Nematol. medit. (1992), *20*: 199-202

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BIOLOGICAL CONTROL OF ROOT-ROT DISEASE COMPLEX OF CHICKPEA CAUSED BY *MELOIDOGYNE INCOGNITA* RACE 3 AND *MACROPHOMINA PHASEOLINA*

by
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Summary. Four biocontrol agents viz. *Paecilomyces lilacinus, Acrophialophora fusispora, Bacillus licheniformis* and *Alcaligenes faecalis* were used in three doses for the control of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* on chickpea (*Cicer arietnum*). All the biocontrol agents except *A. faecalis* were found more effective in improving plant growth when used against *M. incognita* alone or with *M. phaseolina* but less effective against *M. phaseolina* alone. *A. faecalis* had an adverse effect on plant growth. *P. lilacinus* was the best biocontrol agent while *B. licheniformis* and *A. fusispora* were equally effective.

Chickpea (*Cicer arietinum* L.) is an economically important pulse crop in India which is susceptible to root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *Macrophomina phaseolina* (Tassi) Goid. Interaction of *M. incognita* race 3 and *M. phaseolina* has been reported to cause severe damage to the crop (Siddiqui and Husain, 1991).

As an alternative to chemical pesticides, investigations were undertaken to assess the efficacy of some biocontrol agents, namely *Paecilomyces lilacinus* (Thoms.) Samson, *Acrophialophora fusispora* (Saksena) Samson, *Bacillus licheniformis* (Weigmann) Chester emend. Gibson and *Alcaligenes faecalis* Castellani *et* Chalmers, for the control of root-rot disease complex of chickpea.

Materials and methods

Seeds of chickpea cv. P-256 were surface sterilized with 0.1% mercuric chloride for 2 min. and washed three times with sterile distilled water and treated with a chickpea strain of *Rhizobium* before sowing. Sucrose solution was used as sticker for the bacteria. Five bacteria-treated seeds were sown in each 15 cm earthen pot containing 1 kg steam sterilized soil. After germination, the seedlings were thinned to one per pot. One week after germination the pots were inoculated with juveniles of *M. incognita* and *M. phaseolina*. *M. incognita* was collected from a chickpea field and multiplied on eggplant (*Solanum melongena* L.) from a single egg-mass. *M. incognita* race was identified as race 3 using host differential tests (Taylor and Sasser 1978). Egg-masses were hand-picked using sterilized forceps and placed in a 10 cm diameter sieve mounted, with crossed layer of tissue paper. The sieve with egg-masses was placed for hatching in petri dish with water. A suspension of 2000 freshly hatched second stage juveniles was poured on to the finer roots of the seedling that had been exposed by removing the soil carefully.

M. phaseolina was isolated from chickpea roots and maintained on potato dextrose agar (PDA). Inoculum of the fungus was prepared by culturing the isolate in Richard's liquid medium for 15 days at 25°C. Mycelium was collected on blotting sheets and excess of water and nutrients were removed by pressing it between the two folds of the blotting sheets. One hundred g mycelium were macerated in 1 l distilled water and 10 ml of this suspension containing 1 g fungus were inoculated around the root after carefully removing the top layer of soil.

P. lilacinus and *A. fusispora* were also separately cultured on Richards liquid medium and inocula were prepared in the same way as previously described. Both fungi were used in three doses (0.5, 1.0 and 2.0 g). Cultures of *B. licheniformis* and *A. faecalis* were maintained on nutrient agar medium. A bacterial suspension was prepared containing $10x10^8$ bacterial cells. The bacterial cells in suspension were counted by preparing dilutions up to 10^{-7} and 0.1 ml suspension of each was then carefully spread on nutrient agar plates (dilution 10^{-6} and 10^{-7} separately). The plates were inculated at 37°C for 24 hours and bacterial colonies were counted. Five, 10 and 20 ml suspensions of each bacterial culture were inoculated around the exposed roots of the seedlings.

Each treatment was replicated four times and pots were watered when needed. The experiment was terminated 90 days after inoculation. Dry shoot weight, nodule number, root-knot and root-rot indices and nematode multiplication

were recorded. The soil nematode population was extracted by Cobb's sieving and decanting technique followed by Baermann funnel and numbers counted. The roots were cut into small pieces and mixed homogeneously and 1 g root was taken and comminuted for 45 seconds in a Waring blender and juveniles, eggs and females were counted. Root-knot index of Taylor and Sasser (Sasser *et al.*, 1984) on a 0-5 scale was determined where 0 = no gall, 1 = 1-10 galls, 2 = 11-20 galls, 3 = 21-30 galls, 4 = 31-100 galls and 5 = more than 100 galls per root system.

Root-rot index was determined by scoring the severity of disease on a scale ranging from 0 (no disease) to 5 (severe root-rot).

P. lilacinus and *A. fusispora* were re-isolated from eggs and females to determine the per cent infection on the final population. Data were analysed statistically using multifactorial analysis and critical differences were calculated at 5% level.

Results

Treatments of plants with biocontrol agents without pathogens resulted in greater dry shoot weight and nodulation. Plants treated with biocontrol agents and inoculated with *M. incognita* or with *M. phaseolina* had similar dry shoot weights. Plants inoculated with both pathogens together and treated with biocontrol agents had the least dry shoot weights (Table I).

Moderate and high doses of all the biocontrol agents, except *A. faecalis*, caused a significant improvement in dry shoot weight. Moreover, a low dose of *P. lilacinus* was also effective in increasing dry shoot weight. *P. lilacinus* was the best in improving dry shoot weight while *B. licheniformis* and *A. fusispora* were equally effective. *A. faecalis* had an adverse effect on dry shoot weight (Table I).

All three doses of *P. lilacinus* were effective in improving dry shoot weight of plants inoculated with *M. incognita* alone or with *M. phaseolina*, but against *M. phaseolina* alone only a high dose was found effective (Table II). On the other hand, only moderate and high doses of *A. fusispora* and *B. licheniformis* were effective in improving dry shoot weight of plants inoculated with *M. incognita* alone or with *M. phaseolina*. Only a high dose of *B. licheniformis* was effective against *M. phaseolina* alone while neither of the doses of *A. fusispora* were effective against *M. phaseolina* alone. All doses of *A. faecalis* had an adverse effect on plant growth.

The effect of all biocontrol agents, except *A. faecalis*, on nodulations was statistically the same (Table II). The

Table I - *Biological control of* Meloidogyne incognita *race 3 and* Macrophomina phaseolina *on chickpea.*

Treatment		Dry shoot weight (g)	No. of nodules	Final nematode population (10^3)
Control with Treatment		7.8	45	–
M. incognita with Treat.		6.1	30	27.2
M. phaseolina with Treat.		6.0	31	–
M. incognita + *M. phaseolina* with Treat.		4.5	18	15.1
C.D. 5%		0.2	2.5	0.2
Control inoculated-untreated		5.7	28	34.1
P. lilacinus	0.5 g	6.1	32	23.7
	1.0 g	6.6	35	14.8
	2.0 g	7.1	37	9.1
A. fusispora	0.5 g	6.0	31	26.2
	1.0 g	6.3	34	18.9
	2.0 g	6.7	36	13.0
A. faecalis	5 ml	5.5	26	29.4
	10 ml	5.3	25	23.0
	20 ml	5.0	22	18.3
B. licheniformis	5 ml	6.0	31	28.3
	10 ml	6.3	32	20.4
	20 ml	6.7	36	15.9
C.D. 5%		0.3	1.4	0.6

Table II - *Biological control of* M. incognita *(MI) race 3 and* M. phaseolina *(MP) on chickpea.*

Treatments		Dry shoot weight (g)	% reduction	No. of nodules	% reduction	Nematode population (10^3)	% reduction	Root knot Index	Root rot Index
Control		7.7	–	44	–	–	–	–	–
M. incognita (MI)			5.5	28.6	26	40.9	42.3	–	5 –
M. phaseolina (MP)		5.9	23.4	29	34.1	–	–	–	4
MI + MP		3.7	51.9	14	68.2	25.8	–	5	5
P. lilacinus	Control	7.8	–	46	–	–	–	–	–
	MI	6.2	20.5	31	32.6	30.5	27.9	5	–
0.5g	MP	6.0	23.1	31	32.6	–	–	–	4
	MI+MP	4.5	42.3	20	56.5	16.8	34.9	5	5
	Control	7.9	–	47	–	–	–	–	–
	MI	6.8	13.9	35	25.5	18.5	56.3	5	–
1g	MP	6.2	21.5	33	29.8	–	–	–	4
	MI+MP	5.7	27.8	24	48.9	11.1	57.0	5	5
	Control	8.0	–	49	–	–	–	–	–
	MI	7.2	10.0	39	20.4	10.7	74.7	4	–
2g	MP	6.8	15.0	34	30.6	–	–	–	3
	MI+MP	6.4	20.0	27	44.9	7.4	71.3	4	4
A. fusispora	Control	7.8	–	44	–	–	–	–	–
	MI	5.8	25.6	31	29.5	34.4	18.7	5	–
0.5g	MP	6.1	21.8	33	25.0	–	–	–	4
	MI+MP	4.1	47.4	17	61.4	17.9	30.6	5	5
	Control	7.9	–	46	–	–	–	–	–
	MI	6.4	19.0	34	26.1	24.7	41.6	5	–
1g	MP	6.3	20.3	35	23.9	–	–	–	4
	MI+MP	4.7	40.5	20	56.6	13.0	49.6	5	5
	Control	7.9	–	46	–	–	–	–	–
	MI	6.8	13.9	38	17.4	16.0	62.2	5	–
2g	MP	6.4	19.0	35	23.9	–	–	–	4
	MI+MP	5.5	30.4	26	43.5	10.0	61.2	4	5
A. Faecalis	Control	7.6	–	42	–	–	–	–	–
	MI	5.2	31.6	24	42.9	38.8	8.3	5	–
5ml	MP	5.5	27.6	26	38.1	–	–	–	5
	MI+MP	3.5	53.9	10	76.2	20.0	22.5	5	5
	Control	7.5	–	42	–	–	–	–	–
	MI	4.9	34.7	24	42.9	30.2	28.6	5	–
10ml	MP	5.1	32.0	23	45.2	–	–	–	5
	MI+MP	3.4	54.7	10	76.2	15.7	39.1	5	5
	Control	7.5	–	40	–	–	–	–	–
	MI	4.6	38.7	19	52.5	23.4	44.7	5	–
20ml	MP	4.8	36.0	21	47.5	–	–	–	5
	MI+MP	3.2	57.3	8	80.0	13.2	48.8	5	5
B. licheniformis	Control	7.8	–	45	–	–	–	–	–
	MI	5.8	25.6	29	35.6	36.7	13.3	5	–
5ml	MP	6.1	21.8	31	31.1	–	–	–	4
	MI+MP	4.2	36.2	18	60.0	19.8	23.3	5	5
	Control	7.9	–	47	–	–	–	–	–
	MI	6.4	19.0	30	36.2	26.8	36.6	5	–
10ml	MP	6.2	21.5	32	31.9	–	–	–	4
	MI+MP	4.6	42.8	20	57.4	14.0	45.7	5	5
	Control	8.0	–	48	–	–	–	–	–
20ml	MI	7.1	11.3	36	25.0	20.0	52.7	5	–
	MP	6.6	17.5	34	29.2	–	–	–	4
	MI+MP	5.3	33.8	24	50.0	11.8	54.3	5	5
C.D. 5%		0.5		4.9		0.8			

highest reduction in *M. incognita* multiplication, whether present alone or with *M. phaseolina*, was caused by *P. lilacinus* followed by *A. fusispora*, *B. licheniformis* and *A. faecalis* (Table II).

About 40 per cent of the females and 70 per cent of the eggs were infected with *P. lilacinus* while 25 per cent of the females and 50 per cent of the eggs were infected with *A. fusispora*. *B. licheniformis* and *A. faecalis* were not found to be parasitic to nematodes but reduction in the final population was noted due to inhibitory effect.

Discussion

The parasitism of *P. lilacinus* resulted in a lowering of the nematode inoculum potential thereby improving the plant growth. A high dose of *P. lilacinus* was more effective because it parasitized more number of females and eggs of *M. incognita* than a moderate or low dose. The antifungal property of *P. lilacinus* resulted in improved growth of *M. phaseolina*-infected plants. *P. lilacinus* is known to produce ß (1-3) gluconase (Domsch *et al.*, 1980), chitinase (Okafor, 1967) extracellularly which are the key enzymes in the lysis of fungal cell wall (Mitchell and Allexander, 1963). Arai *et al.* (1973) isolated leucostatin and lilacin, two water soluble peptide antibiotics. The improved growth of *M. phaseolina*-infected and *P. lilacinus* treated plants can be attributed to these enzymes and antibiotics. *A. fusispora* improved the growth of only nematode inoculated plants by parasitizing females and eggs of the nematode. *B. licheniformis*, although it did not parasitize nematodes, reduced nematode population by an inhibitory effect. *B. licheniformis* is closely related sp. to *B. subtilis* which has been reported to have inhibitory effect on several plant pathogens (Broadbent *et al.*, 1971, 1977). Although *A. faecalis* reduced nematode populations, it also had an adverse effect on plant growth and thus cannot be used as a biocontrol agent.

Acknowledgement - The authors thank Dr. R. Gherna, American Type Culture Collection Rock-Ville, Maryland, U.S.A. for authentic identification of *Bacillus* sp.

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Accepted for publication on 25 May 1992.