MANAGEMENT OF NEMATODE INDUCED WILT DISEASE COMPLEX IN *CAPSICUM* USING *PSEUDOMONAS FLUORESCENS* AND *PAECILOMYCES LILACINUS*

M.S. Rao, D.S. Sowmya, M.K. Chaya, R. Manoj Kumar, K. Rathnamma, J. Gavaskar, K. Priti and N. Ramachandran

Indian Institute of Horticultural Research, Hessaraghatta Lake, Bangalore 560 089, India

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Summary. Experiments were conducted to standardize a method for the management of nematode induced disease complex in bell pepper (*Capsicum annuum* L.), caused by the interaction of the nematode *Meloidogyne incognita* with the bacterium *Ralstonia solanacearum*, using the combination formulation of *Pseudomonas fluorescens* and *Paecilomyces lilacinus*. The use of this formulation as seed treatment and subsequent application in the nursery and in the field, as a method for the management of this disease complex, was standardized. Ten grams of this formulation was used for the seed (1 kg) treatment, 5 g for treating 1 kg of substrate (coco peat) and 5 kg for the enrichment of a vermi-compost (500 kg) which was applied to the field before transplanting bell pepper seedlings. This method reduced the population of *M. incognita* and the disease incidence. The plant growth components (shoot and root length), nematode egg parasitization and crop yield were also significantly increased. The colonization of the roots by *P. fluorescens* did not affect that by *P. lilacinus*.

Key words: Bio-pesticide, *Capsicum annuum*, *Meloidogyne incognita, Ralstonia solanacearum*.

The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. was found to significantly reduce the yields of bell pepper (*Capsicum annum*) (Di Vito *et al*., 1985). The incidence of wilt disease caused by *Ralstonia solanacearum* Yabuuchi was found to be significantly higher in the presence of root-knot nematodes (Rao *et al.*, 2009). Considering the negative impact of the synthetic chemicals commonly used for the management of nematodes and bacteria on the environment, an evaluation was planned to test the effects of a combination formulation of *Pseudomonas fluorescens* Migula and *Paecilomyces lilacinus* (Thom) Samson developed at Indian Institute of Horticultural Research, Bangalore, India, in the management of wilt disease complex in bell pepper (*Capsicum annuum* L.) under field conditions. The potential of the bio-control fungus *P. lilacinus* has been reported by various researchers (Jatala, 1986; Rao and Parvatha Reddy, 1994; Rao *et al*., 1997a, 1997b, 1998, 1999; Mohd *et al*., 2009; Mucksood and Tabreiz, 2010). Similarly, others have reported the benefits of *P. fluorescens* against various nematodes and fungal and bacterial pathogens (Liu *et al*., 1995; Wei *et al*., 1996; Kloepper *et al*., 1999; Geoffrey *et al*., 2001; Shouan Zhang *et al*., 2002; Rao *et al*., 2009; Manoj Kumar *et al*., 2010; Meyer 2010; Rekha *et al*., 2010). However, there are no reports on the combined use of these two promising bio-agents for the management of root-knot nematodes and this bacterial pathogen on bell pepper.

MATERIALS AND METHODS

The experiment consisted of two steps. In the first step, seeds of bell pepper were treated with both or either bio-agent and raised in seedling trays (with 98 wells about 10 cm^3 per well, maintained at 25-30 °C by covering seedling trays with black mulching sheet). When 30 days old, 15 seedlings (out of 98 seedlings grown) were collected at random from each tray and used to record entire seedling length and weight and assess root colonization by the bio-agents. Then the remaining seedlings were transplanted into field plots amended with vermi-compost to which was added either or both bio-agents.

The local isolates of *P. lilacinus* (IIHR–Pl 2) (ITCC NO. 6887) and *P. fluorescens* (IIHR–Pf 2), (ITCC B0034), maintained in the collection of ITCC (Indian Type culture collection, IARI, New Delhi), were mass produced through liquid fermentation processes (the details of the fermentation process are not revealed here for patent considerations) and prepared as a talc-based combination formulation. In the field experiments, a formulated product of this bio-pesticide containing *P. lilacinus* (2×10^8 cfu/g) and *P. fluorescens* (2×10^9 cfu/g) was used. Seed (1 kg) treatment was done using 10 g of this formulation. Seedlings were produced in seedling trays filled with 1 kg of coco peat treated with 5 g of this formulation. Vermi-compost (500 kg) was enriched by the addition of 5 kg of the combination formulation of *P. lilacinus* and *P. fluorescens* or single formulations of *P. lilacinus* or *P. fluorescens* and left for a period of 15 days, under shade and by maintaining optimum moisture of 25-28%. The bio-pesticide enriched vermi-compost was added to the field plots at different doses. The experiment was conducted at the Indian Institute of Horticultural Research farm in a field infested with *M. incognita* and *R. solanacearum*. The texture of the soil in the experimental plot was sandy-clay-loam, with pH of 6.6-6.8 and organic matter of 0.22-0.24%. During the experimental periods in two seasons there were rainy days with total rain received up to 63 mm

(first season) and 76 mm (second season) and soil temperatures were in the range 25-37 °C. Treated seedlings were transplanted into 2 m × 3 m plots. There were eleven treatments (Table I-III). They consisted of one kg of seeds treated with 10 g of bio-formulation containing either or both of the bio-agents (three treatments), seeds sown in coco-peat substrate - 1 kg substrate added with 5 g of bio-formulation containing one or both of the bio-agents (three treatments), and treatments (three) in which seedlings derived from seed treatment and raised in the treated coco-peat were transplanted in plots amended with 500 kg/ha of vermi-compost enriched with 5 kg/500 kg of the formulation containing both bio-agents. Seedlings derived from non treated seeds or coco-peat and transplanted in plots not amended with vermi-compost served as controls.

Seedlings (30-day-old) of bell pepper were transplanted in the plots with a spacing of 50 × 50 cm in 6 rows (4 plants in each row) and there were five replicates per treatment arranged in a randomized block design (RBD). The crop was maintained by applying recommended dosages of fertilizers and plant protection chemicals. Observations of the root-knot index on a 1-10 scale (Bridge and Page, 1980), nematode infestation, disease incidence, yield of capsicum at harvest (90 days after transplanting), root colonization by *P. lilacinus* and *P. fluorescens* were recorded.

To evaluate the root colonization by *P. lilacinus*, the root system was carefully washed to remove soil, blotted dry, weighed and cut into small pieces of about 3-4 mm each. A one gram sample of roots was taken randomly and root colonization was assessed by plating the root pieces on semi-selective medium following the method given by Mitchell *et al*. (1987). The Petri plates (90 mm diameter) were incubated at 25-27 °C for 15 days.

Root colonization by *P. fluorescens* was assessed by following the standard serial dilution technique. One gram root sub-samples were taken and washed gently to remove the soil. The dilutions were prepared up to 10^{-5} and 0.1 ml aliquots of the 10^{-4} and 10^{-5} dilutions were spread on Petri plates containing King's B medium and 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.

Soil samples were collected at random by taking 100 g soil from five different spots in each plot and thoroughly mixed. Then the nematodes in a 100 cm^3 subsample were extracted using Cobb's sieving and decanting technique combined with the modified Baermann funnel technique (Flegg, 1967).

Five plants per plot were uprooted at random and the nematode infestation of the roots was estimated from the rating chart of Bridge and Page (1980), using 25 g of roots (5 g from each of the five plants per plot collected at random). The roots were stained in boiling 0.1% acid fuchsin solution (acid fuchsin dissolved in a 1:1:1 mixture of glycerol, lactic acid and distilled water) for five minutes, comminuted in a homogenizer and the adult nematodes counted under a stereo-microscope. The data on eggs were not recorded. The reductions in the nematodes density due to the application of *P. lilacinus* or *P. fluorescens* or both were calculated. Further, data on the mortality of the plants in the field, due to wilt disease complex, were recorded by counting the plants wilted in the field (during flowering, the number of plants completely wilted out of total number of plants was expressed as a percentage). The plant death due to wilt disease was confirmed by ooze test (Patrice *et al*., 2009) and re-isolating the pathogen on 2,3,5 TTC medium (2,3,5 Triphenyl Tetrazolium Chloride medium) (Kelman 1954).

Depending upon experimental design, a one-way analysis of variance (ANOVA) was performed using SPSS ver. 10.0. As a follow-up of ANOVA, the treatment means were separated using Fisher's least significant difference (LSD) and Duncan's multiple range tests. Data from repeated trials were pooled after confirming the homogeneity of variances.

Table I. Effect of integration of *P. lilacinus* and *P. fluorescens* on the growth of the seedlings of bell pepper in the field.

Treatment	Seedling length (cm)	Seedling weight (g)	Colonization of *P. lilacinus* (CFU/g root)	Colonization of *P. fluorescens* (CFU/g root)
PL+PF - SD	15.3 e	3.7 c	10843 c	11632 b
PL - SD	13.4 b	3.2 a	10225 b	0.0 a
PF - SD	14.8 d	3.7 c	0.0 a	12878 d
PL+PF - SB	16.2 g	3.9 e	11928 d	12558 c
PL - SB	13.7 c	3.5 b	12438 e	0.0 a
PF - SB	15.7 f	3.8 d	0.0 a	13158 e
Control	12.8 a	3.2 a	0.0 a	0.0 a
C.D. at 5%	1.24	0.33	452.86	696.45

SD = Seed treatment; SB = Substrate treatment.
Means followed by the same letter in each column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

To study egg parasitization, ten egg masses from each plant root system were randomly selected and shaken in a 0.5% sodium hypochlorite solution for five minutes to dissolve the gelatinous matrix. All of the infected eggs in each egg mass were counted under a stereo zoom microscope. The fungus was isolated from adult females and eggs of *M. incognita* by using the semi-selective medium mentioned above and ascertained that the parasitization was due to *P. lilacinus*.

RESULTS AND DISCUSSION

The seedlings were vigorous and there was a significant increase in the growth of those raised after seed and substrate treatments with the combination formulations of *P. lilacinus* and *P. fluorescens* (Table I). The roots of the seedlings were colonized by the bio-agents (Table I) and, after transplanting, colonization persisted until harvest of the crop (Table II). Also, as was evident from soil and root colonization data, root colonization by *P. fluorescens* did not affect that by *P. lilacinus* (Table II).

Bell pepper seedlings obtained from seeds and substrate treated with the combination formulation of *P. lilacinus* and *P. fluorescens* and transplanted into the plots amended with vermi-compost enriched with the combination formulation showed significant reductions in galling index, disease incidence, and numbers of ne-

Table II. Effect of integration of *P. lilacinus* and *P. fluorescens* on their rhizospheric colonization in bell pepper under field conditions.

Treatment	*P. lilacinus*		*P. fluorescens*	
	CFU/g root	CFU/g soil	CFU/g root	CFU/g soil
PL+PF – SD	15453 b	12187 b	18349 c	13754 d
PL – SD	16296 c	12769 c	0.0 a	0.0 a
PF – SD	0.0 a	0.0a	17423b	12685 b
PL+PF – SB	17857 d	22856 d	19343 d	13246 c
PL – SB	18256 e	23254 d	0.0 a	0.0a
PF – SB	0.0 a	0.0 a	19653 e	15349 e
PL+PF - SD+SB + Vermi-compost*	33645 f	24338 f	36342 f	25982 f
PL - SD+SB + Vermi-compost*	34358 g	23667e	0.0 a	0.0a
PF - SD+SB + Vermi-compost*	0.0 a	0.0 a	36895 g	26321 g
Vermi-compost	0.0 a	0.0 a	0.0 a	0.0 a
Control	0.0 a	0.0 a	0.0 a	0.0 a
C.D. at 5%	1276.48	1148.49	1366.56	116.35

Means followed by the same letter in each column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
SD = Seed treatment; SB = Substrate treatment;
*Vermi-compost enriched with the respective individual bio-agents or their combination used in that treatment.

Table III. Effect of the integration of *P. lilacinus* and *P. fluorescens* on the population of *M. incognita* in roots and soil, root-knot index, mortality due wilt disease complex and the yield of bell pepper under field conditions.

Treatment	Nematode population		Root-knot index (1-10)	Mortality due wilt disease complex (%)	Bell pepper yield (kg/6 m2)
	In 100 cc soil	In 5 g root			
PL+PF - SD	74 c	24 a	6.5 f	29.8 g	5.5 a
PL - SD	76 d	26 b	6.3 e	32.6 h	5.4 a
PF - SD	80 e	29 d	6.7 f	28.5 f	5.6 b
PL+PF - SB	63 a	24 a	5.4 b	18.2 c	6.9 d
PL - SB	66 a	25 b	5.8 c	24.4 e	6.5 c
PF - SB	69 a	27 c	6.0 d	22.7 d	6.8 d
PL+PF - SD+SB + Vermi-compost*	62 a	20 a	4.7 a	17.8 b	7.7 f
PL - SD+SB + Vermi-compost*	68 a	22 a	5.0 a	24.5 e	7.3 e
PF - SD+SB + Vermi-compost*	71 b	24 a	5.4 b	16.9 a	7.5 e
Vermi-compost	117 f	32 e	7.8 g	34.7 i	6.4 c
Control	124 g	34 f	8.2 h	37.5 j	5.2 a
C.D. at 5%	4.32	2.35	1.78	5.68	0.35

Means followed by the same letter in each column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
SD = Seed treatment; SB = Substrate treatment.
*Vermi-compost enriched with the respective individual bio-agents or their combination used in that treatment.

matodes in roots and soil compared with individual treatments (Table II). This treatment was also effective in significantly increasing the yield of the crop and reducing the mortality of the plants in the plots (Table III). Reduction in the root-knot nematode population by *P. lilacinus* was responsible for the significant reduction in the mortality of the plants due to wilt disease complex (Table III).

There are several reports on the bio-control of root-knot nematodes by *P. lilacinus* (Jatala, 1986; Parvatha Reddy, 1994; Rao *et al*., 1997a, 1997b, 1998, 1999; Mohd *et al*., 2009; Mucksood and Tabreiz, 2010). Other reports indicate the efficacy of *P. fluorescens* and *P. aeruginosa* Migula for the control of root-knot nematodes and wilt causing fungi and bacteria (Perveen *et al*., 1998; Kloepper *et al*., 1999; Geoffrey *et al*., 2001; Manoj Kumar *et al*., 2010; Meyer, 2010). Perveen *et al*. (1998) reported the combined efficacy of *P. aeruginosa* and *P. lilacinus* in reducing disease complex caused by the root-knot nematode *Meloidogyne javanica* (Treub) Chitwood and the fungi *Macrophomina phaseolina* (Tassi) Goid. and *Fusarium oxysporum* (Synder *et* Hansen) on pumpkin and *F. solani* (Mart.) Sacc. on watermelon.

It is important to develop a suitable delivery system for the application of bio-agents in the field as huge quantities of bio-agents are required to treat the soil. Our study showed that vermi-compost could be easily enriched with *P. fluorescens* and *P. lilacinus*. Delivery of the bio-agents through vermi-compost helps in the application of *P. fluoroscens* and *P. lilacinus* to the rhizosphere of the bell pepper crop. This is the first report on the multiplication of *P. fluorescens* and *P. lilacinus* in vermi-compost.

Integrated use or combined use of *P. fluorescens* and *P. lilacinus* did not affect root colonization of *P. lilacinus* and vice versa (Table I and II). Further, this strain of *P. fluoroscens* was also found to reduce the root-knot nematode infestation (Table III). Hence, the use of a combination formulation of *P. fluoroscens* and *P. lilacinus* for seed, seedling and field treatment would be very useful for the management of the nematode induced disease complex in capsicum.

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