BIO-INTENSIVE MANAGEMENT OF ROOT-KNOT NEMATODES ON BELL PEPPER USING *POCHONIA CHLAMYDOSPORIA* AND *PSEUDOMONAS FLUORESCENS**

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Summary. Formulations of the egg parasitic fungus, *Pochonia chlamydosporia*, and *Pseudomonas fluorescens* were evaluated under field conditions for their efficacy against the root-knot nematode, *Meloidogyne incognita*, infecting bell pepper. In the nursery bed, the formulation of *P. chlamydosporia* was significantly more effective at 50 g/m^2 than at 25 g/m^2 in reducing root galling index and the numbers of nematodes in roots and soil and increasing the percent parasitization of eggs and yield. Seed treatment with *P. fluorescens* alone or nursery treatment with *P. chlamydosporia* were also effective. The individual effect of each organism was maximized when both were added together to the nursery bed.

Excessive use of chemicals in horticultural systems is affecting soil bio-diversity adversely. This has resulted in an increase of populations of nematodes in soil. The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. significantly reduces the yields of bell pepper (Huang *et al.*, 2000; Rao *et al.*, 2002a, b). Surveys also show that this nematode is widespread in most of the bell pepper growing areas of India (Rao *et al.*, 2002a, b).

Keeping in view the hazardous consequences of the use of chemicals for the control of nematodes, the combined efficacy of *Pochonia chlamydosporia* Zare *et al.*, and *Pseudomonas fluorescens* Migula, for the management of *M. incognita* on bell pepper under field conditions was evaluated. The egg parasitic fungus *P. chlamydosporia* has been reported as a promising bio-agent by various researchers (De Leij and Kerry, 1991; Kerry *et al.* 1993; Reddy *et al.*, 1999). Similarly, various researchers have reported the bio-control potential of *Pseudomonas fluorescens* against root-knot and other nematodes (Siddiqui *et al.*, 1999; Perveen *et al.*,1998; Santhi and Sivakumar 1995; Rao *et al.*, 2002a, b). However, there are no reports on the combined use of these two promising bio-agents for the management of root-knot nematodes on bell pepper. Hence, investigations were carried out to study the compatibility of these two bio-agents and their combined effect on the management of *M. incognita* infecting bell pepper, under field conditions.

MATERIALS AND METHODS

A local isolate of *P. chlamydosporia*, the identification of which was confirmed by Professor Brian Kerry, United Kingdom, using a molecular diagnostic test (Beta tubulin gene test), was mass produced by liquid and solid fermentation processes (the details of the fermentation process are not revealed here for patent considerations). The formulation of *P. chlamydosporia*, containing 10^6 chlamydosopores/gram, was evaluated at 25 and 50 g per m^2 in combination with a seed treatment with *P. fluorescens* to provide information that would help design a strategy for the intensive bio-management of *M. incognita* on bell pepper (*Capsicum annuum* var. grossum L.) *cv.* California Wonder under field conditions.

The experiment was conducted at the Indian Institute of Horticultural Research farm in 30 raised nursery beds, each of 1 m^2 and infested with 111 ± 8 J_2 *M. incognita* per 100 g of soil. Ten beds received the formulation of *P. chlamydosporia* at 25 g/m^2 (*Pc* 25) and ten more beds received 50 g/m^2 (*Pc* 50) of the same formulation. Five of the ten beds treated with *P. chlamydosporia* at a dosage of 25 g/m^2 were sown with the seeds of pepper treated with *P. fluorescens* and the remaining five with untreated seeds. Similarly, five of the ten beds that received *P. chlamydosporia* at 50 g/m^2 were sown with seeds of pepper treated with *P. fluorescens* and the remaining five with untreated seeds. Five more beds, not treated with *P. chlamydosporia,* were sown with pepper seeds treated with *P. fluorescens* to evaluate the individual effect of the seed treatment. Five beds maintained as controls received no *P. chlamydosporia* and were sown with untreated seeds of pepper. All the treatments were replicated five times in a randomized block design.

To coat pepper seeds with *P. fluorescens*, 40 ml of log phase of the bacterial (IIHR isolate NO.11$^+$cl2) suspension was added to a centrifuge tube and centrifuged at 10,000 rpm for 15 mins. The supernatant was discarded and about 1 ml of distilled water was added to re-suspend the pellet. Optical Density (O.D.) was read at 560 nm. The value was adjusted to 0.5 Absorbance. Pepper

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seeds treated with *P. fluorescens* were suspended in 40 ml CMC (Carboxy methyl cellulose) solution. CMC with and without the bacterium were evaluated before starting the experiment and, since we did not observe any effect of CMC on the nematodes, we did not include a control of CMC alone. The seeds were allowed to imbibe the bacterial suspension by maintaining the beakers on a rotary shaker at 50 rpm for half an hour to ensure uniform coating of bacterial cells on the seed surface. The solution was decanted and the coated seeds were maintained in the shade. The effect of seed treatment with *P. fluorescens* on pepper seed germination was assessed and found not to affect it. Hence, treated seeds were then sown in the nursery beds.

In each bed, 75 seedlings were retained in five rows (15 seedlings per row) by thinning. After 30 days, five seedlings were randomly selected from each bed to record seedling height and weight and root colonization by *P. chlamydosporia* and *P. fluorescens*.

Subsequently, the seedlings were transplanted into 2 x 3 m plots in a field infested with *M. incognita* at the density of 107 ± 12 J_2 per 100 cm^3 soil. In each plot, 30 seedlings were transplanted at a spacing of 75 x 30 cm. Each treatment was replicated five times in a randomized block design. Shoot and root weights, root galling on a 1-10 scale (Bridge and Page, 1980), root and soil nematode population densities and the yield of bell pepper at harvest (80 days after transplanting) were recorded. The final nematode population in 100 cm^3 soil collected at random from each plot (each replicate) was estimated using Cobb's sieving and decanting technique followed by a modified Baermann funnel (Flegg, 1967) technique.

Colonization of roots by *P. chlamydosporia* and *P. fluorescens* and percent of eggs per egg mass parasitized by *P. chlamydosporia* were also recorded. Root colonization by *P. fluorescens* was assessed by a standard serial dilution technique. A 1-g root subsample, infested by the root knot nematodes, was taken and washed gently to remove the soil. The dilutions were prepared up to 10^{-5} and 0.1 ml of the 10^{-4} and 10^{-5} dilutions were spread on Petri plates containing King's B medium. The plates were incubated at 27 ± 1 °C. The colonies emitting a pale green fluorescent light under UV at 302 nm were counted and calibrated to 10^{-6} cfu/ml. To assess root colonization by *P. chlamydosporia,* the root system was gently washed to remove soil, blotted dry, weighed and cut into small pieces of about 3-4 mm each. One gram subsamples of roots were taken randomly and root pieces plated on a semi-selective medium developed by Kerry *et al.* (1993). The Petri plates were incubated at 25-27 °C for 15 days in the dark. To study egg parasitism, ten egg masses from each plant root system of five plants per plot (collected at random) were dissolved in a 0.05% sodium hypochlorite solution and the number of eggs infected with bio-control fungus were counted under a microscope. The fungus was isolated from adult females and eggs of *M. incognita* by using the semi-selective medium mentioned above. The Petri plates were incubated at 25-27 °C for 15 days in the dark and, on the basis of morphological features of *P. chlamydosporia,* parasitisation of adult females and eggs was confirmed. The population of nematodes in roots was estimated by using 25 g of root (5 g of root sample/plant, stained using acid fuchsin) collected from five plants choosen at random from each plot and homogenized using a homogenizer. Samples were counted under a stereo-microscope. The relative reductions in nematode density due to the application of *P. chlamydosporia*, *P. fluorescens* or both were calculated and the data were analyzed using ANOVA.

RESULTS AND DISCUSSION

The nursery bed treatment with the formulation of *P. chlamydosporia*, *P. fluorescens* seed treatment and the combination of these two treatments significantly increased the growth of bell pepper seedlings and reduced the number of galls per seedling when compared with the control (Table I). A dose of 50 g/m^2 of the formulation of *P. chlamydosporia* was significantly more effective than 25 g/m^2. The seedlings were highly vigorous because there was a significant increase in growth of the seedlings raised on all the nursery beds treated with the formulations of *P. chlamydosporia* or *P. fluorescens* or both. The role of *P. fluorescens* in plant growth promotion has been very well documented (Shouan Zhang *et al.,* 2003; Kishore *et al.,* 2003). Though there are several reports on the bio-control potential of *P. chlamydosporia* (De Leij and Kerry, 1991; Kerry *et al.* 1993; Rao *et al.,* 1997, 2001; Reddy *et al.*, 1999), there are no reports on the effect of *P. chlamydosporia* in increasing plant growth. Increased growth of tomato and egg plant inoculated with *P. chlamydosporia* was observed earlier by us (Rao *et al.*, 1997, 1998, 2003).

The seedlings were colonized by both bio-agents (Table I) and, when transplanted into the field, the bio-agents colonised the field soil as they were recovered from root and soil samples at harvest of the crop (Table II).

Treatment of the nursery bed with the formulation of *P. chlamydosporia* at 50 g/m^2 was significantly more effective than 25 g/m^2 in reducing the number of nematodes in roots and soil, and increasing the percent parasitization of eggs by the bio-control fungus and also the yield of the crop (Table II, III and IV). Seed treatment with *P. fluorescens* alone and the treatment with *P. chlamydosporia* alone were effective and statistically at par in reducing the number of galls in the seedlings (Table I). Though the bio-control potential of *P. fluorescens* against root-knot nematodes is very well documented (Siddiqui *et al.*, 1999; Perveen *et al.*, 1998; Santhi and Sivakumar 1995; Rao *et al.*, 2002a, b), in the present studies, *P. chlamydosporia* alone was found to be significantly better than *P. fluorescens* in reducing the numbers of nematodes in root and soil at crop harvest (Table

Table I. Effects of integration of treatments with *Pochonia chlamydosporia* and *Pseudomonas fluorescens* on *Meloidogyne incognita* root galling and growth of the seedlings of bell pepper in nursery beds.

Treatment	Seedling length (cm)	Seedling weight (g)	No. of galls per seedling	No. colony forming units per gram root	
				P. chlamydosporia	*P. fluorescens*
P. chlamydosporia at 25 g/ m^2 (Pc 25)	12.6	3.6	6.2	15,896	--
P. chlamydosporia at 50 g/ m^2 (Pc 50)	14.6	3.8	5.9	17,459	--
P. fluorescens (Pf)	15.9	4.0	5.5	--	12,563
Pc 25 + Pf	18.5	4.5	5.1	16,256	12,349
Pc 50 + Pf	17.4	4.4	5.3	16,789	11,897
Untreated	11.2	3.2	8.2	--	--
C.D. 5%	1.67	0.34	0.76	959.7	729.6

Values are means of 5 replicates

Table II. Effects of integration of treatments with *P. chlamydosporia* and *P. fluorescens* on the colonization of the bio-agents and parasitisation of *M. incognita* eggs by *P. chlamydosporia* under field conditions.

Treatment	No. colony forming units of *P. chlamydosporia* per gram root	No. colony forming units of *P. chlamydosporia* per gram soil	No. colony forming units of *P. fluorescens* per gram root	No. colony forming units of *P. fluorescens* per gram soil	% eggs parasitised by *P. chlamydosporia*
P. chlamydosporia at 25 g/m^2 (Pc 25)	22,456	19,569	--	--	41.00
P. chlamydosporia at 50 g/m^2 (Pc 50)	25,789	22,845	--	--	45.00
P. fluorescens (Pf)	--	--	22,568	15,987	--
Pc 25 + Pf	21,679	19,234	21,789	16,843	40.00
Pc 50 + Pf	24,587	23,221	21,567	15,359	42.67
Untreated	--	--	--	--	--
C.D. 5%	1246.76	1178.32	1089.86	873.65	3.78

Values are means of 5 replicates.

Table III. Effects of integration of treatments with *P. chlamydosporia* and *P. fluorescens* on *M. incognita* populations in roots and soils.

Treatment	Nematode population in 100 cm^3 soil	Nematode population in 5 g root	Root galling index (1-10)
P. chlamydosporia at 25 g/m^2 (Pc 25)	79	41	6.3
P. chlamydosporia at 50 g/m^2 (Pc 50)	68	35	5.6
P. fluorescens (Pf)	89	57	6.0
Pc 25 + Pf	71	35	4.8
Pc 50 + Pf	60	33	4.4
Untreated	132	68	8.1
C.D. 5%	9.67	7.45	0.45

Values are means of 5 replicates.

Table IV. Effects of integration of treatments with *P. chlamydosporia* and *P. fluorescens* on growth of transplants of bell pepper and crop yield.

Treatment	Mean shoot weight (g)	Root weight (g)	Yield (kg per plot)
P. chlamydosporia at 25 g/m^2 (Pc 25)	267	78	4.4
P. chlamydosporia at 50 g/m^2 (Pc 50)	286	83	4.7
P. fluorescens (Pf)	312	95	5.1
Pc 25 + Pf	340	105	5.3
Pc 50 + Pf	346	103	5.5
Untreated	225	75	4.0
C.D. 5%	35.79	16.42	0.28

Values are means of 5 replicates

III). However, we did not study the effect of these bio-agents beyond 80 days after transplanting as the farmers in India would not retain the crop after 80-90 days since it is not economically viable to do so. Further, *P. fluorescens* treatment alone was found to increase the growth and yield of bell pepper significantly when compared to the treatments with *P. chlamydosporia* (Table IV). This could be due to plant growth promoting activity of *P. fluorescens,* which also had an impact on yield.

Integration of both the bio-agents in the nursery bed has proved significantly effective in reducing the root-galling index and the numbers of nematodes in the roots and soil and in increasing the yield of the crop. Finally, their individual effect was maximized when both organisms were integrated in the nursery bed stage (Table I, II, III and IV). This could be due to the combined effect of both organisms on bell pepper and root-knot nematode. Combined use of *P. fluorescens* and *P. chlamydosporia* did not affect colonization of the root by either species (Table II, III and IV).

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