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Field application of *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* for the control of *Meloidogyne incognita* (Kofoid and White) Chitwood on sugarbeet

J. KAVITHA, E. I. JONATHAN and R. UMAMAHESWARI
Department of Nematology, Tamil Nadu Agricultural University
Coimbatore – 641003, Tamil Nadu, India
E-mail: eijonathan@yahoo.co.in

ABSTRACT: Field experiments were conducted to assess the efficacy of *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* against the root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood infesting tropical sugarbeet cv. Indus. The biocontrol agents were applied at 2.5 kg / ha and compared with carbofuran (1 kg a. i. / ha). There was a significant increase in the plant growth parameters and decrease in nematode population in all the treatments compared to control. Among the biocontrol agents tested, *P. fluorescens* recorded significantly higher growth parameters and lower nematode population and was as effective as carbofuran. Induction of defence enzymes, *viz.*, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, was studied in all the treatments. Enzyme activity was significantly higher in *P. fluorescens* treated plants, followed by *T. viride* and *B. subtilis*.

KEY WORDS: *Bacillus subtilis, Meloidogyne incognita, Pseudomonas fluorescens*, sugarbeet, *Trichoderma viride*

INTRODUCTION

Tropical sugarbeet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Doll) is one of the important sugar crops of the world that provides more than 45 per cent of the world's sugar requirement (Singh *et al.*, 2003). In India, tropical sugarbeet is now being introduced mainly for the production of ethanol as biofuel. Several plant parasitic nematodes, *viz.*, *Meloidogyne incognita* (Kofoid and White) Chitwood, *M. arenaria, M. javanica* (Treub) Chitwood, *M. hapla* Chitwood, *Nacobbus aberrans* (Thorne) Thorne and Allen and *Trichodorus* sp. were recorded in sugarbeet, among which *M. incognita* was considered to be the most important pest causing severe damage to the crop (Jones, 1987). A recent survey conducted by the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, revealed the association of *M. incognita* in the crop causing a yield loss of 31.46 per cent (Kavitha, 2006). Chemical nematicides have been routinely used to control nematodes in high value crops. Their prohibitive cost and associated health hazards lay special emphasis on evolving biological control as a promising and effective alternative. In recent years, plant growth promoting rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus* spp. have been reported to be effective against several plant parasitic nematodes (Ramakrishnan *et al.*, 1998;

Jonathan *et al.*, 2000). The use of *Trichoderma viride* as a biological control agent has been reported as a successful strategy in the management of root knot nematode in various crops (Janowicz *et al.*, 1997; Sobita Devi and Hassan, 2002). However, the effect of biocontrol agents on nematodes affecting sugarbeet has not been studied so far. Hence, the present investigation was carried out to evaluate the efficacy of biocontrol agents, *viz.*, *P. fluorescens*, *B. subtilis* and *T. viride*, on *M. incognita* infesting sugarbeet and the subsequent biochemical changes induced in the crop.

MATERIALS AND METHODS

The experiments were conducted at Tamil Nadu Agricultural University during *rabi* (September, 2004–February, 2005) in two sites (36 E and 37 E, Eastern block) in tropical sugarbeet (cv. Indus) fields with severe infestation of root knot nematode, *M. incognita*. Initial population of *M. incognita* in field 1 was 561 and in field 2 was 602 per 250 cc of soil. Seedlings were maintained in the field as strip planting in a plot of 5 x 4 m^2 with a spacing of 50 x 20 cm. Commercial formulations of *P. fluorescens* and *T. viride*, and talc based formulations of *B. subtilis* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. At the time of application, the population of *P. fluorescens*, *B. subtilis* and *T. viride* was 2.8 x 10^8 cfu / g, 2.6 x 10^8 cfu / g and 3 x 10^8 cells / g talc in the formulations, respectively. These biocontrol agents were applied in soil at 2.5 kg /ha near the root zone on 15 days after sowing, by which time the seeds germinate. Carbofuran (1 kg a. i. / ha) was also applied 15 days after sowing as a standard check. Untreated plants were maintained as control. All the treatments were replicated four times in a randomized block design. The pooled data of both the experiments were statistically analysed and critical differences determined (Gomez and Gomez, 1984).

At the time of harvest, observations were recorded on plant growth parameters, *viz.*, shoot length, shoot weight, root length, root weight (yield) and root girth. Nematode populations in soil and root samples were analysed as per Cobb (1918) and modified Baermann funnel techniques (Schindler, 1961). Number of galls, females and egg masses per plant were also recorded in roots.

For assay of enzyme activity, root samples (1 g) were homogenised in 1 ml of 0.1 M phosphate buffer pH 7.0 at 4°C in a pre-chilled pestle and mortar. The homogenate was centrifuged at 20,000 g at 4°C for 15 minutes and the supernatant used as enzyme sample. Peroxidase activity was analysed spectrophotometrically (Hammerschmidt *et al.*, 1982). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 100μl of enzyme extract and 0.5 ml of H_2O_2 (1%). The reaction mixture was incubated at room temperature (28±2°C). The change in absorbance at 420 nm was recorded at 30 seconds interval for 3 minutes. The enzyme activity was expressed as change in the absorbance min^{-1} g^{-1} of fresh tissue.

Polyphenoloxidase activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 100 μl of the enzyme extract. To start the reaction, 200 μl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} g^{-1} protein. Phenyl alanine ammonia lyase activity was determined as the rate of conversion of L-phenyl alanine to trans-cinnamic acid at 290 nm as described by Dickerson *et al.* (1984). Sample containing 100μl of enzyme extract was incubated with 1.2 ml of 0.1 M borate buffer, pH 8.8 and 1.5 ml of 12 M L-Phenyl alanine in the same buffer for 30 minutes at 30°C. The reaction was stopped by addition of 1 M trichloroacetic acid and after incubation for 5 min at 37°C, the absorbance was read at 290 nm. Enzyme activity was expressed as mmol trans-cimmamic acid min^{-1} g^{-1} of fresh tissue.

RESULTS AND DISCUSSION

In the present study, there was a significant increase in plant parameters and decrease in nematode population in all the treatments compared to untreated control. Amongst the biological control agents tested, *P. fluorescens* at 2.5 kg / ha recorded the maximum shoot length, shoot weight, root

length, tuber weight and tuber girth, followed by *T. viride* at 2.5 kg / ha and *B. subtilis* at 2.5 kg / ha. It also recorded the minimum nematode population in soil and lower number of galls, number of females and number of egg masses in roots and was comparable with carbofuran at 1 kg a. i. / ha (Table 1 and 2).

The results of the present study agree with earlier reports where application of *P. fluorescens* or *B. subtilis* (cultured on nutrient broth) increased the growth and yield of chickpea and reduced the infection of *M. incognita* by minimizing the number of galls per root system, eggmass production and soil population (Khan *et al.*, 2001). Sobita devi and Pandey (2001) studied the field application of *P. fluorescens* and *T. viride* in chickpea against *M. incognita* and observed significant reduction in gall formation.

Table 1. Efficacy of biological control agents on plant growth characters of sugarbeet infested by *M. incognita

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root girth (cm)	Yield (Root weight in g)	Cost-benefit ratio
P. fluorescens (2.5 kg/ha)	40.54 (42.34)	79.31 (87.22)	23.34 (72.37)	24.50 (60.02)	755.11 (79.25)	1: 2.67
B. subtilis (2.5 kg/ha)	32.74 (14.95)	71.58 (68.98)	18.52 (36.77)	20.20 (31.93)	675.10 (60.26)	1: 1.98
T. viride (2.5 kg/ha)	34.23 (20.18)	72.70 (71.62)	20.60 (52.14)	22.67 (48.07)	721.66 (71.31)	1: 2.35
Carbofuran (1 kg a.i./ha)	41.58 (45.99)	81.45 (92.28)	24.48 (80.79)	25.53 (66.75)	780.32 (85.23)	1: 2.61
Control	28.48	42.36	13.54	15.31	421.25	-
CD (P=0.05)	0.27	0.43	0.40	0.40	0.98	-

* Mean of 4 replications; pooled data from two experiments; figures in parentheses are per cent increase over control

Table 2. Efficacy of biological control agents on number of galls, females, egg masses and soil population of *M. incognita* on sugarbeet*

Treatments	No. of galls / plant	No. of females / g root	No. of egg masses / g root	Soil population / 250 cc soil
P. fluorescens (2.5 kg / ha)	325.50 (23.09)	75.25 (34.27)	49.00 (49.48)	830.00 (18.6)
B. subtilis (2.5 kg/ha)	385.50 (8.91)	95.00 (17.03)	76.00 (21.64)	950.50 (6.83)
T. viride (2.5 kg/ha)	355.75 (15.94)	84.00 (26.03)	64.25 (33.76)	900.25 (11.78)
Carbofuran (1 kg a.i./ha)	316.50 (25.22)	64.50 (43.66)	32.25 (66.75)	800.00 (21.58)
Control	423.25	114.50	97.00	1020.25
CD (P = 0.05)	32.70	6.52	4.46	69.26

*Mean of 4 replications; pooled data from two experiments

In the present investigation, studies on induction of defence mechanisms revealed high accumulation of peroxidase, polyphenol oxidase and phenylalanine ammonia lyase acitivity in *P. fluorescens* treated plants, followed by *T. viride* and *B. subtilis*. Similar results were also observed by Anita *et al.* (2004) where treatment with *P. fluorescens* induced the activity of peroxidase, polyphenol oxidase, phelyalanine ammonia lyase, catalase and chitinase in tomato against *M. incognita*. Umamaheswari *et al.* (2004) reported higher activity of defense enzymes coupled with high accumulation of proline, lignin and phenols in green gram treated with *T. viride* against *M. incognita*.

Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Van Peer *et al.* (1991) reported that *P. fluorescens* activates the enzymes including peroxidase and polyphenol oxidase that catalyse the formation of lignin and phenylalanine ammonia lyase that is involved in the synthesis of phytoalexins and phenolics. In the present study, increased enzyme activities were also recorded in carbofuran treated plots. This might be due to the phytotonic effect of carbofuran that had induced the enzyme activities (Choi *et al.*, 1983). Cost: benefit ratio indicated that the application of *P. fluorescens* @ 2.5 kg/ha was more economical than carbofuran @ 1 kg a.i/ha (Table 1). Thus the present study confirms the efficacy of biological control agents for the successful management of *M. incognita* in sugarbeet.

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