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Impact of Bioagents on Growth and Yield Parameters of Root-knot Nematode Infested Mulberry Plants

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Authors' contributions

This work was carried out in collaboration among all authors. Author BKB did formal analysis, performed the statistical analysis, wrote and prepared the original draft of the manuscript. Authors VKS, BKG and SKR designed the study and investigated the work. Author KTR managed treatment selection and provided insights on RKN. Author NCR assisted in recording the observations. All authors read and approved the final manuscript.

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ABSTRACT

Mulberry (*Morus alba* L.) is a vital crop for sericulture and its productivity is crucial for the industry's sustainability. This study investigated the influence of bioagents *viz.*, *Purpureocillium lilacinum*, *Trichoderma harzianum*, *Trichoderma viride*, *Pochonia clamydosporia* and *Pseudomonas*

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fluorescens, alongside control treatments including carbofuran 3G and neem cake on growth and yield parameters in severely root-knot nematode infested mulberry plants. The treatments involved are generally recommended to manage root-knot nematode infestation. The results revealed that *T. viride* @ 5 kg/ha significantly improved plant growth, leaf area and leaf yield leading to enhanced productivity. The findings of this study underscore the potential of bioagents in fostering environmentally sustainable and economically viable sericulture.

Aims: The study aims to assess the impact of bioagents on growth and yield parameters of root-knot nematode, *Meloidogyne incognita* infested mulberry plants.

Study Design: The data collected from the experimental field were analyzed statistically by using one-way RCBD.

Place and Duration of Study: The experiment was carried out in the root-knot nematode infested mulberry garden in Kalyapura Village, Shidlaghatta Taluk, Chikkaballapura District, Eastern Dry Zone (Zone-5) of Karnataka, India between January to September 2022, covering three harvests.

Methodology: The treatments were incorporated near the root zone of mulberry plants within a week after pruning as per the recommendations. The observations were recorded once before the imposition of the treatments (initial) and during the first (60 DAT) and second (120 DAT) shoot harvests in mulberry after imposing the treatments.

Results: Among the bioagents *Trichoderma viride* showed better results for shoot height (120.00 cm), number of leaves per plant (511.75), leaf area (102.10 cm^2) and leaf yield per plant (528.38 g) during the second harvest *i.e.*, 120 DAT. The minimum leaf yield per plant was recorded in the untreated check (216.43 g).

Conclusion: Incorporating *T. viride* @ 5 kg/ha mixed with 5 tons FYM near the root zone of mulberry infested with RKN showed promising results in promoting plant growth and yield.

Keywords: Mulberry; root-knot nematode; bioagents; growth; yield.

1. INTRODUCTION

The silkworm, *Bombyx mori* L. is a monophagous lepidopteran insect that feeds exclusively on mulberry foliage. 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.

Among several factors in obtaining a successful cocoon crop, mulberry leaf alone contributes to around 38.20 per cent that 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 [1]. Apart from soil parameters, the biotic and abiotic stress factors greatly affect the quality of mulberry leaves. The nutritive values get degraded due to diverse biotic stresses *viz*., diseases and pests and the mulberry plants attract these pests and diseases due to their perennial, fast-growing and lush green characteristics. Plant-parasitic nematodes, particularly root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid and White), pose a significant threat to mulberry productivity, causing substantial reductions in herbage yield and leaf quality, as well as curtailing plant longevity. As a major pest of mulberry, *M. incognita* infestations result in quantitative and qualitative losses, underscoring the need for effective management strategies to mitigate their impact on this economically important crop [2]. Root gall formation, accompanied by symptoms of stunted growth, chlorotic discolouration and diminished plant vigour, characterizes the manifestation of this disease. *M. incognita* alone is known to cause 20-50 per cent loss in mulberry leaf yield apart from deteriorated quality [3].

The applications of synthetic pesticides in controlling RKNs have been found to induce highly toxic residual effects on the environment, particularly on non-target organisms. Some are mobile, volatile in the air and soluble in water [4]. Alternately, bio-pesticides have intrinsic application in the control of plant pests and diseases, due to their eco-friendly nature and bioavailability as opposed to synthetic pesticides [5]. The bioagents include parasites, predators, fungi, bacteria, viruses, plant products and others that are specific and safe to non-target species, beneficial insects, higher animals and the man with the least effects on the environment and ecosystem. The use of fungal and bacterial bioagents has great potential in nematode management and has a great influence on growth and yield parameters. The bioagents *viz.*, *Purpureocillium lilacinum, Trichoderma harzianum, Trichoderma viride, Pochonia clamydosporia* and *Pseudomonas fluorescens* recommended against RKN were assessed for their influence on growth and yield parameters of mulberry in the present investigation.

2. EXPERIMENTAL 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 the study 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; the field was irrigated in two-day intervals with drip irrigation facility *i.e.*, based on soil moisture conditions; organic manures and inorganic fertilizers were applied following the recommended package of practice. Except for the management of RKN, the selected field was well maintained.

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) were included in the treatments for comparison of the efficacy.

The treatments were incorporated in four replications each near the root zone of mulberry plants within a week after pruning as per the recommendations.

2.1 Observations

The growth and yield parameters *viz.*, shoot height, number of shoots per plant, number of leaves per plant, leaf area and leaf yield per plant were recorded once before the imposition of the treatments (initial) and during the first (60 DAT) and second (120 DAT) shoot harvests in mulberry after imposing the treatments.

Shoot height was recorded from the base of the shoot to the topmost fully opened leaf in randomly selected five shoots of each replication of every treatment. The mean height of five shoots was worked out to obtain the shoot height of the replication. The number of shoots in each replication (plant) was counted and recorded as the number of shoots per plant. The total number of leaves in selected five shoots of each replication was counted separately and the mean was calculated, then multiplied by the number of shoots per plant. The fully opened third, fifth and seventh leaves from the top were plucked separately from the selected five shoots of each replication and their area was determined using the leaf area meter. The leaf yield per plant was determined by multiplying the number of shoots per plant with the leaf weight in grams per shoot.

List 1. Treatment details

Treatments		Recommendation
T_1	*Purpureocillium lilacinum*	5 kg/ha (with 5 tons FYM) [6]
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) [7]
T_7	Neem cake	2000 kg/ha [8]
T_8	Carbofuran 3G (Standard check)	40 kg/ha [8]
T_9	Control (Untreated check)	-

2.2 Statistical Analysis

The data collected from the experimental field were analyzed statistically by using one-way RCBD for testing of significance by Fisher's method of analysis of variance [9]. The level of significance used in the F-test was P = 0.05.

3. RESULTS AND DISCUSSION

The efficacy of different bioagents on various growth and yield attributes of mulberry *viz.*, shoot height (cm), number of shoots per plant, number of leaves per plant, leaf area (cm^2), leaf weight per shoot (g) and leaf yield per treatment (g) were 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).

3.1 Shoot Height

The observations reveal that the shoot height was found to be unwavering before the imposition of treatments (initial). The observations among the bioagents reveal that on 60 DAT, the lengthiest shoot was recorded in Nemahari (153.50 cm) on par with *T. viride* (152.75 cm) followed by *P. lilacinum* (145.25 cm), *T. harzianum* (140.75 cm), *P. fluorescens* (139.50 cm), neem cake (135.75 cm), *P. clamydosporia* (132.25 cm) and carbofuran 3G (125.50 cm). The shortest shoot was observed in the untreated check (108.00 cm).

The lengthiest shoot among the treatments on 120 DAT was recorded in Nemahari (193.00 cm) on par with *T. viride* (192.00 cm) followed by *P. lilacinum* (181.25 cm), *T. harzianum* (176.25 cm), *P. fluorescens* (174.50 cm), *P. clamydosporia* (166.75 cm), neem cake (166.50 cm) and carbofuran 3G (152.75 cm). The shortest height of the shoot was recorded in the untreated check (109.75 cm) (Table 1).

3.2 Number of Shoots Per Plant

Among the treatments, except for the control the number of shoots per plant was found to be consistent before imposition of treatments (initial) and also on 60 DAT without any significant difference. The maximum number of shoots per plant among the treatments on 120 DAT was recorded in the untreated check (22.00) followed by Nemahari (20.50), *P. clamydosporia* (18.25), *P. lilacinum* (17.75), carbofuran 3G (17.75), *T. harzianum* (17.50), neem cake (17.00), *T. viride* (16.25) and *P. fluorescens* (15.75) (Table 1).

It was observed that the control showed stunted plant growth which might have resulted in yielding higher number of stunted shoots. The increase in the number of healthy shoots in the treatment plot involving Nemahari might be a result of its manurial composition.

3.3 Number of Leaves Per Plant

The number of leaves per plant was found to be consistent in all treatments before the imposition of treatments (initial). The observations reveal that on 60 DAT, the maximum number of leaves per plant was recorded in Nemahari (448.50) on par with *T. viride* (445.75) followed by *P. lilacinum* (439.75), *T. harzianum* (431.00), neem cake (413.50), *P. clamydosporia* (409.25), *P. fluorescens* (380.50) and carbofuran 3G (327.75). The least was observed in the untreated check (236.00).

The maximum number of leaves per plant among the treatments on 120 DAT was recorded in *T. viride* (511.75) on par with Nemahari (515.50) followed by *P. lilacinum* (486.25), *T. harzianum* (463.50), neem cake (461.25), *P. clamydosporia* (452.25), *P. fluorescens* (450.00) and carbofuran 3G (401.75). The least number of leaves per plant was observed in the untreated check (265.25) (Table 1).

3.4 Leaf Area

The leaf area of mulberry was found to be consistent in all treatments before the imposition of treatments (initial). The observations reveal that on 60 DAT, the maximum leaf area was recorded in *T. viride* (78.72 cm^2) on par with Nemahari (79.39 cm^2) followed by *P. lilacinum* (63.52 cm^2), *T. harzianum* (62.98 cm^2), *P. fluorescens* (60.42 cm^2), neem cake (58.38 cm^2), *P. clamydosporia* (58.14 cm^2) and carbofuran 3G (54.94 cm^2). The minimum leaf area was observed in the untreated check (36.68 cm^2).

The maximum leaf area among the treatments on 120 DAT was recorded in Nemahari (104.08 cm^2) on par with *T. viride* (102.1 cm^2) followed by *P. lilacinum* (96.43 cm^2), *T. harzianum* (94.80 cm^2), *P. fluorescens* (90.44 cm^2), neem cake (89.39 cm^2), *P. clamydosporia* (87.21 cm^2) and carbofuran 3G (74.08 cm^2). The minimum leaf area was observed in the untreated check (35.50 cm^2) (Table 2).

Table 1. Effect of bioagents used against RKN on the growth parameters of mulberry

Treatments	Shoot height (cm)			Number of shoots per plant			Number of leaves per plant		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T_1	109.25	145.25	181.25	18.50	18.75	17.75	235.75	439.75	486.25
T_2	109.75	140.75	176.25	17.00	18.25	17.50	236.00	431.00	463.50
T_3	109.00	152.75	192.00	17.25	17.75	16.25	235.75	445.75	511.75
T_4	109.50	132.25	166.75	17.50	18.50	18.25	237.00	409.25	452.25
T_5	109.75	139.50	174.50	17.00	17.25	15.75	237.75	380.50	450.00
T_6	109.75	153.50	193.00	17.25	21.25	20.50	236.00	448.50	515.50
T_7	109.00	135.75	166.50	18.25	18.25	17.00	238.75	413.50	461.25
T_8	109.25	125.50	152.75	17.50	17.75	17.75	236.75	327.75	401.75
T_9	109.25	108.00	109.75	18.25	21.50	22.00	238.25	236.00	265.25
F-test	NS	*	*	NS	NS	*	NS	*	*
SEm ±	-	6.09	5.88	-	-	0.74	-	44.96	31.49
CD $_{0.05}$	-	17.77	17.16	-	-	2.18	-	131.24	91.92

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

Table 2. Effect of bioagents used against RKN on yield parameters of mulberry

Treatments	Leaf area (cm2)			Leaf yield per treatment (g)		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T_1	39.76	63.52	96.43	222.15	471.97	489.07
T_2	38.52	62.98	94.8	220.94	435.28	509.32
T_3	38.57	78.72	102.1	221.16	454.12	528.38
T_4	38.24	58.14	87.21	218.85	380.16	456.27
T_5	37.29	60.42	90.44	217.22	384.62	467.28
T_6	38.9	79.39	104.08	219.38	478.78	531.92
T_7	38.18	58.38	89.39	224.51	410.25	465.13
T_8	37.06	54.94	74.08	219.9	339.71	397.96
T_9	37.26	36.68	35.5	220.68	217	216.43
F-test	NS	*	*	NS	*	*
SEm ±	-	1.78	2.14	-	44.2	33.12
CD $_{0.05}$	-	5.21	6.25	-	129.02	96.68

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

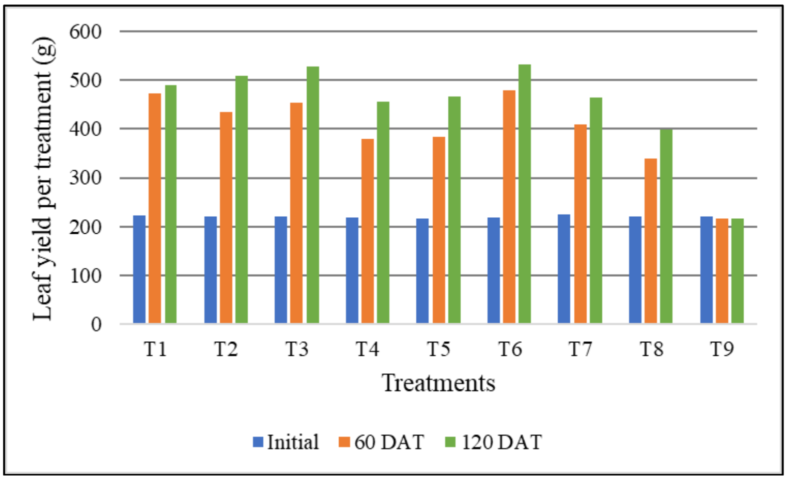

Fig. 1. Effect of bioagents on mulberry leaf yield per plant (g)

3.5 Leaf Yield Per Plant

The mulberry leaf yield per plant was found to be consistent before the imposition of treatments (initial). The observations reveal that on 60 DAT, the maximum leaf yield per plant was recorded in Nemahari (478.78 g) on par with *P. lilacinum* (471.97 g) followed by *T. viride* (454.12 g), *T. harzianum* (435.28 g), neem cake (410.25 g), *P. fluoroscens* (384.62 g), *P. clamydosporia* (380.16 g) and carbofuran 3G (339.71 g). The minimum leaf yield per plant was recorded in the untreated check (217.00 g).

The maximum leaf yield per plant among the treatments on 120 DAT was recorded in Nemahari (531.92 g) on par with *T. viride* (528.38 g) followed by *T. harzianum* (509.32 g), *P. lilacinum* (489.07 g), *P. fluoroscens* (467.28 g), neem cake (465.13 g), *P. clamydosporia* (456.27 g) and carbofuran 3G (397.96 g). The minimum leaf yield per plant was recorded in the untreated check (216.43 g) (Table 2).

The increased plant growth and yield parameters might be due to the suppression of RKN population at the infection site preventing the nematode entry and multiplication in the mulberry root [10]. The positive impact of *T. viride* on growth and yield parameters of mulberry might be due to its inhibitory effect and reduction of galls and egg masses [11,12]. *T. viride* in combination with organic amendments was also known to produce growth hormones, which were observed to have an added response in boosting the plant vigour [13]. The observations on shoot length, root length, shoot weight and root weight in cowpeas treated with bioagents revealed that *T. harzianum* @ 10 g/kg seed recorded maximum compared to *T. viride* and *P. fluorescens* @ 5, 7.5 and 10 g/kg seed [14]. The evaluation of the combination of chemical nematicide, bioagent, oilcake and trap crop in managing the RKN in mulberry revealed that carbofuran + *T. viride* performed better than all other combinations in terms of nematode population reduction and enhancement of growth and yield parameters of mulberry [15].

4. CONCLUSION

The study demonstrated the efficacy of bioagents used against RKNs in enhancing growth and yield parameters of mulberry. Among the bioagents, incorporating *T. viride* @ 5 kg/ha mixed with 5 tons FYM near the root zone showed promising results in promoting plant growth and yield. The integration of bioagents with organic amendments can further enhance their effectiveness. Future research should focus on exploring the potential of bioagents in combination with other compatible bioagents, to develop sustainable management strategies for RKNs in mulberry. Additionally, investigating the mechanisms of action of bioagents and their impact on soil microbiome can provide valuable insights into their role in plant health and ecosystem functioning. The findings of this study have significant implications for the development of environmentally friendly and sustainable sericulture practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, *etc*) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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