Evaluation of Bioagents for their Efficacy in Management of Root-knot Nematode Infesting Mulberry

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Received:- 01 July 2024/ Revised:- 10 July 2024/ Accepted:- 17 July 2024/ Published: 31-07-2024

Abstract— *Mulberry is integral to sericulture, with its foliage being crucial for silk quality. The root-knot nematode, Meloidogyne incognita, significantly impacts mulberry by causing considerable leaf yield losses and degrading leaf quality. This study evaluated the efficacy of various bioagents for the eco-friendly management of RKN. The bioagents tested were Purpureocillium lilacinum, Trichoderma harzianum, Trichoderma viride, Pochonia clamydosporia, and Pseudomonas fluorescens, alongside control treatments including carbofuran 3G and neem cake. Results demonstrated that T. viride markedly reduced the nematode population in both soil and roots, achieving a nematode reduction of 79.82% in soil and 85.21% in roots at 120 days post-treatment. These reductions were comparable to those obtained with the chemical standard carbofuran 3G. The application of T. viride, in conjunction with farmyard manure, emerged as a highly effective strategy, offering a sustainable alternative to chemical nematicides. This approach not only controls RKN populations but also enhances soil health, thereby supporting sustainable sericulture practices. These findings underscore the potential of bioagents in fostering environmentally sustainable and economically viable sericulture.*

Keywords— Bioagents, Mulberry, Population, Root-knot nematode, Rhizosphere.

I. INTRODUCTION

The perennial plant mulberry (*Morus alba* L.) is highly adaptable to varied climatic conditions ranging from temperate to tropics and thrives well under different soils. Foliage is the major economic part of mulberry that ultimately decides the quality of raw silk since the silkworm feeds on mulberry leaf alone to derive its nutrients for growth and productivity. The quantity of quality leaf produced per unit area has a direct bearing on cocoon production and raw silk quality.

Several factors contribute in obtaining a successful cocoon crop, among them mulberry leaf alone contributes to around 38.20 per cent which is followed by microclimate in the rearing house (37.00%), silkworm rearing techniques (9.30%) and the breed (4.20%), which signifies the role of quality foliage in cocoon production. Apart from soil parameters, the biotic and abiotic stress factors greatly affect the quality of mulberry leaf. The nutritive values get degraded due to diverse biotic stresses *viz*., diseases (pathogens) and pests (insect/ non-insect) and the mulberry plants attract these pests and diseases due to its perennial, fast-growing and lush green characteristics (Miyashita, 1986). The biotic stressors affect both above and below ground parts of the mulberry plant, the symptoms of which can be easily traced through the foliage.

A markedly higher population of fungi and nematodes could be observed in the mulberry rhizosphere (Nandi *et al.*, 2004). Around forty-two species of nematodes belonging to 24 genera were associated with mulberry from almost all countries, where sericulture is being practiced (Swamy and Govindu, 1965). Among them, the nematodes belonging to five genera *viz.*, *Meloidogyne*, *Rotylenchulus*, *Helicotylenchus*, *Hoplolaimus* and *Xiphinema* were reported from India. The root-knot nematode

(RKN), *Meloidogyne incognita* species alone is known to cause 20-50 per cent mulberry leaf yield loss apart from deteriorated quality (Arunakumar *et al.*, 2018).

The infestation of *M. incognita* (Kofoid and White) Chitwood on mulberry was first reported in India by Narayan *et al*. (1966). The infestation is more commonly noticed in sandy to loamy soils and under irrigated conditions. The RKNs are parasites of underground roots, which is difficult to recognize and hence the damage symptoms very often go unnoticed (Sengupta and Govindaiah, 1991). The affected plants show stunted growth, marginal necrosis and yellowing of leaves. The characteristic knots or galls appear on the roots and affect the utilization of water and nutrients resulting in poor plant growth (Govindaiah *et al.*, 1991).

The RKNs have three life stages – egg, larva with four juvenile stages (J_1, J_2, J_3, J_4) and adult of which, the J_2 infects the host plants (Biligrami and Dube, 1976). The female larva enters into roots, harbours in the sub-epidermal layer and starts feeding on the parenchymatous cells causing hypertrophy and hyperplasia that induce characteristic knots. The larvae undergo four moults in the roots and develop into mature oval or spherical egg-laying females. Each female lays 200-300 ellipsoidal eggs covered with a gelatinous substance. The eggs hatch and larvae are liberated into the soil under favourable conditions. The life cycle is completed in 30-40 days and 2-3 such cycles are noticed per annum. A temperature of 15-30˚C and soil moisture of 40-60 per cent are more favourable for the growth and development of RKNs (Padma *et al.*, 2008). The size and number of galls induced by RKNs keep on increasing as the generations are repeated in the root tissues. Apart from damaging the parenchyma tissue, the cracks and holes in the galls invite secondary root infections. Thus, the root-knot infected plants show symptoms of both nutrient deficiency and other root diseases like root rot (Babu *et al.*, 1996).

The studies reveal that the infestation of RKNs not only affects the growth and development of the crop but also affects the physiology ultimately resulting in inferior quality of foliage. Further, since mulberry leaf is the sole source of nutrition for silkworm, *B. mori*, the cocoon crop and quality of raw silk are severely affected causing huge economic loss to the farmer. Hence, effective management of RKNs is imminent for sustained productivity in sericulture. Though synthetic nematicides are available for the management of RKNs, environmental concerns, mammalian toxicity and longer safety periods limit their utility in sericulture. Alternately, the use of bioagents antagonistic to the nematodes serves the purpose of nematode management apart from improving soil health, which is an apt strategy to pace with the increasing environmental concerns.

With this background, an experiment was planned to identify suitable bioagents for eco-friendly management of RKNs infesting mulberry.

II. MATERIALS AND METHODS

2.1 Experiment site details:

The experiment was carried out in the RKN infested mulberry garden in Kalyapura Village, Shidlaghatta Taluk, Chikkaballapura District i.e., in the Eastern Dry Zone (Zone-5) of Karnataka, India at 13°14'20"N latitude and 77°52'17"E longitude, at an altitude of 904m above mean sea level.

The mulberry plantation selected for isolation of nematodes had red sandy loam type of soil with six years old V1 variety plants, planted at the spacing of 90×90cm; bottom pruning (Kolar method) was followed; field was irrigated in two-days interval with drip irrigation facility *i.e.*, based on soil moisture conditions; organic manures and inorganic fertilizers were applied in accordance with the recommended package of practice. Except for the management of RKN, the selected field was well maintained.

2.2 Bioagents:

The following bioagents were selected based on the reviews and were used for the management of RKN in mulberry. The selected bioagents were,

a) *Purpureocillium lilacinum*

b) *Trichoderma harzianum*

c) *Trichoderma viride*

d) *Pochonia clamydosporia*

e) *Pseudomonas fluorescens*

Along with the five bioagents, Nemahari (bio-nematicide developed by CSRTI, Mysore), neem cake, carbofuran 3G and control (untreated check) are included in the treatments for comparison of the efficacy.

2.3 Treatment details:

TABLE 1
TREATMENT DETAILS

Treatments		Recommendation	Reference
T_1	*Purpureocillium lilacinum*	5 kg/ha (with 5 tons FYM)	Saxena *et al.*, 2021
T_2	*Trichoderma harzianum*		
T_3	*Trichoderma viride*		
T_4	*Pochonia clamydosporia*		
T_5	*Pseudomonas fluorescens*		
T_6	Nemahari	40 kg/ha (with 400 kg FYM)	Nishita and Prateeshkumar, 2015
T_7	Neem cake	2000 kg/ha/yr	Dandin and Giridhar, 2014
T_8	Carbofuran 3G (Standard check)	40 kg/ha/yr	Dandin and Giridhar, 2014
T_9	Control (Untreated check)	-	-

The treatments were incorporated near the root zone of the mulberry within a week after pruning by calculating the dosage per plant as per the recommendations.

2.4 Observations recorded:

In the soil, the initial (before imposing the treatment), intermediary (30, 60 and 90 days after treatment imposition - DAT) and final (120 DAT) nematode population were estimated using combined Cobb sieving and decanting method followed by Modified Baermann's funnel method whereas, in mulberry roots, the number of galls and number of egg masses per root system was counted based on visual observations while the initial, intermediary (*i.e.*, first shoot harvest - 60 DAT) and final (*i.e.*, second shoot harvest - 120 DAT) nematode population were estimated using root incubation method (Ayoub, 1977).

The soil and root samples of the infested garden were collected from the rhizosphere soil of each replication of every treatment using a spade and shovel from the root zone of mulberry. Later, the samples each weighing 200 g of soil and 5 g of roots were collected in a polythene bag. The collected samples were preserved under refrigerated conditions and then analysed for the nematode population before imposing the treatments. The same procedure was repeated to collect the soil and root samples for intermediary and final estimations.

2.4.1 Estimation of root-knot nematode population in soil:

The soil sample of 200 cc was washed thoroughly and processed through combined "Cobb's sieving and decantation method followed by Modified Baermann's funnel method" (Ayoub, 1977) as given below:

a) 200 cc of soil was taken in a 1000 ml beaker and a sufficient quantity of water was added to make soil solution

b) This was stirred thoroughly and allowed to stand for heavier particles to settle down

c) Then the soil solution was passed through a set of sieves of 100, 250, 325 and 400 mesh sizes, respectively

d) Residue from 325 and 400 mesh sieves was collected and poured over a tissue paper spread on a wire gauge and placed on Baermann's funnel

e) Level of water in the Baermann's funnel was maintained to keep the tissue paper wet and left undisturbed for 48 hours

f) After incubation for 48 hours, the volume of suspension was made to 200 ml, out of which 10 ml was pipetted out and used for counting the presence of root-knot nematodes using a stereo-zoom binocular microscope

g) The nematode population from this was finally estimated for 200 cc of soil

2.4.2 Estimation of root-knot nematode population in mulberry roots:

The RKN population in 5 g of roots was estimated using the root incubation method (Ayoub, 1977) as follows:

a) Root samples were washed with water

b) The washed roots were cut into bits of 2.5 cm and then sliced longitudinally

c) The cut bits were then placed over tissue paper spread on the wire gauge and then kept on the Petri plate

d) Water level was maintained in the Petri plate and was kept undisturbed for 48 hours

e) Later, the suspension in the Petri plate was collected and observed for nematodes using a stereo-binocular microscope

f) The nematodes counted were finally estimated for 5 g of roots

III. RESULTS AND DISCUSSION

3.1 Nematode population in the rhizosphere soil:

The observation on the effect of different bioagents on the population of root-knot nematode per 200 cc soil was recorded once before the imposition of treatments (initial), intermediary (30, 60 and 90 days after treatment imposition - DAT) and final (120 DAT). The recorded observations are presented in Table 2 and Fig. 1.

FIGURE 1: RKN infested mulberry root with root-knots or galls

TABLE 2

EFFECT OF DIFFERENT BIOAGENTS ON THE POPULATION OF RKN IN THE RHIZOSPHERE SOIL OF MULBERRY

Treatments		Initial	Nematode population per 200 g root							
			30 DAT	Per cent decrease over control	60 DAT	Per cent decrease over control	90 DAT	Per cent decrease over control	120 DAT	Per cent decrease over control
T_1	***Purpureocillium lilacinum***	209.25	184.25	23.78	147.75	46.07	115.50	59.58	69.00	76.51
T_2	***Trichoderma harzianum***	210.25	174.25	27.92	136.25	50.27	100.25	64.91	64.00	78.21
T_3	***Trichoderma viride***	209.50	168.25	30.40	127.25	53.55	89.50	68.67	59.25	79.82
T_4	***Pochonia clamydosporia***	209.75	186.50	22.85	155.00	43.43	119.00	58.35	69.50	76.34
T_5	***Pseudomonas fluorescens***	210.75	171.50	29.05	132.50	51.64	97.00	66.05	60.00	79.57
T_6	**Nemahari**	210.50	160.75	33.50	111.75	59.21	81.25	71.56	51.75	82.38
T_7	**Neem cake**	209.25	164.00	32.16	112.25	59.03	84.50	70.42	54.25	81.53
T_8	**Carbofuran 3G (standard check)**	209.00	139.75	42.19	81.25	70.34	54.25	81.01	27.50	90.63
T_9	**Control (untreated check)**	209.75	241.75	0	274.00	0	285.75	0	293.75	0
	F-test	NS	*	-	*	-	*	-	*	-
	SEm ±	-	10.63	-	11.32	-	10.59	-	9.69	-
	CD $_{0.05}$	-	31.03	-	33.05	-	30.93	-	28.30	-

DAT – Days after treatment imposition; NS – Non-significant; * - Significant

The observations revealed that the nematode population (J2 stage) in the rhizosphere soil was consistent in all the treatment plots before the imposition of treatments (initial). The analysis of rhizosphere soil samples 30 DAT revealed a significant per cent reduction of nematode population in all the individual treatments as compared to the untreated check. Among the treatments, the least nematode population of 139.75 (42.19% reduction) was recorded in the standard check, carbofuran 3G. Among the bioagents, the least nematode population of 168.25 (30.40% reduction) was recorded in *T. viride*, which was on par with Nemahari 160.75 (33.50% reduction) followed by neem cake 164.00 (32.16% reduction), *P. fluorescens* 171.50 (29.50% reduction), *T. harzianum* 174.25 (27.92% reduction), *P. lilacinum* 184.25 (23.78% reduction) and *P. clamydosporia* 186.50 (22.85% reduction). The highest nematode population was recorded in the control (241.75).

On 60 DAT, the least nematode population among the bioagents was recorded in *T. viride* 127.25 (53.55% reduction) which was on par with Nemahari 111.75 (59.21% reduction). This was followed by neem cake 112.25 (59.03% reduction), *P. fluorescens* 132.50 (51.64% reduction), *T. harzianum* 136.25 (50.27% reduction), *P. lilacinum* 147.75 (46.07% reduction) and *P. clamydosporia* 155.00 (43.43% reduction). The nematode population increased from 241.75 to 274.00 in the untreated check.

Among the bioagents, the least nematode population of 89.50 (68.67% reduction) was recorded in the case of *T. viride* on 90 DAT which was on par with Nemahari 81.25 (71.56% reduction) followed by neem cake 84.50 (70.42% reduction), *P. fluorescens* 97.00 (66.05% reduction), *T. harzianum* 100.25 (64.91% reduction), *P. lilacinum* 115.50 (59.58% reduction) and *P. clamydosporia* 119.50 (58.35% reduction). The highest nematode population was recorded in check (285.75).

Among the bioagents, the least nematode population at 120 days after treatment imposition was recorded in *T. viride* 59.25 (79.82% reduction) which was on par with Nemahari 51.75 (82.38% reduction) followed by neem cake 54.25 (81.53% reduction), *P. fluorescens* 60.00 (79.57% reduction), *T. harzianum* 64.00 (78.21% reduction), *P. lilacinum* 69.00 (76.51% reduction) and *P. clamydosporia* 69.50 (76.34% reduction). The nematode population increased to 293.75 from 209.75 during the 120 days period in the untreated check. The highest per cent reduction in the nematode population was observed in the standard check, carbofuran 3G (90.63% reduction).

The similar results were obtained with *T. viride* applied @ 30 g/m^2 (2×10^6 cfu/g) in the pot culture experiments (Narasimhamurthy, 2010). The exact mechanism resulting in the reduction of the nematode population was not studied. The

reduction of the nematode population might be due to the parasitism of nematode juveniles and the production of nematotoxic metabolites by the antagonistic fungi and bacteria (Popal, 2020). The manurial attributes of Nemahari might have enhanced the plant's immune responses against the root-knot nematodes in the rhizosphere soil of mulberry (Kshirsagar, 2017).

3.2 Nematode population in mulberry roots:

The observation on the effect of different bioagents on the population of root-knot nematode per 5 g root samples was recorded once before the imposition of treatments (initial), intermediary (*i.e.*, first shoot harvest - 60 DAT) and final (*i.e.*, second shoot harvest - 120 DAT). The recorded observations are presented in Table 3.

TABLE 2
EFFECT OF DIFFERENT BIOAGENTS ON THE POPULATION OF RKN IN THE ROOTS OF MULBERRY

Treatments		Nematode population per 5 g root				
		Initial	60 DAT	Per cent decrease over control	120 DAT	Per cent decrease over control
T_1	***Purpureocillium lilacinum***	170.25	115.75	37.34	66.00	64.17
T_2	***Trichoderma harzianum***	169.75	112.75	38.97	64.75	64.85
T_3	***Trichoderma viride***	170.00	92.75	49.79	27.25	85.21
T_4	***Pochonia clamydosporia***	168.50	123.50	33.15	66.75	63.77
T_5	***Pseudomonas fluorescens***	169.00	121.25	34.37	64.00	65.26
T_6	**Nemahari**	170.75	102.25	44.65	35.25	80.86
T_7	**Neem cake**	169.00	104.25	43.57	39.00	78.83
T_8	**Carbofuran 3G (standard check)**	168.75	74.75	59.53	16.50	91.04
T_9	**Control (untreated check)**	169.00	184.75	0	184.25	0
	F-test	NS	*	-	*	-
	SEm ±	-	10.68	-	6.03	-
	CD $_{0.05}$	-	31.19	-	17.61	-

DAT – Days after treatment imposition; NS – Non-significant; * - Significant

In the experimental plot, the nematode population in the mulberry roots was almost invariable before the imposition of treatments (initial). The analysis of root samples after imposition of treatments *i.e.*, 60 DAT revealed a significant per cent reduction of nematode population in all the treatments when compared to the untreated check. Among the treatments, the least nematode population of 74.75 (59.53% reduction) was recorded in the standard check, carbofuran 3G. Among the bioagents, *T. viride* 92.75 (49.79% reduction) recorded the least nematode population on par with Nemahari 102.25 (44.65% reduction) followed by neem cake 104.25 (43.57% reduction), *T. harzianum* 112.75 (38.97% reduction), *P. lilacinum* 115.75 (37.34% reduction), *P. fluorescens* 121.25 (34.37% reduction) and *P. clamydosporia* 123.50 (33.15% reduction). The highest nematode population was recorded in the control (184.75).

The least nematode population at 120 days after treatment imposition among the bioagents was recorded in *T. viride* 27.25 (85.21% reduction) on par with Nemahari 35.25 (80.86% reduction) followed by neem cake 39.00 (78.83% reduction), *P. fluorescens* 64.00 (65.26% reduction), *T. harzianum* 64.75 (64.85% reduction), *P. lilacinum* 66.00 (64.17% reduction) and *P. clamydosporia* 66.75 (63.77% reduction). The nematode population increased from 169.00 to 184.25 during the 120 days period in the untreated check. The highest per cent reduction in nematode population was observed in the standard check, carbofuran 3G (91.04% reduction).

On comparing different bioagents, Ravichandra and Somasekhara (2010) obtained similar results wherein *T. viride* was very effective in reducing the nematode population both in soil and roots compared to other bioagents like *T. harzianum*, *P. fluorescens* and *P. lilacinum*. The production of chitinase enzyme by *Trichoderma* spp. might have caused the premature hatching of nematode eggs and thereby controlled the nematode population (Narasimhamurthy, 2010). The capability of the bioagents in colonising the RKN-infested regions might reduce the feeding sites for the nematodes, thereby reducing the nematode population in the roots.

IV. CONCLUSION

From the results obtained in the present studies, it may be stated that the bio-management of RKN serves to be a better approach when the nematode density is considerably low and it may be suggested for the application of *T. viride* @ 5 kg/ha with 5 tons FYM in the mulberry field along the drip line in the furrows after pruning once every year. This will help in reducing the root-knot nematode population, subsequently improving the growth and yield parameters of mulberry plants.

ACKNOWLEDGEMENTS

The authors are thankful to AICRP (Nematodes) Laboratory, UAS-Bangalore, GKVK for providing required laboratory tools and assistance. They are gratified by the farmer's cooperation in conducting the experiment.

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