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Assessment of the performance of silkworm (*Bombyx mori* L.) on feeding with mulberry raised using different bioagents

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Abstract
Silk production significantly relies on the quality of mulberry foliage, which is the sole food source for the silkworm, *Bombyx mori* L. This study explores the impact of feeding the silkworms with mulberry raised with different bioagents *viz.*, *Purpureocillium lilacinum*, *Trichoderma harzianum*, *Trichoderma viride*, *Pochonia chlamydosporia* and *Pseudomonas fluorescens* recommended for the management of root-knot nematode infestation. The bioagents were assessed for their impact on various silkworm development and cocoon parameters. Results showed that *T. viride* treated mulberry leaves significantly enhanced fifth instar larval weight (3.11 g), reduced larval duration (7.19 days), improved cocoon weight (2.02 g), shell weight (0.39 g) and pupal weight (1.67 g). Furthermore, the average filament length (1097.16 m) and denier (3.07) of the cocoons were superior in the *T. viride* treatment compared to all other treatments. The findings highlight the crucial role of mulberry leaf quality in sericulture and suggest that bioagents, particularly *T. viride* can enhance silkworm productivity, mitigating the adverse effects of root-knot nematodes.

Keywords: Bioagents, silkworm, larval parameters, cocoon parameters, reeling parameters

Introduction
Silk, a highly valued agricultural commodity, accounting for about 0.2 per cent of the total global textile fibre production, is basically a product of the silkworm. India stands as the only country worldwide producing four kinds of commercially exploited natural silks, mulberry, tasar, muga and ERI of which mulberry silk contributes maximum (74%). 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. Hence, the quantity of quality leaf produced per unit area has a direct bearing on cocoon production and raw silk quality.

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. 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) [4].

Among the major disease causing pathogens, the root-knot nematode (RKN) species *Meloidogyne incognita* alone is known to cause 20-50 per cent loss in leaf yield apart from deteriorated quality (Arunakumar *et al.*, 2018) [1]. The RKNs are parasites of underground roots, which is difficult to recognize and hence the damage symptoms very often go unnoticed. The infestation is more commonly noticed in sandy to loamy soils and under irrigated conditions (Sengupta and Govindaiah, 1991) [7].

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 country's stand in increasing environmental concerns.

With this background, an experiment was planned to assess the performance of silkworm (*B. mori* L.) on feeding with mulberry raised with different bioagents against the RKN.

Materials and Methods

A. 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.

B. Different bioagents used against root-knot nematode in mulberry

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

1. *Purpureocillium lilacinum*
2. *Trichoderma harzianum*
3. *Trichoderma viride*
4. *Pochonia clamydosporia*
5. *Pseudomonas fluorescens*

Along with the five bioagents, Nemahari (bio-nematicide developed by CSRTI, Mysore), neem cake and carbofuran 3G (chemical nematicide recommended by Dandin and Giridhar, 2014) [2] and control (untreated check) are included in the treatments for comparison of the efficacy.

Treatments		Recommendation	Reference
T_1	*Purpureocillium lilacinum*	5 kg/ha (with 5 tons FYM)	Saxena *et al.*, 2021[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)	Nishita and Prateeshkumar, 2015 [5]
T_7	Neem cake	2000 kg/ha/yr	Dandin and Giridhar, 2014 [2]
T_8	Carbofuran 3G (standard check)	40 kg/ha/yr	Dandin and Giridhar, 2014 [2]
T_9	Control (untreated check)	-	-

The listed bioagents were obtained from IIHR, Bengaluru. The bioagents were incorporated into the plant rhizosphere soil as per the treatment details near the root zone of the mulberry within a week after pruning by calculating the dosage per plant.

C. Silkworm rearing

The III instar larvae of commercial hybrid (PM × CSR2) were procured from the registered Chawki Rearing Centre and reared until the third moult the silkworms were fed with untreated mulberry leaves. Post-third moult a total of 30 larvae were reared in each replication under recommended rearing conditions (Dandin and Giridhar, 2014) [2]. The late-age silkworms were reared by following three feed schedules with the leaves harvested from treated mulberry plants. Trays were cleaned and faeces were removed daily in the morning, before feeding the silkworms.

The ripe worms were hand-picked from each replication and were mounted separately on the bamboo mountages for cocoon spinning. The mountages were kept in the mounting hall to provide optimum environmental conditions for spinning. Later on, the cocoons were harvested manually on the fifth and sixth days of mounting.

D. Observations

I. Fifth instar larval weight (g)

The weight of ten grown-up silkworms was recorded by randomly picking silkworms from each replication of respective treatments on the fifth day of the fifth instar.

II. Fifth instar duration (days)

The total number of days taken from the first day of the fifth instar till the time when 50 per cent of the worms were mature was recorded in each replication of every treatment, in their respective wise and the mean duration was worked out.

III. Larval progression (%)

The larval progression was calculated in the fifth instar with the following formula:

$$\text{Larval progression (\%)} = \frac{\text{Number of larvae alive per treatment}}{\text{Total number of larvae per treatment}} \times 100$$

IV. Effective rate of rearing (ERR) (%)

The number of cocoons harvested at the end of rearing in each replication of every treatment was documented and the ERR was calculated by using the formula:

$$\text{ERR (\%)} = \frac{\text{Number of cocoons harvested}}{\text{Total number of worms brushed}} \times 100$$

V. Single cocoon weight (g)

Ten cocoons were randomly selected from each replication of every treatment and weight was recorded on the fifth day of

mounting.

VI. Single cocoon shell weight (g)
After taking the cocoon weight, ten cocoons were cut open and the cocoon shell weight was recorded for each replication and the average was calculated to get the mean shell weight.

VII. Pupal weight (g)
Ten pupae were obtained from cutting the ten weighed cocoons in each replication and the average was calculated to obtain the mean pupal weight.

VIII. Cocoon shell ratio (%)
The shell ratio was calculated by using the formula:

$$\text{Shell Ratio (\%)} = \frac{\text{Cocoon shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

IX. Average filament length (m)
Three cocoons from each replication of the treatments will be randomly drawn, cooked separately and reeled on an eprouvette. The filament length is determined by,

$$L = R \times 1.125 \text{ m}$$

Where, L-Length of the filament (m); R-Number of revolutions; 1.125 m-Circumference of the eprouvette reel

X. Non-breakable filament length (NBFL) (m)
The non-breakable filament length was calculated using the formula:

$$\text{NBFL (m)} = \frac{\text{Total filament length (m)}}{1 + \text{Number of breaks}}$$

XI. Filament denier
The filament denier is calculated using the formula:

$$\text{Denier} = \frac{\text{Weight of the filament (g)}}{\text{Filament length (m)}} \times 9000$$

E. Statistical analysis
The data collected from the experimental field, laboratory analysis and silkworm rearing were analyzed statistically by using one-way RCBD for testing of significance by Fisher's method of analysis of variance (Snedecor and Cochran, 1979). The level of significance used in the F-test was P = 0.05. The critical difference (CD) values were computed to compare the significance of the treatments.

Results and Discussion
The effect of different bioagents on the *B. mori* L. larval parameters on feeding with the mulberry leaves raised with different bioagents against the root-knot nematode was recorded based on the results of silkworm rearing conducted during the second harvest after imposition of the treatments.
Among the bioagents, the fifth instar larval weight recorded on the fifth day was found to be highest in *T. viride* treatment (3.11 g) on par with Nemahari (3.18 g) followed by *T. harzianum* (3.07 g), *P. fluoroscens* (3.06 g), *P. lilacinum* (3.05 g), neem cake (3.01 g) and *P. clamydosporia* (2.99 g). The least larval weight was recorded in carbofuran 3G treatment (2.98 g) and control (2.97 g) (Fig. 1) (Table 1).
The fifth instar larval duration was found to be least in the treatment having *T. viride* (7.19 days) which was on par with *T. harzianum* (7.24 days) followed by *P. clamydosporia* (7.29 days), *P. fluoroscens* (7.36 days), *P. lilacinum* (7.39 days), neem cake (7.45 days) and Nemahari (7.47 days). The control (7.20 days) was on par with *T. viride*. Carbofuran 3G treatment recorded longer fifth instar duration (7.58 days). The fifth instar larval progression was recorded to be 100 per cent in all the treatments as all the larvae were alive till the age of spinning, counting from the day of brushing. The ERR was 100 per cent in all the treatments except for carbofuran 3G (99.15%) and the control (98.30%). The reduction in ERR was due to the mishandling of worms during mounting (Table 1).
The *T. viride* treatment recorded the maximum cocoon weight of 2.02 g among the bioagents and was on par with Nemahari (2.21 g) followed by *P. clamydosporia* (1.90 g), neem cake (1.81 g), *P. fluoroscens* (1.79 g), *P. lilacinum* (1.77 g) and *T. harzianum* (1.72 g). The minimum single cocoon weight was recorded in the treatment having carbofuran 3G (1.57 g) and in control (1.54 g) (Fig. 2). Among the bioagents, the cocoon shell weight was found to be highest in *T. viride* treatment (0.39 g) on par with Nemahari (0.43 g) followed by *P. clamydosporia* (0.32 g), *P. lilacinum* (0.31 g), *T. harzianum* (0.31 g), neem cake (0.31 g) and *P. fluoroscens* (0.29 g). The least cocoon shell weight was recorded in carbofuran 3G (0.26 g) and control (0.23 g) treatments (Table 2).
Treatment with *T. viride* recorded the pupal weight of 1.67 g among the bioagents and was on par with Nemahari (1.82 g) followed by *P. clamydosporia* (1.54 g), *P. fluoroscens* (1.48 g), neem cake (1.44 g), *P. lilacinum* (1.43 g) and *T. harzianum* (1.38 g). The minimum pupal weight was recorded in the treatments having carbofuran 3G (1.34 g) and control (1.29 g). Among the treatments, the cocoon shell ratio was found to be highest in Nemahari (19.53%) on par with *T. viride* (19.42%) followed by *T. harzianum* (18.01%), *P. lilacinum* (17.77%), *P. clamydosporia* (16.99%), neem cake (16.91%), carbofuran 3G (16.72%), *P. fluoroscens* (16.25%) and the least was recorded in control (15.12%) (Table 2).
The lengthiest average filament length among the bioagents was recorded in the treatment having *T. viride* (1097.16 m) which was on par with Nemahari (1108.69 m) followed by *T. harzianum* (1080.84 m), *P. lilacinum* (1076.63 m), *P. fluorescens* (1074.94 m), neem cake (1055.25 m) and *P. clamydosporia* (1054.41 m). The shortest filament length was recorded in the untreated check (982.68 m). The non-breakable filament length (NBFL) was found to be unwavering among the treatments. Among the treatments, the denier was highest in Nemahari (3.26) on par with *T. viride* (3.07) followed by *T. harzianum* (2.91), *P. lilacinum* (2.84), *P. fluoroscens* (2.74), *P. clamydosporia* (2.62), neem cake (2.60), carbofuran 3G (2.51) and the least was recorded in control (2.40) (Table 3).

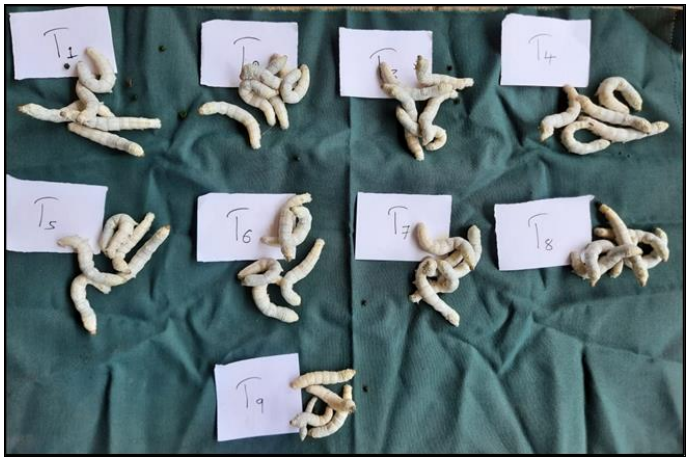

Fig 1: Fifth instar fifth day silkworm

Fig 2: Cocoons harvested on the sixth day of mounting

Table 1: Effect of different bioagents used against mulberry RKN on *Bombyx mori* L. (PM × CSR2) larval parameters

Treatments		Fifth instar			ERR (%)
		Larval weight (g)	Larval duration (days)	Larval progression (%)	
T_1	*Purpureocillium lilacinum*	3.05	7.39	100.00	100.00
T_2	*Trichoderma harzianum*	3.07	7.24	100.00	100.00
T_3	*Trichoderma viride*	3.11	7.19	100.00	100.00
T_4	*Pochonia clamydosporia*	2.99	7.29	100.00	100.00
T_5	*Pseudomonas fluorescens*	3.06	7.36	100.00	100.00
T_6	Nemahari	3.18	7.47	100.00	100.00
T_7	Neem cake	3.01	7.45	100.00	100.00
T_8	Carbofuran 3G (Standard check)	2.98	7.58	100.00	99.15
T_9	Untreated check (control)	2.97	7.20	100.00	98.30
	F-Test	*	*	NS	NS
	S.E.M ±	0.04	0.03	-	-
	CD $_{0.05}$	0.12	0.09	-	-

ERR-Effective rate of rearing; NS-Non significant; *-Significant

Table 2: Effect of different bioagents used against mulberry RKN on *Bombyx mori* L. (PM × CSR2) cocoon parameters

Treatments		Single cocoon weight (g)	Cocoon shell weight (g)	Pupal weight (g)	Cocoon shell ratio (%)
T_1	*Purpureocillium lilacinum*	1.77	0.31	1.43	17.77
T_2	*Trichoderma harzianum*	1.72	0.31	1.38	18.01
T_3	*Trichoderma viride*	2.02	0.39	1.67	19.42
T_4	*Pochonia clamydosporia*	1.90	0.32	1.54	16.99
T_5	*Pseudomonas fluorescens*	1.79	0.29	1.48	16.25
T_6	Nemahari	2.21	0.43	1.82	19.53
T_7	Neem cake	1.81	0.31	1.44	16.91
T_8	Carbofuran 3G (Standard check)	1.57	0.26	1.34	16.72
T_9	Untreated check (control)	1.54	0.23	1.29	15.12
	F-test	*	*	*	*
	S.E.M ±	0.10	0.01	0.11	0.76
	CD $_{0.05}$	0.31	0.04	0.31	2.22

NS-Non significant; *-Significant

Table 3: Effect of different bioagents used against mulberry RKN on *Bombyx mori* L. (PM × CSR2) silk reeling parameters

Treatments		Average filament length (m)	NBFL (m)	Denier
T_1	*Purpureocillium lilacinum*	1076.63	940.92	2.84
T_2	*Trichoderma harzianum*	1080.84	947.67	2.91
T_3	*Trichoderma viride*	1097.16	958.64	3.07
T_4	*Pochonia clamydosporia*	1054.41	793.41	2.62
T_5	*Pseudomonas fluorescens*	1074.94	940.92	2.74
T_6	Nemahari	1108.69	969.18	3.26
T_7	Neem cake	1055.25	923.20	2.60
T_8	Carbofuran 3G (Standard check)	1013.63	635.62	2.51
T_9	Untreated check (control)	982.68	615.37	2.40
	F-Test	*	NS	*
	SEm ±	7.05	-	0.10
	$CD_{0.05}$	20.59	-	0.30

NS-Non significant; *-Significant

The silkworm larval, cocoon and reeling parameters were significant for larval weight, larval duration, single cocoon weight, shell weight, pupal weight, cocoon shell ratio, average filament length and denier except for larval progression, ERR and NBFL. The silkworms fed with mulberry leaves from *T. viride* treated plants exhibited better results for larval, cocoon and reeling parameters as they might be efficient in controlling the root-knot nematode population which not only deters the productivity but also the quality attributes. These results were contrary to that reported by Govindaiah *et al.* (1996) [3] who conducted the bioassay using organic manures, mulches and intercropping against RKN, *M. incognita*, and the report of high silkworm larval mortality on exposure to the culture filtrate of *T. harzianum* and *P. chlamydosporia* at higher concentrations (Sharma, 1999) [8].

Limited reports have been found with respect to the effect of bioagents on mulberry plant growth and leaf quality and the subsequent impact on the performance of silkworm (*B. mori* L.).

Conclusion

From the results obtained in the present studies, it may be stated that the application of *Trichoderma viride* @ 5 kg/ha with 5 tons FYM in the mulberry field in the furrows after pruning once every year would help in improving the performance of the silkworm besides reducing the effect of root-knot nematode on growth and yield parameters of mulberry.

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Conflict of Interest

None

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