis	1500	638[a]	55[a]	53[a]
Azospirillum lipoferum	1500	304[a]	59[a]	57[a]
Beijerinkia indica	1500	659[a]	54[a]	53[a]
Fenamiphos	1500	505[a]	47[a]	46[a]
L.S.D. at P = 0.05 *F*- value		**258.76**	**8.55**	**6.2**
Microorganisms (df = 9)		**400.6[b]**	**101.62[b]**	**142.21[b]**

[a] significantly different from the respective controls at P = 0.05;
[b] significant at P = 0.05;
NS not significant at P = 0.05.

dica Banerjee) were obtained from the Institute of Microbial Technology, Chandigarh, India. Massculture of these microorganisms for field inoculation were made in culture tubes containing specific media broth recommended for these microorganisms (Subba Rao, 1975). Just before sowing the seed 1 ml of bacterial broth in 100 ml of water was added to each of the eighteen sites in microplots designated to receive the bacterial culture.

The nematode fungal parasites, *Arthrobotrys oligospora* Fres., *Cylindrocarpon destructans* (Zinssmeister) Scholten and *Verticillium chlamydosporium* Goddard were obtained from the Institute of Microbial Technology, Chandigarh and *Paecilomyces lilacinus* (Thoms.) Samson from the Indian Agricultural Research Institute, New Delhi. Sub-culturing of *P. lilacinus*, *A. oligospora*, *V. chlamydosporium* and *C. destructans* was done in culture tubes with potato dextrose agar, corn meal agar, potato carrot agar and potato sucrose agar, respectively. Inoculum for field application was prepared in appropriate liquid media, except *P. lilacinus* which was reared in Richard's liquid medium. Liquid suspensions of fungi containing 1 g spores + mycelium were mixed in 100 ml of water and added to each of the eighteen sites in microplots and mixed in the soil as done with nematode suspensions just before sowing seed.

Green gram cv. T-44 seeds treated with commercial green gram strain of *Rhizobium* were sown at 18 sites (3-4 seeds/sites) in each of the sixty microplots. Immediately after sowing 0.75 kg diammonium phosphate and 0.25 kg urea was broadcast in the entire field (33 x 5 m). Fifteen days after sowing, the emerged seedlings were irrigated. Thereafter, plants were thinned to one seedling at each site i.e., 6 seedlings/row, 18 seedlings/microplot. Three and a half months after sowing, nine alternate plants from each microplot were harvested to determine the length of shoot, fresh shoot weight (excluding pods), shoot dry weight and number of functional (pink) or non functional(brown), galls and egg masses/root system. Soil populations of *M. incognita* juveniles were estimated from the top soil (rhizospheric) collected from the nine plants harvested (Southey, 1986).The experiment was conducted during two consecutive years under identical conditions.

Nine plants were randomly harvested from each microplot to provide three replicates for each treatment. Since the experiment was conducted during two consecutive years, data from one year (27 plants/treatment, 9 plants/microplot) were averaged and considered as one replicate. Thus, there were two replicates which were analysed for variance. The data on plant growth variables and nodules were subjected to a two factor analysis of variance (ANOVA), and galls, egg masses and soil population were analysed by a single factor analysis of variance. Critical Difference (CD) was calculated for each variable at the probability level of P 0.05 (Dospekhov, 1984).

RESULTS AND DISCUSSION

Nematode inoculation alone resulted in extensive galling on roots of green gram. Treatments with the experimental microorganisms except *A. oligospora* and *C. destructans* suppressed gall formation (Table I). Decline in gall formation was greatest due to the nematicide

treatment (43.4%), followed by *B. indica* (34.90%), *B. subtilis* (33.7%), *A. lipoferum* (28.9%), *A. chroococcum* (25.3%), *P. lilacinus* (14.5%) and *V. chlamydosporium* (10.8%) compared to untreated control. Egg mass production was significantly inhibited due to application of all the microorganisms. Fenamiphos treatment resulted in maximum decline in the egg masses, followed by *B. indica* and *Bacillus subtilis* (36,1%) and lowest due to *A. oligospora* or *C. destructans* (8.4%) (Table I). Soil populations of root-knot nematodes were adversely and significantly affected due to application of the microorganisms, except *A. oligospora*. Percent decrease in soil populations was greater than the decrease in galls or egg masses. Highest decrease in nematode juveniles/kg soil occurred with *A. lipoferum* (83.6%), followed by *A. chroococcum* (55.5%), *V. chlamydosporium* (34.8%), *C. destructans* (24.6%) and *P. lilacinus* (14.2%) compared to the control.

Application of all the rhizobacteria significantly promoted the dry weight of shoot of uninoculated plants (Table II). Shoot length was, however, increased only with the application of *B. indica*. Nematode infection alone suppressed the length (13.6%), fresh weight (15.9%) and dry weight (14.0%) of green gram plants. Shoot length, fresh and dry weights were significantly increased with application of rhizobacteria and nematicide compared with the inoculated control (Table II). Treatment with *P. lilacinus* significantly promoted the shoot length of nematode

factor ANOVA revealed individual significant effects of nematode for all the variables and of microorganisms for fresh and dry weights. The interactive effect of microorganisms and nematode was also significant for fresh and dry weights of shoot (Table II). Significant decrease in the root-knot disease and increase in root nodulation due to application of antagonists led to a corresponding increase in the biomass production of green gram. Similar effects of *B. subtilis*, *Pseudomonas*

Table II. Effect of soil application of rhizobacteria and antagonistic fungi on plant growth and dry matter podulation of green gram in the presence and abscence of *M. incognita*.

Microorganisms	Nematode	Shoot length cm	Total weight g fresh	dry
Control	-	102.7	165.9	31.5
Arthrobotrys oligospora	-	102.8	162.0	31.4
Verticillium chlamydosporium	-	101.5	163.1	31.2
Cylindrocarpon destructans	-	101.9	162.9	31.0
Paecilomyces lilacinus	-	102.0	164.0	30.2
Azotobacter chroococcum	-	108.5	168.0	34.4[a]
Bacillus subtilis	-	107.5	170.8	36.9[a]
Azospirillum lipoferum	-	109.5	169.5	34.5[a]
Beijerinkia indica	-	110.5[a]	167.8	35.7[a]
Nematicide	-	100.9	159.3	29.6
Control	1500	88.7[a]	139.5[a]	27.1[a]
A. oligospora	1500	91.0	143.0	28.6
V. chlamydosporium	1500	93.7	145.0	29.0
C. destructans	1500	94.9	144.9	28.0
P. lilacinus	1500	96.2[a]	145.8	28.9
A. chroococcum	1500	97.5[a]	165.0[a]	32.5[a]
B. subtilis	1500	102.0[a]	169.2[a]	33.7[a]
A. lipoferum	1500	98.9[a]	165.0[a]	32.1[a]
B. indica	1500	96.0[a]	164.3[a]	33.0[a]
Nematicide	1500	96.5[a]	155.4[a]	30.8[a]
L.S.D. at P = 0.05 *F*- value		**7.3**	**14.3**	**2.25**
Microorg.anisms (df = 9)		**NS**	**8.66[b]**	**4.15[b]**
Nematode (df = 1)		**57.34[b]**	**59.22[b]**	**18.70[b]**
Interaction (df = 9)		**NS**	**3.65[b]**	**3.89[b]**

[a] significantly different from the respective controls at P = 0.05;
[b] significant at P = 0.05;
NS not significant at P = 0.05.

stutzeri and *P. fluorescens* on the root-knot disease of tomato have also been recorded (Khan and Tarannum, 1999; Khan and Akram, 2000).

Nodulation induced by *Rhizobium* sp. was promoted by the rhizobacteria, leading to 13.8 (*A. chroococcum*) and 8.3% (*A. lipoferum*) increase in nodules/root system of uninoculated plants (Table III). Significant increase in functional nodules was recorded with *A. chroococcum*, *Azospirillum lipoferum* and *B. indica*. Nematicide application decreased the total and functional nodules by 11.1 and 17.2%, respectively (Table III). Nematode infection caused 34.4 and 30.5% decrease in functional and total nodules/root system, respectively compared to the uninoculated control. Infection by *M. incognita* on green gram roots resulted in 30% decrease in the rhizobial nodules. A similar adverse effect of root-knot nematodes on nodule formation has been recorded on chickpea (Khan *et al.*, 1996), cowpea (Khan and Khan, 1996), soybean (Kabi, 1983) and pigeon pea (Taha, 1993). Root-knot nematodes also invade nodules and form galls on them (Melakebrhan and Webster, 1993). Such nodules degenerate and are shed much earlier than the normal ones (Ali *et al.*, 1981). In the present study, 5-10% nodules/root system were invaded by the nematode as evident by the presence of a small gall. This would have been an explanation for lower number of nodules on nematode infected plants. Application of the microorganisms, however, greatly enhanced the nodulation on nematode inoculated plants. The greatest increase in functional nodules was recorded with *A. lipoferum* (57.8%), followed by *A. chroococcum* (47.3%), *B. indica* (42%), *B. subtilis* (36.8%), *V. chlamydosporium* (26.3%), *P. lilacinus* and A. *oligospora* (21.1%) in comparison to nematode inoculated control. Total number of nodules was significantly increased due to the application of the microorganisms used, except *C. destructans* and P. *lilacinus*. The number of non-functional nodules was significantly decreased due to the ap-

Table III. Effect of soil application of rhizobacteria and antagonistic fungi on the root nodulation of green gram in the presence and abscence of *M. incognita*.

Microorganisms	Nematode	Number of Nodules per root system		
		Functional	Non-functional	Total
Control	-	29	7	36
Arthrobotrys oligospora	-	29	7	36
Verticillium chlamydosporium	-	29	6	35
Cylindrocarpon destructans	-	27	7	34
Paecilomyces lilacinus	-	29	6	35
Azotobacter chroococcum	-	34[a]	7	41[a]
Bacillus subtilis	-	28	6	34
Azospirillum lipoferum	-	33[a]	6	39[a]
Beijerinkia indica	-	33[a]	5[a]	38
Nematicide	-	24[a]	8[a]	32[a]
Control	1500	19[a]	6[a]	25[a]
A. oligospora	1500	23[a]	5	28[a]
V. chlamydosporium	1500	24[a]	5	29[a]
C. destructans	1500	22[a]	5	27
P. lilacinus	1500	23[a]	4[a]	27
A. chroococcum	1500	28[a]	4[a]	32[a]
B. subtilis	1500	26[a]	3[a]	29[a]
A. lipoferum	1500	30[a]	3[a]	33[a]
B. indica	1500	27[a]	4[a]	31[a]
Nematicide	1500	20	6	26
L.S.D. at P = 0.05 *F*- value		**2.0**	**1.14**	**3.0**
Microorg.anisms (df = 9)		**13.57[b]**	**NS**	**25.4[b]**
Nematode (df = 1)		**33.39[b]**	**NS**	**50.4[b]**
Interaction (df = 9)		**6.50[b]**	**NS**	**17.6[b]**

[a] significantly different from the respective controls at P = 0.05;
[b] significant at P = 0.05;
NS not significant at P = 0.05.

plication of bacteria and *P. lilacinus*. According to ANOVA, single and interactive effects of the microorganisms and nematode were significant for functional and total nodules (Table III).

The study has demonstrated the potential scope of growth-promoting rhizobacteria in nematode disease management. Application of *B. subtilis* or *B. indica* greatly suppressed the nematode disease and its reproduction and significantly promoted the plant growth and dry matter production of green gram. Both these bacteria performed better overall than the nematicide (fenamiphos), suppressing the galling by 33-34% and increasing dry matter production of green gram by 22-24% against 44% and 13.6% of the nematicide, respectively.

LITERATURE CITED

Ali M.A., Trabulsi J.Y. and Abd-Elsamea M.E., 1981 - Antagonistic interaction between *Meloidogyne incognita* and *Rhizobium leguminosarum* on cowpea. *Plant Disease, 65*: 432-435.

Bridge J., 1981 - Nematodes. Pp. 111-25. In : *Nematode Pest Control in Tropical Grain Legumes Center for Overseas Pests Research*, Ward, A., Mercer, S.L. and Howe, V. (eds), London.

Dospekhov B.A., 1984 - *Field Experimentation.* Mir Publishers Moscow, Russia, 352 pp.

Gaur C. 1990 - *Phosphate Solubilising Microorganisms as Biofertilizers.* Omega Scientific Publishers, New Delhi, 176 pp.

Kabi M.S., 1983 - Rhizobial interaction in the rhizosphere of soybean for nodule sites. *Zentralblat Mikrobialogie, 138*: 269-275.

Khan M.R. and Akram M. 2000 - Effect of certain antagonistic fungi and rhizobacteria on wilt disease complex caused by *Meloidogyne incognita* and *Fusarium oxysporium* f. sp. *lycopersici* on tomato. *Nematologia Mediterranea, 28*: 139-144.

Khan M.R. and Khan M.W., 1996 - Development of root-knot nematodes on cowpea as influenced by sulphur dioxide. *Nematologia Mediterranea, 24*: 33-35.

Khan M.R., Khan M.W. and Sing K., 1996 - Effects of nickel and root-knot nematode on the growth and protein content of chickpea. *Nematologia Mediterranea, 24*: 87-90.

Khan M.R. and Tarranum Z., 1999 - Effect of field application of various microorganisms on the root-knot disease of tomato. *Nematologia Mediterranea, 27*: 33-38.

Mankau R., 1980 - Biological control of nematode pests by natural enemies. *Annual Review of Phytopathology, 18*: 415-440.

Melakeberhan H. and Webster L.M., 1993 - The phenology of plant-nematode interaction and yield loss. Pp. 26-41. In: *Nematode Interactions.* Khan M.W. (ed.), Chapman and Hall, London, U.K.

Rao A.B., 1990 - Role of microorganisms in plant nutrition under acid condition. Pp. 67-84. In: *Biofertilizers.* Vyas L.L. (ed.), Scientific Publ. Jaudpur.

Sasser J.N., 1979 - Economic importance of *Meloidogyne* in tropical countries. Pp. 359-374. In: *Root-knot nematodes (Meloidogyne species)-Systematics, Biology and Control.* Lamberti F. and Taylor C.E. (eds), Academic Press, NY, U.S.A.

Sikora R.A. and Greco N., 1990 - Nematode parasites of grain legumes. Pp. 181-236. In: *Plant Parasitic Nematodes in Subtropical and Trioical Agriculture.* Luc M., Sikora R.A. and Bridge J. (eds), C.A.B. International, Oxon, London, U.K.

Southey J.F., 1986 - *Plant Nematology.* Her Majestiy's Stationary office, London, U.K., 440 pp.

Stirling G.R., 1991 - *Biological Control of Plant Parasitic Nematodes.* C.A.B. International, Oxon, London, U.K., 282 pp.

Subba Rao N.S., 1975 - *Soil Microorganisms and Plant Growth.* Oxford and IBH, New Delhi, 335 pp.

Taha Y.A., 1993 - Nematode intractions with root-nodule bacteria. Pp. 170-202. In: *Nematode Interactions.* Khan, M.W. (ed.), Chapman and Hall, London, U.K.

Vasudeva R.S., Subbaiah T.V., Satry M.L.N., Rangaswamy G. and Iyengar M.R.S., 1958 - Bulbiformi, an antibiotic produced by *Bacillus subtilis. Annals of Applied Biology, 46*: 336-45.

Accepted for publication on 6 February 2002