Biocontrol of root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in mulberry (*Morus alba* L.)

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ABSTRACT

Field experiments conducted for the evaluation of efficacy of biocontrol agents *viz.*, *Pseudomonas fluorescens* and *Trichoderma viride*, against root knot nematode, *Meloidogyne incognita* in mulberry (V1 variety) revealed that soil application of both *P. fluorescens* and *T. viride* alone or in combination was able to control the nematode population and improve the mulberry leaf yield and nutritional standards. Combined soil application of *P. fluorescens* (@ 10 g/plant) + *T. viride* (@ 10 g/plant) as soil application was effective to check the root knot nematode disease and to improve growth of mulberry with increased leaf yield and reduced nematode population.

Keywords: *Pseudomonas fluorescens, Trichoderma viride, Meloidogyne incognita,* mulberry, crop pest.

INTRODUCTION

Mulberry (*Morus alba* L.), the sole food plant of silkworm (*Bombyx mori* L.), is cultivated both in tropical and temperate countries of the world. India is the second largest country in the world having 3.42 lakh hectares under mulberry cultivation (Govindaiah and Sharma, 1994). In India, due to the prevalence of favourable climatic conditions, mulberry is cultivated mainly in the states of Andhra Pradesh, Jammu and Kashmir, Karnataka, Tamil Nadu, Uttar Pradesh, West Bengal and North-Eastern states. These states collectively account for 97 per cent of the total area under mulberry cultivation and 95 per cent of raw silk production in the country.

Several pests and diseases reduce the nutritive value of mulberry leaves and influence the growth and development of silkworm ultimately leading to poor cocoon production. Plant parasitic nematodes play an important role in reducing herbage yield and quality of leaves besides the life span of mulberry plants. Among mulberry nematodes, root knot nematode, *Meloidogyne incognita* (Kofoid and white) is economically important as they affect the crop quantitatively and qualitatively. Root knot nematode disease is found world wide but it is most serious in tropical and subtropical countries. The nematode has got a wide range of host plants and cause economic damage to many agricultural crops (Sasser, 1989). The disease is manifested by the formation of galls in the root accompanied by stunted growth, chlorosis and loss of vigour of the plant (Babu *et al.*, 1999). The efficacy of the antagonistic microorganisms, *P. fluorescens* and *T. viride* against the root knot nematode in mulberry was assessed in the present investigation.

MATERIALS AND METHODS

Two field experiments were conducted for the management of root knot nematode to evaluate the biocontrol potential of *Pseudomonas fluorescens* and *Trichoderma viride*, in comparison with standard chemical check of carbofuran 3G and an untreated control, in an established mulberry garden with V1 variety in field at Thondamuthur village of Coimbatore district. The experiments were conducted in a completely randomized block design with three replications. The treatments were T_1 - Soil application of *P. fluorescens* @ 10g/plant, T_2 - Soil application of *P. fluorescens* @ 5g/plant, T_3 - Soil application of *T. viride* @ 10g/plant, T_4 - Soil application of *T. viride* @ 5g/plant, $T_5 – T_1 + T_3$, $T_6 – T_2 + T_4$, T_7 – Carbofuran 3G @ 1kg a.i./ha and T_8 – untreated control. The experiments were terminated five months after initiation and observations were made on plant growth parameters *viz.*, shoot length, root length, shoot and root weight, number of branches per plant, number of leaves per plant, leaf area (mean of five leaves), nematode population and colonizing ability of bioagents.

Colonization of *P. fluorescens* and *Trichoderma viride*

At the time of termination of the experiment soil samples were collected from the non-rhizophere region of 5-6 cm away from root base of mulberry plant. One g of the soil sample was taken and it was dissolved in

9ml of sterile distilled water to make a dilution of 10^{-1}. One ml of 10^{-1} dilution was pipetted out using a sterile pipette and transferred to 9ml sterile distilled water in test tubes. It gave a dilution of 10^{-2}. Similarly, serial dilution was continued up to 10^{-4}. From 10^{-4} dilution, one ml of suspension was transferred to Petri dish containing *Trichoderma* selective medium (Elad and Chet, 1983) and incubated at 28 ± 2°C for 5 days and colonies of fungus were counted. Similarly serial dilution was continued upto 10^{-6}. One ml of 10^{-6} diluted suspension was transferred to Petri dish containing King's B medium (King *et al.*, 1954) and incubated at 28 ± 2°C for 5 days and then colonies of *P. fluorescens* were counted.

RESULTS AND DISCUSSION

The bioagents, *P. fluorescens* and *T. viride*, and the chemical carbofuran 3G tested in the present investigation were found to improve the mulberry plant growth characters and reduce the population of *M. incognita* compared to the untreated control (Tables 1, 2 and Fig. 1). The explanations for these results may be due to the antagonistic activity of *P. fluorescens* (Santhi and Sivakumar, 1995) and higher activity of defense enzymes in the plants treated with *T. viride* (Umamaheswari *et al.*, 2004).

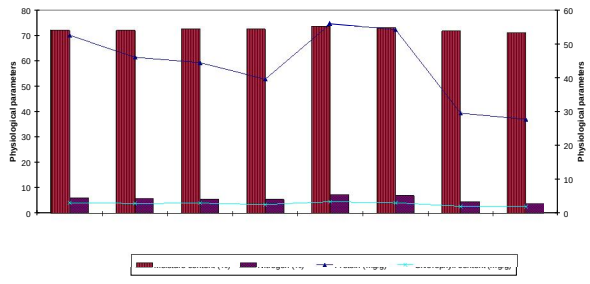

Figure 1. Efficacy of biocontrol agents on physiological changes in mulberry leaves (VI variety) infested by *Meloidogyne incognita*

T_1 - Soil application of *Pseudomonas fluorescens* @ 10g/ plant, T_2 - Soil application of *P. fluorescens* @ 5g/plant, T_3 - Soil application of *Trichoderma viride* @ 10g/plant, T_4 - Soil application of *T. viride* @ 5g/plant, T_5 – T_1 +T_3 T_6 – T_2 +T_4, T_7 – Carbofuran 3G @ 1kg a.i./ha, T_8 – Untreated control.

Combination of the bacterium, *P. fluorescens* and the fungus, *T. viride* at higher dose of 10 g/plant was the

Table 1. Effect of biocontrol agents on plant growth characters of mulberry in *Meloidogyne incognita* infested field (Pooled data from two experiments)

Treatments	Number of branches	Number of leaves	Leaf area (cm^2)	Plant height (cm)	Branch length (cm)	Branch weight (cm) 5 nos. (g)	100 leaf /weight (g)
Pseudomonas fluorescens @ 10g/ plant	15.40(+45.28)	294.16(+43.11)	169.17(+28.87)	270.45(+25.42)	195.36(+39.18)	1325.37(+31.94)	533.26(+35.29)
P. fluorescens @ 5g/ plant	13.50(+27.36)	270.37(+31.54)	145.96(+11.19)	260.35(+20.74)	190.34(+35.61)	1425.94(+41.95)	482.98(+22.54)
Trichoderma viride @ 10g/ plant	14.60(+37.73)	290.64(+41.40)	163.29(+24.39)	265.65(+23.19)	195.64(+39.38)	1450.13(+44.36)	532.36(+35.07)
T. viride @ 5g/plant	13.40(+26.41)	251.15(+22.19)	143.64(+9.42)	240.76(+11.65)	180.38(+28.51)	1350.89(+34.48)	482.54(+22.43)
P. fluorescens @ 10g/plant + *T. viride* @10g/plant	15.80(+49.06)	352.43(+71.46)	235.29(+79.24)	290.27(+34.61)	210.39(+49.89)	1600.56(+59.33)	639.04(+62.13)
P. fluorescens @ 5g/plant + *T. viride* @ 5g/plant	15.70(+48.11)	300.56(+46.23)	231.14(+76.08)	275.48(+27.75)	200.83(+43.08)	1500.75(+49.39)	606.44(+53.86)
Carbofuran 3G @ 1 kg a.i./ha	11.40(+7.55)	230.82(+12.29)	136.59(+4.05)	240.58(+11.57)	180.57(+28.65)	1200.02(+19.52)	415.48(+5.41)
Control	10.60	205.54	131.27	215.63	140.36	1004.52	394.14
CD(p = 0.05)	0.069	1.601	1.446	0.833	0.745	19.670	2.985

Table 2. Effect of biocontrol agents on nematode population in mulberry field infested with *Meloidogyne incognita* (Pooled data from two experiments)

Treatments	Nematode population (250g of soil)	Number of females/g root	Number of egg masses/g root	Number of eggs /egg mass	Gall index	Soil antagonistic population (cfu/g soil)
Pseudomonas fluorescens @ 10g/ plant	121.56(-42.15)	14.44(-55.44)	13.44(-48.58)	230.51(-29.67)	2	$2.2x10^6$
P. fluorescens @ 5g/ plant	135.72(-35.41)	15.72(-51.49)	13.72(-47.51)	245.34(-25.15)	2	$1.4x10^6$
Trichoderma viride @ 10g/plant	156.85(-25.35)	14.39(-55.60)	12.93(-50.53)	256.67(-21.69)	2	$1.2x10^4$
T. viride @ 5g/plant	172.13(-18.08)	15.83(-51.16)	13.38(-48.81)	265.42(-19.02)	2	$1.2x10^4$
P. fluorescens @10g/plant + *T. viride* @10g/plant	99.54(-52.63)	12.45(-61.58)	11.54(-55.85)	186.98(-42.95)	2	$3.1x10^6$* $4.6x10^4$**
P. fluorescens @5g/plant + *T. viride* @5g/plant	110.02(-47.64)	13.65(-57.88)	12.56(-51.95)	203.94(-37.78)	2	$2.2x10^6$* $4.1x10^4$**
Carbofuran 3G @ 1 kg a.i./ha	125.58(-40.23)	18.10(-44.15)	14.10(-46.06)	216.43(-33.97)	3	-
Control	210.12	32.41	26.14	327.76	5	-
CD(p=0.05)	1.278	0.224	0.163	1.532	-	-

Figures in parentheses are per cent increase (+) over control, * *Pseudomonas fluroscens* in soil, ** *Trichoderma viride* in soil.

most effective treatment. Similar combination treatment (*P. fluorescens*, *T. viride* and *T. harzianum*) has been reported to reduce the nematode population and increase the yield when tried along with neem cake and FYM (Murugesh and Mahalingam, 2008). Our results suggest that the dose of 10 g/plant each of *P. fluorscens* and *T. viride* could provide significant reduction in *M. incognita* population and effective growth of mulberry crop.

According to Cronin *et al*. (1997), the antibiotics 2-4 diacetyl phloroglucinal, produced by *P. fluorescens* was inhibitory to *Globodera rostochiensis*. Treatment with *P. fluorescens* induced the activity of peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, catalase and chitinase in tomato against *M. incognita* (Anita *et al.*, 2004). Bokhari (2009) reported that all culture filtrate of the *Trichoderma* species was highly significant in controlling reniform nematode (*Rotylenchulus reniformis*) and root knot nematode (*Meloidogyne javanica*) genera on eggplant. *Trichoderma harazianum*, *T. hamatum* and *T. koningii* culture filtrates gave a significant reduction *in vitro* and decreased the female and egg-masses of reniform and root knot nematodes. *Trichoderma* species led to inhibition of the nematode activity and movements *in vitro* during one week exposure. *Trichoderma viride* in combination with organic amendments was also known to produce growth hormones, which were observed to have added response in boosting the plant vigour (Chang *et al*., 1986). Application of *P. fluorescens* with other management practices has been proved more effective in many crops for different nematodes. Devrajan *et al*. (2004) reported that the combination of *P. fluorescens* and neem cake and mustard as an inter crop reduced the population of potato cyst nematode and increased tuber yield. The highest reduction of root knot nematode population in soil was observed in *P. fluorescens* and FYM treated vines (Senthilkumar and Rajendran, 2004). Kavitha *et al*. (2007) reported that *P. fluorescens* (2.5 kg/ha) recorded significantly higher growth parameters and lower nematode population in sugarbeet. They observed enhanced activities of enzymes in *P. fluorescens* treated sugarbeet plant roots.

The results of the estimation of physiological parameters in mulberry leaves indicated that there was significant reduction in moisture content, protein, nitrogen and chlorophyll content in nematode infested plants. The treatment with the bioagents or carbofuran improved the physiological status of the infested plants. The protein deficient leaves adversely affected the growth and silk production of silkworms. Sharma *et al*. (1998) reported that application of carbofuran 3G @ 40kg/ha/year reduced the disease severity due to the root knot nematode by 72.1 to 76.1 per cent in mulberry.

Carbofuran treatment did not show any favourable influence on larval growth and their silk production although it improved plant growth and leaf protein content significantly. Paul *et al*. (1995) opined that the leaves of treated plants might have contained the chemical residues which had toxic effect on silkworm larvae or carbofuran was metabolized by mulberry plants into a substance toxic to silkworms. These findings suggest that application of carbofuran might be lethal to silkworms and the application of biological agents is safe. Present results showed that soil application of *P. fluorscens* and *T. viride* each at 10 g/plant gave significant reduction in *M.*

incognita infestation and also to increase the growth of mulberry crop compared with carbofuran.

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