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INTEGRATED CONTROL OF A ROOT-ROT DISEASE COMPLEX OF CHICKPEA BY FUNGAL FILTRATES AND GREEN MANURING

by
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Summary. Seed treatment by fungal filtrates and green manuring were used for the control of a root-rot disease complex of chickpea (*Cicer arietinum*) caused by *Meloidogyne incognita* race 3 and *Macrophomina phaseolina*. Treatments by fungal filtrates and green manuring were more effective against *M. incognita* alone or with *M. phaseolina* but less effective against *M. phaseolina* alone. Individually green manuring of *Cymbopogon citratus* was best against both test pathogens while in filtrates *Paecilomyces lilacinus* gave better results than *Aspergillus niger*. In integrated control, combination of *C. citratus* and *P. lilacinus* was more effective than the combination of *P. lilacinus* and *A. niger*.

Chickpea, *Cicer arietinum* L., is an economically important pulse crop in India. It is susceptible to *Meloidogyne incognita* (Kofoid *et* white) Chitw. and *Macrophomina phaseolina* (Tassi) Goid. Both pathogens cause a root-rot disease complex which results in severe damage to the crop (Siddiqui and Husain, 1991a; 1992).

In the present study integrated control of root-rot disease complex of chickpea was undertaken using *Cymbopogon citratus* Stapf, *Eicchornia crassipes* Solms as green manure and fungal filtrates of *Paecilomyces lilacinus* (Thom.) Samson and *Aspergillus niger* Van Tiegh.

Materials and methods

Twenty grams of fresh chopped leaves of *C. citratus* and *E. crassipes* were incorporated into 1 kg steam sterilized soil contained in 15 cm diameter earthen pots. Pots were watered daily and after one month 5 chickpea cv. P-256 seeds were sown in each pot. Before sowing the seeds were surface sterilized with 0.1% mercuric chloride and then immersed in S/100 concentration of fungal filtrate for 10 hrs. The seeds were then treated with the chickpea strain of *Bradyrhizobium japonicum*. After germination only one seedling per pot was maintained.

P. lilacinus and *A. niger* were cultured in Richards liquid medium. Mycelial mats of 15 day old cultures were removed and liquid medium was filtered through Whatman No. 1 filter paper. Culture filtrates were centrifuged at 6000 rpm for 15 minutes and diluted 100 times with distilled water to prepare an S/100 concentration.

M. incogntia was collected from a chickpea field and multiplied on eggplant (*Solanum melongena* L.) from a single egg-mass. *M. incognita* race was identified as 3 by using host differentials (Taylor and Sasser, 1978). For the inoculum, egg-masses were hand picked with sterilized forceps and placed on layers of tissue paper in 8 cm diameter sieves immersed in water. A suspension of 2000 freshly hatched second stage juveniles was inoculated in the root zone of 1 week old plants.

M. phaseolina was isolated from infected chickpea roots and grown in Richards liquid medium for 15 days at 25 °C. The mycelium in 10 g quantities was macerated in 100 ml distilled water and 10 ml suspension was inoculated at the base of 1 week old seedlings to give 1 g mycelium per pot.

Each treatment (Table I) was replicated four times and plants were watered periodically as required. Ninety days after inoculation data on dry shoot weight, number of nodules, root-knot galls and root-rot indices and nematode density were recorded. Nematodes in the soil were extracted by Cobb's sieving and decanting technique followed by Baermann funnel. Numbers of juveniles, eggs and females were estimated by taking a 1 g root sample from a homogenous mixture and macerated for 45 seconds in a Waring blender. Nematode density in the roots was calculated by multiplying the number of nematodes with total weight of root. The root-rot index was determined on a scale ranging from 0 (no disease) to 5 (severe root-rot). Data were analysed by multifactorial analysis.

TABLE I - *Integrated control of* Meloidogyne incognita *and* Macrophomina phaseolina *by green manuring and fungal filtrates on chickpea.*

Treatments		Dry shoot weight (g)	% reduction	No. of nodules	% reduction	Nematode population in 100 g soil	% reduction	No. of galls	Root-rot index
Control	(C)	6.2	–	43	–	–	–	–	–
M. incognita	(MI)	4.5	27	22	49	46.3	–	281	–
M. phaseolina	(MP)	4.7	24	25	42	–	–	–	4
MI + MP		2.7	56	12	72	26.7	–	207	5
	C	6.6	–	45	–	–	–	–	–
	MI	5.5	17	33	27	24.4	47	120	–
C. citratus	MP	5.2	21	31	31	–	–	–	4
	MI+MP	4.4	33	26	42	11.4	57	64	5
	C	6.5	–	44	–	–	–	–	–
	MI	5.3	18	33	25	27.1	41	141	–
E. crassipes	MP	5.1	22	30	32	–	–	–	4
	MI+MP	4.2	35	23	48	13.6	49	88	5
	C	6.3	–	45	–	–	–	–	–
	MI	5.0	21	28	38	29.4	37	167	–
P. lilacinus	MP	4.9	22	30	33	–	–	–	4
	MI+MP	3.9	38	21	53	15.7	41	126	5
	C	6.3	–	43	–	–	–	–	–
	MI	4.9	22	30	30	31.8	31	181	–
A. niger	MP	4.8	24	27	37	–	–	–	4
	MI+MP	3.7	41	19	56	18.2	32	136	5
C. citratus	C	6.7	–	48	–	–	–	–	–
+	MI	6.1	9	36	25	16.3	65	118	–
E. crassipes	MP	6.0	10	35	27	–	–	–	3
	MI+MP	5.8	13	32	33	8.1	70	63	3
C. citratus	C	6.6	–	45	–	–	–	–	–
+	MI	6.4	3	40	11	5.1	90	28	–
P. lilacinus	MP	6.2	6	39	13	–	–	–	2
	MI+MP	5.9	11	35	22	2.3	91	16	3
C. citratus	C	6.6	–	46	–	–	–	–	–
+	MI	6.2	6	38	17	7.4	84	43	–
A. niger	MP	6.0	9	37	20	–	–	–	2
	MI+MP	5.8	12	35	24	3.9	85	19	3
E. crassipes	C	6.5	–	43	–	–	–	–	–
+	MI	6.1	6	38	12	11.6	75	70	–
P. lilacinus	MP	6.0	8	39	9	–	–	–	2
	MI+MP	5.7	12	33	23	5.7	79	32	3
E. crassipes	C	6.5	–	44	–	–	–	–	–
+	MI	6.0	8	36	18	14.2	69	79	–
A. niger	MP	5.8	11	34	23	–	–	–	3
	MI+MP	5.6	14	32	27	6.8	75	44	3
P. lilacinus	C	6.4	–	42	–	–	–	–	–
+	MI	5.2	19	28	33	25.3	45	106	–
A. niger	MP	5.1	20	29	31	–	–	–	5
	MI+MP	4.2	34	25	40	12.7	54	58	5
L.S.D. (P=0.05)		0.25	–	3.2	–	0.7	–	10	–
F value for									
Control treatments (df=10)		182.8	–	64.9	–	3099.2	–	83.4	–
Pathogens (df=3)		69.7	–	462.1	–	9815.3*	–	6619.1*	–
Interaction (df=30)		20.3	–	7.1	–	221.7**	–	11.2**	–

* = d f 1; ** = d f 10.

Results

All of the treatments using green manures and fungal filtrates either individually or concomitantly resulted in significant improvement in dry shoot weight and nodulation compared with the untreated and pathogen inoculated plants. The largest increase in dry shoot weight occurred when *C. citratus* and fungal filtrates of *P. lilacinus* were used together. The use of *C. citratus* together with *A. niger* or *E. crassipes* gave a similar improvement in dry shoot weight. The least effective treatment was *P. lilacinus* combined with *A. niger* (Table I).

The uninoculated control treatment provided the highest shoot growth and nodulation. Plants inoculated with *M. incognita* and treated with fungal filtrates and green manures resulted in higher dry shoot weight compared with plants similarly treated but inoculated with *M. phaseolina*. The smallest dry shoot weight increase occurred with plants inoculated with both pathogens concomitantly and treated with fungal filtrates and green manures.

Significant improvements in nodulation were observed when plants were inoculated with pathogens and treated with green manures and fungal filtrates together. The least improvement in nodulation occurred when plants inoculated with both pathogens were treated with *P. lilacinus* plus *A. niger* filtrates. Nematode multiplication and galling was inhibited with *M. phaseolina* (Table I).

M. incognita alone caused 27% reduction in dry shoot weight. The reduction was only 3% when *M. incognita* - inoculated plants were treated with *C. citratus* green manure and *P. lilacinus* filtrate. Other treatments gave reductions from 6 to 22% (Table I). *M. phaseolina* alone caused 24% reduction in dry shoot weight and this was reduced by various treatments, the most effective being *C. citratus* plus *P. lilacinus* (Table I). Both pathogens together caused 56% reduction in dry shoot weight which was reduced to 11% in the *C. citratus* plus *P. lilacinus* treatment. Other treatments were less effective (Table I).

M. phaseolina adversely affected nematode multiplication and galling compared with plants inoculated with *M. incognita* alone. The largest reduction in nematode multiplication (90 and 91%) occurred in the *C. citratus* plus *P. lilacinus* treatment when inoculated with nematode alone or with *M. phaseolina*, respectively. The smallest reduction in nematode multiplication was caused by *P. lilacinus* and *A. niger* (Table I).

Discussion

Green manuring suppresses nematode populations by increasing organic matter and natural enemies as well as improving crop tolerance. In the present study green manuring with *C. citratus* was found more effective than with *E. crassipes*. The *C. citratus* contains citral (54-87%) as a principal constituent. It also contains citronellol, geraniol and mycene. High nematicidal and antifungal activity of *C. citratus* can be attributed to these chemical constituents as reported by Sangwan *et al.* (1985) and Siddiqui and Husain (1990). The nematicidal and antifungal property of *E. crassipes* was probably due to its high potash and chlorine content (Siddiqui and Husain, 1990). *A. niger* is known to produce oxalic acid in the culture medium (Mankau, 1969a; b) and to have nematicidal activity (Mankau, 1969a, b). *P. lilacinus* filtrate has also been shown to have antifungal and nematicidal activity (Siddiqui and Husain, 1991 b) due to the release of toxic metabolites or enzymes such as gluconase, chitinase, leucostatin and lilacin (Mitchell and Allexander, 1963; Okafor, 1967; Arai *et al.*, 1973; Domsch *et al.*, 1980). Similarly, the antibiotic (P-168) produced by this fungus has wide antimicrobial activity against fungi, yeast and gram positive bacteria (Isogai *et al.*, 1980).

A combination of *C. citratus* and *P. lilacinus* is best because both have high nematicidal and antifungal activity both as a seed treatment and a green manure. The *P. lilacinus* and *A. niger* filtrates together were least effective because both were used as seed treatment alone and effect on pathogens were limited.

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