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Management of *Meloidogyne incognita* by *Pseudomonas fluorescens* and *Trichoderma viride* in mulberry

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ABSTRACT

The efficacy of commercial formulations of the plant growth promoting rhizobacterium *Pseudomonas fluorescens* and the antagonistic fungus *Trichoderma viride* on the root-knot nematode, *Meloidogyne incognita*, infesting mulberry, *Morus alba*, were evaluated under field conditions. The effects of applications to the soil of formulations of the bio-control agents, alone and in combination at the rates of 3 and 6 g/plot, were compared with those of the nematicide carbofuran 3G at the rate of 1 kg a.i./ha and controls. The combination of *P. fluorescens* (6 g/plot) and *T. viride* (6 g/plot) was effective in reducing the population of *M. incognita* in soil and root and suppressing root-galling of mulberry. This treatment also improved shoot and root development and increased leaf area, leaf contents of protein, nitrogen and chlorophyll, silkworm larval weight, cocoon weight, shell weight and shell ratio of silkworm larvae fed on leaves of mulberry infested with *M. incognita*.

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INTRODUCTION

Mulberry (*Morus alba* L.), the sole food plant of silkworm (*Bombyx mori* L.), is cultivated both in tropical and temperate countries. India is the second largest producer of silk in the world with an annual silk production of around 16,500 million tons from 3.42 lakh ha mulberry gardens (Govindaiah and Sharma, 1994). The world-wide demand for silk is increasing steadily and it goes without saying that moriculture is an inevitable associate of sericulture. In recent years, much emphasis has been given to produce superior quality raw silk to compete with international market. To achieve this goal, efforts are being diverted to increase the production of good quality mulberry leaves, which has a direct influence on the quality and quantity of raw silk production.

The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. is a major constraint in mulberry cultivation and plays an important role in reducing herbage yield and quality of leaves besides the life span of mulberry plants. It causes formation of galls in the root accompanied by stunted growth, chlorosis and loss of vigour of the plant (Babu *et al*., 1999). Application of carbofuran 3 G (Furadon®) @ 40 kg/ha/year reduced the population of *M. incognita* by 72-76 per cent (Sharma *et al.*, 1998). However, application of carbofuran for nematode management in mulberry is reported to have toxic effect on silkworm larvae (Paul *et al*., 1995). Hence, the need for an alternative to nematicides to combat nematodes in mulberry. The potential of the plant growth promoting rhizobacterium *Pseudomonas fluorescens* Migula (Siddiqui and Shaukat, 2003) and of the antagonistic fungus *Trichoderma viride* Weindling *et* Fawcett (Dababat *et al*., 2006) to control the root-knot nematode on various crops has already

been demonstrated. Therefore, in the present study, the efficacy of commercial formulations of *P. fluorescens* and *T. viride* was evaluated against *M. incognita* in mulberry under glass-house and field conditions.

MATERIAL AND METHODS

Biocontrol formulations :

Commercial formulations of *Pseudomonas fluorescens* (6×10^8 cfu/g) and *T. viride* (6×10^8 cfu/g) were supplied by the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India.

Field experiments :

The two field experiments were conducted on five year old established mulberry garden, of the S36 variety in the Thondamuthur village of Coimbatore district. The fields were naturally infested by *M. incognita* with the mean population density of 187.9 ± 3/ 250 cm^3 soil. Each field was divided in 24 plots, each of 4×3 m size contained 15 plants/plot established in a spacing of 90×90 cm. Trials laid in a Randomized Block Design with three replicates. Treatment details are following the below tables. The bio-agents and carbofuran were mixed with sterile sand (1:10 v/v) and applied for Trail I and Trail II. A basin of 15 cm width and 10 cm depth was formed around the plants leaving 15 cm space from the main trunk and sand mixed bioagents and carbofuran were uniformly broadcasted, then basins were closed and irrigated. Standard agronomic practices were followed for raising the crop as per Dandin *et al.* (2000).

The experiments were concluded five months after treatment. The soil nematode population density in each plot was determined before treatment and five months after treatment. Each sample consisted of ten cores (17 mm core size), randomly collected at a depth of 15-20 cm in the *Rhizosphere* of the plants. The soil cores were pooled together into a composite sample and a 250 cm^3 sub-sample was collected by coning and quartering. The samples were processed using Cobb's decanting and sieving technique, followed by the modified Baermann's funnel technique (Southey, 1986). A gram of root from each replicate was stained with boiling acid fuschin lactophenol solution and kept for one day and then number of egg masses was recorded.

Gall index :

The following gall index with 1-5 scale rating was used in the present study (Table A).

Assessment of root colonization by *P. fluorescens* and *T. viride* and estimation of soluble protein, nitrogen, and chlorophyll contents of the leaves was made as in the green-house experiment. Plant growth components *viz.*, number of branches, plant height, and weight of 100 leaves/plot were also recorded.

Table A : Gall index with 1-5 scale rating

Percentage of root system with galls/plant	Gall index
No galls	1
1-25	2
26-50	3
51-75	4
≥75	5

Physiological study :

Estimation of moisture content in mulberry leaves (Anonymous, 1970) :

The plants were selected at random and 10 leaves each from top, middle and bottom of the plant were collected. Then the fresh leaf weight and dry weight (dried in an oven at 70ºC for 48h) were estimated. Leaf moisture was calculated by the following formula :

$$\text{Leaf moisture content (\%)} = \frac{\text{Fresh weight – dry weight}}{\text{Fresh weight}} \times 100$$

Estimation of chlorophyll :

The chlorophyll content (a, b and total chlorophyll /g of tissue) was estimated by the method modified and described by Yoshida *et al.* (1971).

Two hundred and fifty mg of fresh leaf sample was taken in a pestle and mortar. The sample was macerated with 10ml of 80 per cent acetone. The contents were centrifuged at 3000 rpm for 10 minutes. Then the supernatant was collected and the volume was made upto to 25 ml by using 80 per cent acetone. The optical density was measured at 645, 652, 663 nm by a Spectrophotometer.

The amount of chlorophyll present in 250 mg of tissue was calculated using the following equations :

$$\text{Chorophyll a} = (12.7 \times \text{OD at } 663) - (2.69 \times \text{OD at } 645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = (22.9 \times \text{OD at } 645) - (4.68 \times \text{OD at } 663) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = \frac{\text{OD at } 652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

where, OD – optical density; V – final volume of supernatant (25 ml);

W – Weight of the leaf sample taken in gram.

The chlorophyll content of the samples expressed as mg/g of fresh leaf.

Estimation of soluble protein in mulberry leaf :

The quantitative estimation of soluble protein was done by the method of Lowry *et al.* (1951). Two hundred and fifty mg of leaf sample was macerated with 10 ml of phosphate buffer solution, centrifuged at 3000 rpm for 10 minutes, the supernatant collected and made upto 25 ml. One ml of the

supernatant was taken in a test tube and 5ml of Alkaline copper tartarate (ACT) and 0.5 ml of folin reagent were added and allowed for 30 minutes for colour development. The OD was measured at 660 nm in Spectrophotometer.

Preparation of standard :

Fifty mg of Bovine serum albumin (BSA) was dissolved in 100 ml of distilled water which will give a stock solution of 500 ppm. Different concentrations of BSA standard solutions *viz.*, 100, 200, 300, 400 and 500 ppm were prepared by diluting the stock solution. The series of standards were run in similar way as that of test sample and a standard graph was drawn. Sample OD was plotted in the standard graph and the corresponding concentration (X µg) was recorded. From that, the amount of soluble protein present in the given sample was calculated by using following formula :

$$\text{Amount of soluble protein} = \frac{X \times 25}{1 \times 250} \times 1000$$

Amount of soluble protein present in the given sample was expressed in mg/g.

Estimation of nitrogen :

Nitrogen content of the plant was estimated by Micro-Kjeldhal method described by Sadasivam and Manickam (1992). The leaf portions of the mulberry plants were shade dried and powdered. About 0.5 g of the sample was added to a 25 ml digestion flask. To this sample, 15 ml of diacid mixture was added (H_2SO_4 + $HClO_4$ in 5:2 proportions) and digested till the solution become colourless.

After cooling the digest, it was diluted with distilled ammonia free water and the volume was made upto 100 ml. From that, 10 ml of aliquot was transferred to a distillation apparatus. A conical flask (50 ml) containing 10 ml of boric acid solution along with few drops of mixed indicator was placed such that the tip of the condenser was dipped in the solution. Then 10 ml of sodium hydroxide–sodium thiosulphate solution was added to the sample in the distillation apparatus. The ammonia released from the sample was collected on boric acid, which was indicated by the colour change from red to green. This solution was titrated with standard acid till the red colour was noticed which indicated the end point of titration. The nitrogen content of the sample was calculated by the following formula :

$$\text{N content (\%)} = \frac{X \times 0.00028 \times 250 \times 100}{10 \times 0.5}$$

Further, to study the effect of bioagent treatments on the leaf quality, bioassay studies were conducted with a commercial cross breed silkworm race of (CSR6×CSR26) × (CSR2×CSR27) at Farmers Silk Worm Rearing Unit located at Thondamuthur village of Coimbatore district, India. The leaves from different bio-agents treated plots were used and the rearing was carried out by following the improved silkworm rearing technology (Dandin *et al*., 2000). The leaves were taken on 45–75 days after treatment and used to rear different stages of the silkworms. Fifty larvae per replication were maintained for each treatment. Observations on larval growth and cocoon characters such as mature larvae weight (g), single cocoon weight, single shell weight, and shell ratio (%) were recorded separately for each treatment. Ten fifth instar larvae randomly taken from each replication were used for recording larval weight. Ten randomly taken cocoons during harvest were taken for recording cocoon and shell weight. Shell ratio was calculated by using the formula :

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \times 100$$

Statistical analysis :

All the data were analyzed using analysis of variance and means separated with Duncan's Multiple Range Test following Panse and Sukhatme (1989).

RESULTS AND DISCUSSION

Field experiment effect of bio-control agents on the infestation and development of *M. incognita* and plant growth components showed in Table 1. Application of the bio-agents reduced the nematode population in the soil. The greatest reduction of *M. incognita* (73.48 % over untreated control) was observed in plots with combined application of *P. fluorescens* and *T. viride* each at 6 g/plot. This treatment also reduced the number of egg masses (56.8%) of the nematode on the roots significantly more than carbofuran. There was a significant reduction of gall index (2.0) in all treatments involving the bio-control agents as compared to carbofuran (3.0) (Table 1).

Re-isolation of the bio-agents from mulberry roots revealed that both *P. fluorescens* and *T. viride* colonized simultaneously more the plants when they had been applied each at 6 g/plot. The numbers of branches (15.56/plant) was higher in plants treated with *P. fluorescens* + *T. viride* each at 6 g/plot. The plants subjected to this treatment also had significantly more plant height (288.37 cm) and weight of 100 leaves (628.56 g) than other treatments, including untreated control (Table 1).

Protein, nitrogen and chlorophyll contents of mulberry leaves were significantly increased by application of the two bio-control agents in mulberry plants infested with *M. incognita* (Table 2). Combined application of *P. fluorescens* and *T. viride*, each at 6 g/plant, increased protein content by 101.1 per cent, nitrogen content by 15.8 per cent and total chlorophyll content by 64.4 per cent over the untreated control. The next best treatment was the combined application of *P. fluorescens* at 3 g/plot + *T. viride* at 3 g/plot which was superior to carbofuran. Combined treatment with *P. fluorescens* and *T.*

viride each at 6 g/plot was also found to improve the silkworm larval weight, cocoon weight, shell weight and shell ratio of silkworm larvae fed on leaves of mulberry infested with *M. incognita* (Table 3).

There is increasing interest in the use of microbial inoculants to suppress nematodes in several crops against plant-parasitic nematodes (Rodriguez-Kabana and Morgan-Jones, 1988; Kerry and Jaffee, 1997; Stirling *et al*., 1998 and Dong and Zhang, 2006). However, the bio-control agents are not universally effective on all crops and against all nematodes. In this investigation, *P. fluorescens* and *T. viride* were found to be potential biocontrol agents against *M. incognita* in mulberry under both glass-house and field conditions. Jonathan *et al.* (2006) reported increased yield of banana subsequent to application of *P. fluorescens* to control *M. incognita*. The presence of *P. fluorescens* in roots and *Rhizosphere* is known to suppress plant parasitic nematodes by alteration of root exudates, which influence nematode egg hatch, attraction and penetration behaviour (Oostendorp and Sikora, 1989). This bacterium was reported to produce antibiotics, such as 2,4 diacetyl phloroglucinol (DAPG), which reduce the mobility of *Globodera rostochiensis* J2 (Cronin *et al*., 1997), toxic metabolites that cause mortality of infective juveniles of *Hirschmanniella gracilis* (Seenivasan and Lakshmanan, 2001) and induces systemic resistance by synthesis and accumulation of peroxidase, chitinase and

Table 1 : Effect of bio-control agents on population densities of *M. incognita* and plant growth parameters in mulberry fields

Treatments	J_2/250 cm^3 soil		Egg masses/ g root	Gall index	Bio-agent population (cfu/g root)	Number of branches/ Plant	Plant height (cm)	Weight of 100 leaves (g)
	Before treatment	5 months after treatment						
P. fluorescens 6 g/ plant	180.14	119.56	12.15	2	$2.2 \times 10^{6*}$	14.32	265.54	530.14
	(-6.26)	(-66.66)	(-52.76)			(49.79)	(24.25)	(35.65)
P. fluorescens 3 g/ plant	184.51	130.72	12.32	2	$1.4 \times 10^{6*}$	13.01	258.13	479.34
	(-3.99)	(-63.55)	(-52.99)			(36.09)	(20.78)	(22.65)
T. viride 6 g/ plant	186.32	151.15	11.53	2	$1.2 \times 10^{6**}$	14.45	260.56	529.73
	(-3.04)	(-57.85)	(-55.17)			(51.15)	(21.92)	(35.54)
T. viride 3 g/ plant	182.15	169.53	12.01	2	$1.2 \times 10^{6**}$	13.95	236.58	476.61
	(-5.21)	(-52.73)	(-53.30)			(45.92)	(10.69)	(21.95)
P. fluorescens 6 g/ plant + *T.*	186.32	95.10	11.10	2	$3.1 \times 10^{5*}$	15.56	288.37	628.56
viride 6 g/ plant	(-3.04)	(-73.48)	(-56.84)		$4.6 \times 10^{5**}$	(62.76)	(34.93)	(60.83)
P. fluorescens 3 g/ plant + *T.*	185.15	108.70	12.12	2	$2.2 \times 10^{4*}$	15.32	270.61	602.45
viride 3 g/ plant	(-3.65)	(-69.69)	(-52.88)		$4.1 \times 10^{4**}$	(60.25)	(26.62)	(54.15)
Carbofuran 3G 1 kg a.i./ha	180.16	122.12	13.89	3	-	10.98	239.13	401.62
	(-6.25)	(-65.95)	(-45.99)			(14.85)	(11.89)	(2.76)
Control	192.17	358.63	25.72	5	-	9.56	213.72	390.82
C.D. (P = 0.05)	0.1396	2.9700	0.1682	-	-	0.0757	0.8188	2.9953

Figures in parentheses are per cent increase (+) or decrease (-) over control; * *Pseudomonas fluroscence* in soil; ** *Trichoderma viride* in soil

Table 2 : Effects of the bio-control agents on protein, nitrogen and chlorophyll contents of mulberry leaves in fields infested by *M. incognita*

Treatments	Protein (mg/g)	Nitrogen (%)	Chlorophyll content (mg/g)		
			Chlorophyll A	Chlorophyll B	Total chlorophyll
P. fluorescens 6 g/ plant	52.58 (+88.86)	6.22 (14.44)*	2.21 (+148.31)	0.74 (+60.87)	3.21 (+52.13)
P. fluorescens 3 g/ plant	46.12 (+65.66)	5.99 (14.17)*	1.61 (+80.89)	0.64 (+39.13)	2.99 (+41.71)
T. viride 6 g/ plant	44.57 (+60.09)	5.73 (13.85)*	1.52 (+70.79)	0.75 (+63.04)	3.09 (+46.44)
T. viride 3 g/ plant	39.63 (+42.35)	5.57 (13.65)*	1.40 (+57.30)	0.51 (+108.69)	2.71 (+28.44)
P. fluorescens 6 g/ plant + T. viride 6 g/ plant	55.98 (+101.08)	7.39 (15.79)*	2.33 (+161.79)	0.77 (+67.39)	3.47 (+64.45)
P. fluorescens 3 g/ plant + T. viride 3 g/ plant	54.26 (+94.89)	7.09 (15.44)*	2.29 (+157.30)	0.75 (+63.04)	3.21 (+52.13)
Carbofuran 3G 1 kg a.i./ha	29.64 (+6.46)	4.77 (12.61)*	0.93 (+4.49)	0.47 (+2.17)	2.16 (+2.37)
Control	27.84	4.00 (11.54)*	0.89	0.46	2.11
C.D. (P=0.05)	0.378	0.026	0.020	0.004	0.017

Figures in parentheses are per cent increase (+) over control; * Figures in parentheses indicate arc sine transformed values.

Table 3 : Quality of silk worms fed with leaves of mulberry plants grown in fields infested by *M. incognita* and treated with the bio-agents

Treatments	5th instar larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
P. fluorescens 6 g/ plant	3.85 (+10.00)	1.628 (+7.67)	0.332 (+14.09)	20.39 (26.84)*
P. fluorescens 3 g/ plant	3.95 (+12.86)	1.635 (+8.13)	0.306 (+5.15)	21.00 (27.27)*
T. viride 6 g/ plant	3.85 (+10.00)	1.676 (+10.85)	0.361 (+24.05)	19.25 (26.02)*
T. viride 3 g/ plant	3.66 (+4.57)	1.743 (+15.28)	0.349 (+19.93)	20.37 (26.83)*
P. fluorescens 6 g/ plant + T. viride 6 g/ plant	4.11 (+17.43)	1.875 (+24.01)	0.375 (+28.86)	21.79 (27.83)*
P. fluorescens 3 g/ plant + T. viride 3 g/ plant	4.10 (+17.14)	1.841 (+21.76)	0.374 (+28.52)	21.54 (27.65)*
Carbofuran 3G 1 kg a.i./ha	3.75 (+7.14)	1.714 (+13.36)	0.361 (+24.05)	18.72 (25.64)*
Control	3.50	1.512	0.291	16.69 (24.11)*
CD(p=0.05)	0.133	0.059	0.012	0.693

Figures in parentheses are per cent increase (+) over control; * Figures in parentheses indicate arc sine transformed values

glucanase enzymes in plant root systems (Kalaiarasan *et al*., 2006). These results agree with our findings as *P. fluorescens* and *T. viride* suppressed the root galling and egg mass production by *M. incognita* and resulted in increased yield in both glass-house and field experiments. The reduction of root galling confirms earlier findings by Sharon *et al.* (2001) in green-house tomatoes inoculated with *M. incognita* and treated with *T. viride*. Direct pathogenicity of fungal bio-control agents is one of the main mechanisms responsible for the control of plant-parasitic nematodes (Kerry, 1988; Stirling, 1991). However, secondary metabolites from these fungi also contain compounds which are toxic to plant parasitic nematodes (Hallmann and Sikora, 1996 and Sikora *et al*., 2003).

Mulberry leaf yield was greater when both bio-control agents were used together than either alone. The development and reproduction of some of the juveniles that penetrated mulberry roots might have been affected further by the root colonization by *P. fluorescens* (Santhi and Sivakumar, 1995). *Pseudomonas fluorescens* produces nematoxic metabolites that may be involved in the nematode suppression (Khan and Akram, 2000). Pyrollnitrin (Leyns *et al*., 1990) and phenazin (Gurusiddaiah *et al.*, 1986) produced by *P. fluorescens* could also alter the development of *M. incognita* in roots. Greater reduction of *M. incognita* infestation in the treatment with *P. fluorescens* and *T. viride* might be due to supplementary effect of *T. viride*.

In conclusion, the present study suggests that combined application of *P. fluorescens* and *T. viride*, each at 6 g/plant, can be recommended for the management of root-knot nematode, *M. incognita* in mulberry cultivation.

REFERENCES

Anonymous (1970). Methods of Analysis. Association of official Agricultural Chemists, Washington. 789pp.

Babu A.M., Kumar, Vineet and Philip, Tomy (1999). Root knot nematode – A hard to kill parasite – study. *Indian Silk*, **38** : 11-12.

Cronin, D., Loccoz, Y.M., Fehton, A., Dunne, C., Dowling, D.N., and Gara, F.O. (1997). Role of 2, 4-diacetyl phloroglucinol in the interactions of the biocontrol *Pseudomonas* strain F113 with potato cyst nematode, *Globodera rostochiensis. Appl. & Environ. Microbio.*, **63** (4) : 1357-1361.

Dababat, A.A., Sikora, R.A. and Hauschild, R. (2006). Use of *Trichoderma harzianum* and *Trichoderma viride* for the biological control of *Meloidogyne incognita* on tomato. *Communi. Agric. Appl. Biol. Sci.*, **71** (3 Pt) : 953-961.

Dandin, S.B., Jayaswal, J. and Giridhar, K. (2000). *Hand Book of Sericulture Technologies***,** Central Silk board, Bangalore, India. 185 pp.

Dong, L.Q. and Zhang, K.Q. (2006). Microbial control of plant parasitic nematodes a five party interaction. *Plant & Soil.*, **288** : 31-34.

Govindaiah and Sharma, D.D. (1994). Root knot nematode, *Meloidogyne incognita* infesting mulberry - A review. *Indian J. Sericulture*, **33** : 110-113.

Gurusiddaiah, S., Weller, D.M., Sarkar, A. and Cook, R.J. (1986). Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. tritici and *Pythium* spp. Antimicrobial Agents Chemother, **29** : 488-495pp.

Hallmann, J. and Sikora, R.A. (1996). Toxicity of fungal endophyte secondary metabolites to plant- parasitic nematodes and soil-borne plant-pathogenic fungi. *European J. Plant Pathol.*, **102** (2) : 155-162.

Jonathan, E.I., Sandeep, A., Cannayane, I. and Umamaheswari, R. (2006). Bioefficacy of *Pseudomonas fluorescens* on *Meloidogyne incognita* in banana. *Nematologia Mediterranea*, **34** : 19-25.

Kalaiarasan, P., Lakshmanan, P.L. and Samiyappan, R. (2006). Biotization of *Pseudomonas fluorescens* isolates in groundnut (*Arachis hypogaea* L.) against root knot nematode, *Meloidogyne arenaria*. *Indian J. Nematol.*, **36** (1) : 1-5.

Kerry, B.R. (1988). Two microorganisms for the biological control of plant parasitic nematodes. In: Proceedings of the broighton crop protection conference, **2** : 603-607.

Kerry, B.R. and Jaffee, B.A. (1997). Fungi as biological control agents for plant-parasitic nematodes. In: The mycota: A comprehensive treatise on fungi as experimental systems for basic applied research (Wicklow D.T. and Soderstrom B.E., eds). Springer-Verlag, Berlin Heidelberg, Germany. 203-218pp.

Khan, M.R. and Akram, M. (2000). Effects of certain antagonistic fungi and rhizobacteria on wilt disease complexes of tomato caused by *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici*. *Nematologia Mediterranea*, **28** (2) : 139-144.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. *J. Biol. Chem.*, **193** : 265-275.

Leyns, F., Lambert, B., Joos, H. and Swings, J. (1990). Antifungal bacteria from different crops. In: Biological Control of Soil-Borne Plant Pathogens (Hornby D., Ed.). CAB International, Wallingford, UNITED KINGDOM. 437-444pp.

Oostendorp, M. and Sikora, R.A. (1989). Seed treatment with antagonistic rhizobacteria for the suppression of *Heterodera schachtii* early root infection of sugar beet. *Revue de Nématologie,* **12** (1) : 77-83.

Panse, V.C. and Sukhatme, V.P. (1989). *Statistical Methods for Agricultural Workers*. ICAR, New Delhi (India).

Paul, A.S., Babu, P.S. and Sukul, N.C. (1995). Effect of nematode infected mulberry plants on the growth and silk production of *Bombyx mori* L. *Indian J. Sericulture,* **34** (1) : 18-21.

Rodriguez Kabana, R., Morgan Jones, G. (1988). Potential for nematode control by microfloras endemic in the tropics. *J. Nematol.*, **20**(2) : 191-203.

Sadasivam, S. and Manickam, A. (1992). Biochemical methods for agricultural sciences. Wiley Eastern Limited, New Delhi, India, 246pp.

Santhi, A. and Sivakumar, C.V. (1995). Biocontrol potential of Pseudomonas fluorescens (Migula) against root knot nematode, Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 on tomato. *J. Biological Control*, **9** : 113-115.

Seenivasan, N. and Lakshmanan, P.L. (2001). Effect of culture filtrates of *Pseudomonas fluorescens* on rice root nematode, *Hirschmanniella gracilis*. *Pestol.*, **25**: 11-12.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O. and Spiegel, Y. (2001). Biological control of the root-knot *Nematode Meloidogyne javanica* by *Trichoderma harzianum*. *Phytopathology*, **91** (7) : 687-693.

Siddiqui, I.A. and Shaukat, S.S. (2003). Suppression of root-knot disease by Pseudomonas fluorescens CHAO in tomato: importance of bacterial secondary metabolite, 2, 4-diacetylphloroglucinol. *Soil Biol. & Biochem.*, **35** (12) : 1615-1623.

Sikora, R.A., Niere, B. and Kimenju, J. (2003). Endophytic microbial biodiversity and plant nematode management in African Agriculture, In: Neuenschwander, P., Borgermeister, C. and Langewald, J. (editors), Biological control in IPM systems in Africa, 179-192pp.

Southey, J.F. (ed.) (1986). Laboratory methods for work with plant and soil nematodes. Her Majesty's Stationery Office, London.

Stirling, G.R. (1991). Biological control of plant parrasitic nematodes, Wallingford, U.K.–CAB International.

Stirling, G.R., Licastro, K.A., West, L.M. and Smith (1998). Development of commercially acceptable formulations of the nematophagus fungus *Vertificillium chlamydosperium*. *Biological Control*, **11** : 215-221.

Yoshida, S., Forno, D.A., Cook, J.H. and Gomez, K.A. (1971). Laboratory manual for physiological studies of rice. IRRI, Manila, Philippines, 65 pp.