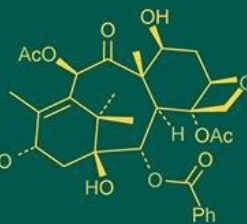
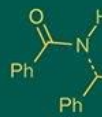
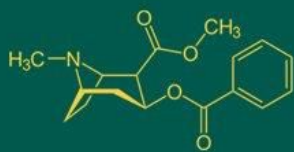


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Impact of bioagents on biochemical and elemental composition of mulberry foliage infested with root-knot nematode

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Abstract

Mulberry is a pivotal crop for sericulture and optimizing its nutritional quality is essential for sustainable industry growth. This study investigated the impact of bioagents, viz., *Purpureocillium lilacinum*, *Trichoderma harzianum*, *Trichoderma viride*, *Pochonia chlamydosporia* and *Pseudomonas fluorescens*, on the biochemical and elemental composition of mulberry foliage under severe root-knot nematode (*Meloidogyne incognita*) infestation. The treatments, also including carbofuran 3G and neem cake, aimed to mitigate nematode-induced stress while enhancing leaf nutritional quality. Results revealed that bioagents applied @ 5 kg/ha significantly augmented leaf moisture content, total chlorophyll, carbohydrate, protein, crude fibre, ash and elemental (macro- and micronutrient) contents. This study underscores the potential of bioagents in fostering environmentally sustainable and economically viable sericulture by optimizing mulberry leaf nutritional quality.

Keywords: Bioagents, root-knot nematode, biochemical composition, elemental composition

Introduction

Mulberry is a fast-growing perennial plant belonging to the genus *Morus* of the family Moraceae. It exhibits several desirable traits, including high foliage yield, a relatively short gestation period and robust environmental adaptability. Foliage is the major economic part of mulberry as the silkworm, *Bombyx mori* L. 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.

The quality of mulberry leaf is the predominant factor influencing cocoon crop success, contributing approximately 38.20% to the overall outcome, followed by microclimate conditions in the rearing house (37.00%), silkworm rearing techniques (9.30%) and breed selection (4.20%). This underscores the critical role of optimal foliage quality in sericulture. Moreover, apart from various other edaphic factors, biotic and abiotic stressors significantly impact mulberry leaf quality. The nutritional value of mulberry plants is compromised by various biotic stresses, including diseases caused by pathogens and infestations by insect and non-insect pests, which are attracted to the plant's perennial, fast-growing and lush green characteristics (Miyashita, 1986) [13]. These biotic stressors impact both the aerial and subterranean parts of the mulberry plant, with symptoms manifesting prominently in the foliage.

Rhizosphere soil is the more intensified zone of microbial activity where the population of microorganisms is comparatively high (Katznelson, 1965) [12]. While studying rhizosphere organisms of mulberry, a markedly higher population of fungi and nematodes was observed (Nandi *et al.*, 2004) [14]. The interaction between microorganisms and the plant root system is largely mutualistic, but can sometimes shift towards pathogenesis. The root-knot nematode (RKN), *Meloidogyne incognita* is one such detrimental association with mulberry known to cause 20-50 percent loss in leaf yield apart from deteriorated quality (Aruna kumar *et al.*, 2018) [2]. Infected plants exhibit symptoms of stunted growth, marginal necrosis and foliar yellowing, accompanied by the formation of characteristic knots or galls on the roots, which impede water and nutrient uptake, ultimately leading to impaired plant growth (Govindaiah *et al.*, 1991) [8].

The employment of synthetic pesticides for the management of RKNs has been associated with deleterious environmental consequences, including the persistence of toxic residues, mobility, volatility and water solubility, thereby posing a significant threat to non-target organisms (Gapasin *et al.*, 2011) [7]. Conversely, bio-pesticides offer a sustainable alternative for plant pest and disease management, leveraging their inherent bioavailability, specificity and eco-friendly profile (Helen *et al.*, 2016) [16]. The utilization of bioagents, comprising parasites, predators, fungi, bacteria, viruses and plant products, provides a targeted approach, ensuring the safety of non-target species, beneficial insects, higher animals, and humans, while minimizing environmental impact. Specifically, fungal and bacterial bioagents, including *Purpureocillium lilacinum*, *Trichoderma harzianum*, *Trichoderma viride*, *Pochonia chlamydosporia* and *Pseudomonas fluorescens* have demonstrated considerable potential in nematode management, significantly influencing growth and yield parameters (Bharath, 2022) [3]. This investigation aimed to elucidate the effects of these bioagents on the biochemical and elemental composition of mulberry foliage.

Materials and Methods

The experiment was carried out in the RKN infested mulberry garden in Kalyapura Village, Shidlaghatta Taluk, Chikkaballapura District, 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 and used for the management of RKN in mulberry

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

Alongside, 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.

Treatment details

Treatments		Recommendation	Reference
T ₁	<i>Purpureocillium lilacinum</i>	5 kg/ha (with 5 tons FYM)	Saxena <i>et al.</i> , 2021 [18]
T ₂	<i>Trichoderma harzianum</i>		
T ₃	<i>Trichoderma viride</i>		
T ₄	<i>Pochonia chlamydosporia</i>		
T ₅	<i>Pseudomonas fluorescens</i>		
T ₆	Nemahari	40 kg/ha (with 400 kg FYM)	Nishita and Prateeshkumar, 2015 [16]
T ₇	Neem cake	2000 kg/ha	Dandin and Giridhar, 2014 [5]
T ₈	Carbofuran 3G (standard check)	40 kg/ha	Dandin and Giridhar, 2014 [5]
T ₉	Control (untreated check)	-	-

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

Observations

The following parameters 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.

A composite sample of forty leaves was collected from each replication, air-dried and then dried in the hot-air oven at 60

°C for 18 hours. The samples were then powdered and stored in polythene bags. These samples were analyzed for total carbohydrates, proteins, crude fibre, ash, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur contents.

Biochemical constituents

Leaf moisture content (%)

The moisture content of mulberry leaves in each replication was determined by the hot air oven method, where the fresh weight of the samples was recorded and then the samples were placed in the hot air oven for 24 hours at 105±1°C, again the dry sample weight was recorded. The percent moisture content was determined by the following formula:

$$\text{Leaf moisture content (\%)} = \frac{(\text{Fresh weight of sample}) - (\text{Dry weight of sample})}{\text{Fresh weight of the sample}} \times 100$$

Moisture retention capacity (%)

To determine the leaf moisture retention capacity after six hours of harvest, a composite sample of thirty leaves was collected for each replication and the fresh weight was taken. The leaves were kept open under laboratory conditions and the weight was recorded after 6 hours of

harvest. The percent moisture retention capacity was calculated by using the formula (Shivashankar, 2015) [19]:

$$\text{Moisture retention capacity (\%)} = 100 - \text{Moisture loss (\%)}$$

where, fresh weight is considered to be 100 percent

$$\text{Moisture loss (\%)} = \frac{(A - B)}{A} \times 100$$

Where, A - fresh weight of leaf; B - leaf weight after six hours of harvest

iii. Chlorophyll content (mg/g)

Chlorophyll content in mulberry leaf was determined by the following procedure described. The leaf's total chlorophyll contents were computed using the formula suggested by Arnon (1949) [1].

$$\text{Total chlorophyll (mg/g)} = \frac{(20.2 A_{645} - 8.02 A_{663}) \times X}{1000 \times n}$$

Where, A₆₆₃ - Absorbance at a wavelength of 663 nm; A₆₄₅ - Absorbance at a wavelength of 645 nm; X - Total volume of the filtrate; n - Sample weight

Total carbohydrates (%)

The total carbohydrates in the leaf samples were estimated using the Anthrone method (Hedge and Hofreiter, 1962) [9]. The amount of carbohydrates in the sample was determined by plotting a standard curve of absorbance at a wavelength of 630 nm (A₆₃₀) on the Y-axis and concentration of glucose on the X-axis. The total carbohydrates is expressed in percentage.

Total protein (%)

The total proteins in the leaf samples were estimated using Lowry's method. The amount of protein in the sample was determined by plotting a standard absorbance curve at a wavelength of 660 nm (A₆₆₀) along the Y-axis and protein

concentration along the X-axis. The total protein is expressed in percentage

Crude protein (%)

The percent crude protein was estimated using the nitrogen content determined by the micro Kjeldahl method involving digestion, distillation and titration of the sample using the formula:

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Crude fibre (%)

The crude fibre of the sample was estimated by using moisture and fat-free sample by Fibra plus apparatus. 2 g of moisture and fat-free sample was transferred to fibre estimating thimbles and placed in the digestion chamber. 200 ml of sulphuric acid was added and the temperature was set at 500 °C for 10 min, the temperature then was reduced to 400 °C and left for 30 min., after that the acid was drained by using a suction pump and washed with boiled distilled water. After an acid wash, the digestion chamber was filled with 150 ml of boiling sodium hydroxide solution for about 30 min at 400 °C. Then the alkali solution was drained out by using the suction pump and again the tubes were washed with boiled distilled water. After that, the crucibles were placed in an oven at 100 °C for about one hour and the weight of the crucible was taken after which the crucible was placed in a muffle furnace at 550 °C for 3 hours or until the white ash was formed and the final weight was taken.

The fibre content of the sample was calculated by:

$$\text{Crude fibre (\%)} = \frac{[100 - (\text{Moisture} + \text{Fat content of the sample})] \times W_e - W_a}{\text{Weight of the sample}} \times 100$$

Where, W_e - Pre-weighed ash (g); W_a - Weight of dish after washing (g)

Ash (%)

The ash content in the samples was determined using the formula (Windham *et al.*, 1991) [22]:

$$\text{Ash content (\%)} = \frac{(Z - X)}{Y} \times 100$$

where, X - Weight of empty crucible (g); Y - (Weight of sample) (g); Z - (Weight of crucible + ash) (g)

Elemental constituents

The powdered leaf samples (1 g) were treated with 10 ml of concentrated HNO₃ and kept overnight for pre-digestion.

$$\text{Nitrogen (\%)} = \frac{\text{TV} \times \text{Normality of acid} \times 0.014 \times \text{Volume of digested sample}}{\text{Weight of the sample} \times \text{aliquot taken}} \times 100$$

where, TV - Titration value

Phosphorous (%)

Phosphorus content in the di-acid digested extract was estimated by the vanadomolybdo-phosphoric yellow colour method in nitric acid medium and the colour intensity was measured at 420 nm wavelength as described by Piper (1966) [17].

Then the samples were digested with 10 ml of the di-acid mixture (9:4 ratio of HNO₃ and HClO₄) until the snow-white residue remained. The residue was cooled and diluted to 100 ml using distilled water, filtered and used to analyse all the elements except nitrogen.

Nitrogen (%)

The nitrogen percentage in the leaf samples was determined by the Kjeldahl digestion-distillation method. The plant sample (1g) was digested using the digestion mixture and sulphuric acid in a digestion flask. After complete digestion, the digested material was distilled in an alkaline medium and the liberated ammonia was trapped in a 2 percent boric acid solution containing mixed indicator. The trapped ammonia was titrated against standard sulphuric acid (Piper, 1966) [17].

Potassium (%)

Potassium in the plant sample was determined by atomizing the diluted di-acid extract in a flame photometer as outlined by Piper (1966) [17].

Secondary nutrients

The calcium and magnesium contents in the mulberry leaves were estimated by the EDTA titration or the versanate-

titration method. The sulphur content in the di-acid digested sample was estimated by the turbidimetric method as outlined by Jackson (1973) [11].

Estimation of micronutrients

The micronutrients were estimated using the specified reagents in the atomic absorption spectrophotometer (AAS).

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 (Snedecor and Cochran, 1979) [20]. 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

Leaf moisture content

Among the treatments, the mulberry leaf moisture content was found to be consistent before the imposition of treatments (initial), in the intermediary (60 DAT) and also during the final observations on 120 DAT (Table 1).

Moisture retention capacity

The moisture retention capacity was found to be unwavering among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that among the bioagents, the maximum moisture retention capacity was recorded in *T. viride* (97.83%) which was on par with Nemahari (97.92%) followed by *T. harzianum* (97.44%), *P. lilacinum* (97.13%), *P. fluoroscens* (97.05%), *P. clamydosporia* (96.81%), neem cake (96.40%) and carbofuran 3G (96.39%). The minimum moisture retention capacity was recorded in the untreated check (90.81%).

The maximum moisture retention capacity among the treatments on 120 DAT was recorded in Nemahari (98.5%) on par with *T. viride* (98.32%) followed by *P. lilacinum* (97.98%), *T. harzianum* (97.41%), *P. fluoroscens* (97.26%), *P. clamydosporia* (97.24%), neem cake (97.13%) and carbofuran 3G (96.93%). The minimum moisture retention capacity was recorded in the control (90.08%) (Table 1).

Total chlorophyll content

The total chlorophyll content was almost consistent among the treatments before the imposition of treatments (initial). Among the bioagents, the intermediary (60 DAT) observations reveal that the highest total chlorophyll content was recorded in *T. viride* (0.116 mg/g) on par with Nemahari (0.123 mg/g) followed by *T. harzianum* (0.114 mg/g), *P. fluoroscens* (0.112 mg/g), *P. lilacinum* (0.104 mg/g), *P. clamydosporia* (0.102 mg/g), neem cake (0.101 mg/g) and carbofuran 3G (0.099 mg/g). The lowest total chlorophyll content was recorded in the untreated check (0.062 mg/g).

The highest total chlorophyll content among the treatments on 120 DAT was recorded in Nemahari (0.176 mg/g) on par with *T. viride* (0.170 mg/g) followed by *T. harzianum* (0.162 mg/g), *P. fluoroscens* (0.159 mg/g), *P. lilacinum* (0.158 mg/g), *P. clamydosporia* (0.155 mg/g), neem cake (0.137 mg/g) and carbofuran 3G (0.132 mg/g). The lowest total chlorophyll content was recorded in the control (0.062 mg/g) (Table 1).

Total carbohydrates

Among the treatments, the total carbohydrates in mulberry leaf was almost invariable before the imposition of treatments (initial). The intermediary (60 DAT) observations revealed that the highest carbohydrate content among the bioagents was recorded in *T. viride* (1.281%), on par with Nemahari (1.498%) followed by *T. harzianum* (1.246%), *P. lilacinum* (1.218%), *P. fluoroscens* (1.19%), *P. clamydosporia* (1.176%), neem cake (1.127%) and carbofuran 3G (1.113%). The lowest carbohydrate content was recorded in the untreated check (0.616%).

The highest carbohydrate content among the treatments on 120 DAT was recorded in Nemahari (2.184%) on par with *T. viride* (1.967%) followed by *T. harzianum* (1.96%), *P. lilacinum* (1.869%), *P. clamydosporia* (1.841%), *P. fluoroscens* (1.806%), neem cake (1.757%) and carbofuran 3G (1.631%). The lowest carbohydrate content was recorded in the control (0.602%) (Table 1).

Total protein

The total protein content was found to be unwavering among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the highest protein content was recorded in Nemahari (27.15%) on par with *T. viride* (24.1%) followed by *T. harzianum* (23.22%), *P. lilacinum* (21.8%), *P. fluoroscens* (21%), *P. clamydosporia* (19.5%), neem cake (19.25%) and carbofuran 3G (18.25%). The lowest protein content was recorded in the untreated check (13.35%).

The highest protein content among the bioagents on 120 DAT was recorded in *T. viride* (27.65%) on par with Nemahari (28.6%) followed by *T. harzianum* (25.3%), *P. lilacinum* (24.07%), *P. fluoroscens* (23.82%), *P. clamydosporia* (23.12%) and neem cake (19.5%). The lowest protein content was recorded in carbofuran 3G (16.77%) and the control (14.7%) (Table 2).

Crude protein

The crude protein content was found to be almost consistent among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the highest crude protein content was recorded in *T. viride* (8.46%) on par with Nemahari (7.93%) followed by *P. lilacinum* (7.39%), *T. harzianum* (7.28%), *P. fluoroscens* (6.84%), neem cake (6.54%), *P. clamydosporia* (6.45%) and carbofuran 3G (5.64%). The lowest crude protein content was recorded in the untreated check (4.06%). The highest crude protein content among the treatments on 120 DAT was recorded in *T. viride* (13.03%) on par with Nemahari (12.31%) followed by *P. lilacinum* (11.92%), *T. harzianum* (11.65%), *P. fluoroscens* (10.93%), neem cake (10.43%), *P. clamydosporia* (9.14%) and carbofuran 3G (6.03%). The lowest crude protein content was recorded in the control (3.84%) (Table 2).

Crude fibre

The crude fibre content was found to be invariable among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the highest crude fibre content was recorded in Nemahari (6.34%) on par with *T. viride* (6.07%) followed by *P. lilacinum* (5.89%), *T. harzianum* (5.71%), *P. fluoroscens* (5.57%), neem cake (5.29%), *P. clamydosporia* (5.18%) and

carbofuran 3G (5.10%). The lowest crude protein content was recorded in the untreated check (4.22%).

The highest crude fibre content among the bioagents on 120 DAT was recorded in *T. viride* (8.23%) was on par with Nemahari (8.47%) followed by *P. lilacinum* (7.27%), *T. harzianum* (7.24%), *P. fluorescens* (7.15%), neem cake (6.96%) and *P. clamydosporia* (6.82%). The lowest crude fibre content was recorded in carbofuran 3G (5.85%) followed by control (4.09%) (Table 2).

Ash

The ash content was found to be invariable among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum ash content was recorded in Nemahari (8.00%) on par with *T. viride* (7.75%) followed by *T. harzianum* (6.75%), *P. lilacinum* (6.50%), *P. fluorescens* (6.25%), neem cake (5.50%), *P. clamydosporia* (5.25%) and carbofuran 3G (4.50%). The minimum ash content was recorded in the untreated check (3.00%).

The maximum ash content among the bioagents on 120 DAT was recorded in *T. viride* (9.50%) was on par with Nemahari (10.25%) followed by *P. lilacinum* (9.25%), *T. harzianum* (9.00%) and *P. fluorescens* (8.50%). The treatment having neem cake and *P. clamydosporia* recorded the same values for ash content (8.00%) followed by carbofuran 3G (5.00%). The minimum ash content was recorded in the control (2.75%) (Table 2).

Nitrogen

The mulberry leaf nitrogen content was found to be unwavering among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum nitrogen content was recorded in *T. viride* (1.35%) on par with Nemahari (1.27%) followed by *P. lilacinum* (1.18%), *T. harzianum* (1.16%), *P. fluorescens* (1.09%), neem cake (1.05%), *P. clamydosporia* (1.02%) and carbofuran 3G (0.90%). The minimum nitrogen content was recorded in the untreated check (0.65%).

The maximum nitrogen content among the treatments on 120 DAT was recorded in *T. viride* (2.08%) followed by Nemahari (1.97%), *P. lilacinum* (1.91%), *T. harzianum* (1.86%), *P. fluorescens* (1.75%), neem cake (1.67%), *P. clamydosporia* (1.46%) and carbofuran 3G (0.96%). The minimum nitrogen content was recorded in the control (0.62%) (Table 3).

Phosphorous

The mulberry leaf phosphorous content was found to be invariable among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum phosphorous content was recorded in *P. fluorescens* (2.464%) on par with Nemahari (2.454%) followed by *T. viride* (2.436%) *P. lilacinum* (2.425%), *T. harzianum* (2.409%), *P. clamydosporia* (2.391%), neem cake (2.336%) and carbofuran 3G (2.262%). The minimum phosphorous content was recorded in the untreated check (2.206%).

The maximum phosphorous content among the treatments on 120 DAT was recorded in *P. fluorescens* (2.987%) on par with Nemahari (2.957%) followed by *T. viride* (2.941%), *P. lilacinum* (2.927%), *T. harzianum* (2.915%), *P. clamydosporia* (2.901%), neem cake (2.875%) and

carbofuran 3G (2.444%). The minimum phosphorous content was recorded in the control (2.192%) (Table 3).

Potassium

The mulberry leaf potassium content was found to be consistent among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum potassium content was recorded in *T. viride* (0.231%) on par with Nemahari (0.233%), *P. lilacinum* (0.194%), *T. harzianum* (0.190%), *P. fluorescens* (0.181%), *P. clamydosporia* (0.172%), neem cake (0.170%) and carbofuran 3G (0.161%). The minimum potassium content was recorded in the untreated check (0.153%). The maximum potassium content among the treatments on 120 DAT was recorded in *T. viride* (0.302%) on par with Nemahari (0.323%) followed by *P. lilacinum* (0.266%), *T. harzianum* (0.262%), *P. fluorescens* (0.259%), *P. clamydosporia* (0.253%), neem cake (0.220%) and carbofuran 3G (0.189%). The minimum potassium content was recorded in the control (0.147%) (Table 3).

Calcium

The calcium content in mulberry leaves was found to be unwavering in all the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum calcium content was recorded in Nemahari (2.230%) on par with *T. viride* (2.085%) followed by *P. lilacinum* (2.060%), *P. fluorescens* (2.042%), *T. harzianum* (2.032%), *P. clamydosporia* (1.997%), carbofuran 3G (1.985%) and neem cake (1.942%). The minimum potassium content was recorded in the untreated check (1.915%).

The maximum calcium content among the treatments on 120 DAT was recorded in Nemahari (3.072%) on par with *T. viride* (2.940%) followed by *P. lilacinum* (2.932%), *T. harzianum* (2.862%), *P. fluorescens* (2.710%), *P. clamydosporia* (2.515%), neem cake (2.160%) and carbofuran 3G (2.042%). The minimum calcium content was recorded in the control (1.835%) (Table 4).

Magnesium

The magnesium content in mulberry leaves was invariable in all the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum magnesium content was recorded in Nemahari (0.262%) on par with *T. viride* (0.217%) followed by *T. harzianum* (0.177%), *P. lilacinum* (0.155%), *P. clamydosporia* (0.125%). The treatments having *P. fluorescens* and neem cake recorded the same values for the magnesium content (0.122%) followed by carbofuran 3G (0.117%). The minimum magnesium content was recorded in the untreated check (0.107%).

The maximum magnesium content among the treatments on 120 DAT was recorded in Nemahari (0.282%) on par with *T. viride* (0.225%) followed by *T. harzianum* (0.192%), *P. lilacinum* (0.180%), *P. fluorescens* (0.175%), *P. clamydosporia* (0.145%), neem cake (0.137%) and carbofuran 3G (0.127%). The minimum magnesium content was recorded in the control (0.085%) (Table 4).

Sulphur

The sulphur content in mulberry leaves was consistent in all the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations revealed that the

maximum sulphur content was recorded in Nemahari (0.134%) on par with *T. viride* (0.118%) followed by *P. lilacinum* (0.109%), *T. harzianum* (0.107%), *P. fluorescens* (0.100%), *P. clamydosporia* (0.096%), neem cake (0.091%) and carbofuran 3G (0.088%). The minimum sulphur content was recorded in the untreated check (0.069%).

The maximum sulphur content among the treatments on 120 DAT was recorded in Nemahari (0.185%) on par with *T. viride* (0.179%) followed by *P. lilacinum* (0.169%), *T. harzianum* (0.157%), *P. fluorescens* (0.146%), *P. clamydosporia* (0.138%), neem cake (0.134%) and carbofuran 3G (0.106%). The minimum sulphur content was recorded in the control (0.066%) (Table 4).

Micronutrients

The zinc content in mulberry leaves was found to be consistent in all the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum zinc content was recorded in Nemahari (18.90 ppm) on par with *T. viride* (18.40 ppm) followed by *P. lilacinum* (17.50 ppm), *P. fluorescens* (17.15 ppm), *T. harzianum* (17.00 ppm), *P. clamydosporia* (16.85 ppm), carbofuran 3G (16.90 ppm) and neem cake (16.80 ppm). The final (120 DAT) observations reveal that the maximum zinc content was recorded in Nemahari (23.75 ppm) on par with *T. viride* (23.10 ppm) followed by *P. lilacinum* (22.45 ppm), *T. harzianum* (21.15 ppm), *P. fluorescens* (20.65 ppm), *P. clamydosporia* (20.00 ppm), neem cake (19.90 ppm) and carbofuran 3G (17.75 ppm). The minimum zinc content in the intermediary and final observation was recorded in the untreated check (14.80 and 13.95 ppm, respectively) (Table 5).

The manganese content in mulberry leaves was found to be consistent in all the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum manganese content was recorded in Nemahari (31.10 ppm) on par with *T. viride* (30.70 ppm) followed by *P. lilacinum* (28.40 ppm), *P. fluorescens* (27.95 ppm), *T. harzianum* (27.80 ppm), *P. clamydosporia* (26.65 ppm), neem cake (25.90 ppm) and carbofuran 3G (24.90 ppm). The final (120 DAT) observations reveal that the maximum manganese content was recorded in Nemahari (52.55 ppm) on par with *T. viride* (51.70 ppm) followed by *P. lilacinum* (49.95 ppm), *T. harzianum* (49.15 ppm), *P. fluorescens* (48.65 ppm), *P. clamydosporia* (47.05 ppm), neem cake (45.85 ppm) and carbofuran 3G (27.25 ppm). The minimum manganese content in the intermediary and final observation was recorded in the untreated check (22.00 and 20.70 ppm, respectively) (Table 5).

The copper content in mulberry leaves was invariable in all the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum copper content was recorded in Nemahari (25.00 ppm) on par with *T. viride* (24.70 ppm) followed by *P. lilacinum* (23.80 ppm), *T. harzianum* (23.20 ppm), *P. fluorescens* (22.40 ppm), *P. clamydosporia* (21.60 ppm), neem cake (21.30 ppm) and carbofuran 3G (20.10 ppm). The final (120 DAT) observations reveal that the maximum copper content was recorded in Nemahari (37.75 ppm) on par with *T. viride* (36.40 ppm) followed by *P. lilacinum* (32.30 ppm), *T. harzianum* (31.30 ppm), *P. fluorescens* (30.75 ppm), *P. clamydosporia* (27.75 ppm), neem cake (24.80 ppm) and carbofuran 3G (22.45 ppm). The minimum

copper content in the intermediary and final observation was recorded in the untreated check (18.30 and 17.75 ppm, respectively) (Table 5).

The iron content in mulberry leaves was found to be invariable among the treatments before the imposition of treatments (initial). The intermediary (60 DAT) observations reveal that the maximum iron content was recorded in Nemahari (208.75 ppm) on par with *T. viride* (205.95 ppm) followed by *T. harzianum* (205.10 ppm), *P. lilacinum* (204.80 ppm), *P. clamydosporia* (204.10 ppm), *P. fluorescens* (204.05 ppm), neem cake (201.20 ppm) and carbofuran 3G (201.05 ppm). The final (120 DAT) observations reveal that the maximum iron content was recorded in Nemahari (232.40 ppm) on par with *T. viride* (228.35 ppm) followed by *P. lilacinum* (226.05 ppm), *T. harzianum* (225.70 ppm), *P. fluorescens* (224.85 ppm), *P. clamydosporia* (220.60 ppm), neem cake (219.35 ppm) and carbofuran 3G (209.70 ppm). The minimum iron content in the intermediary and final observation was recorded in the untreated check (192.55 and 191.25 ppm, respectively) (Table 5).

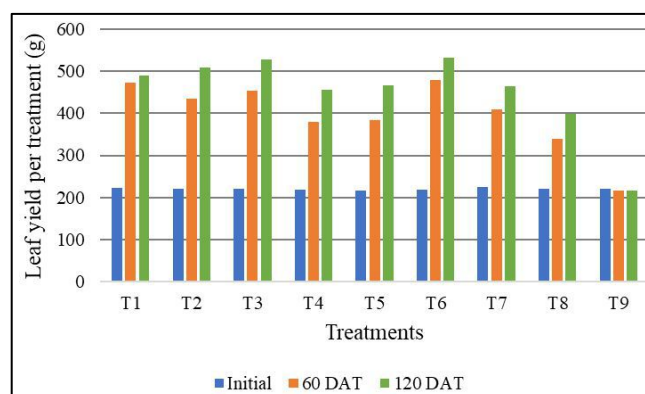


Fig 1: Effect of bioagents on mulberry leaf yield per plant

Discussion

The moisture content varied considerably during the observation intervals but was consistent among the treatments. The bioagents might have prevented the nematodes from entering and blocking the conducting tissues which increased the total chlorophyll content, total carbohydrates, total proteins, crude fibre, crude protein, ash content and the moisture retention capacity (6 hours after harvest) in the mulberry leaves. The influence of bioagents on the elemental composition of mulberry leaves is highly dependent on the availability of nutrients to the plant system without any hindrance from the root zone or the rhizosphere. The fixed form of nutrients might have converted to exchangeable or available forms due to the action of bioagents while the standard and untreated check without any catalysts resulted in reduced elemental composition. The enhanced leaf yield and other biochemical and elemental parameters observed in this study can be attributed to the production of growth-promoting substances by bioagents or the generation of toxic metabolites that inhibit nematodes and suppress other deleterious microorganisms. The findings of this investigation corroborate the results reported by Sobitha Devi and Datta (2002) [21] and Dube and Smart (1987) [6], who observed significant increases in growth and yield of tomato, soybean, tobacco and capsicum in pot and field experiments following inoculation with *Trichoderma* spp. and

Pseudomonas spp. The influence of *Trichoderma* spp. could be attributed to the production of enzyme chitinase which might have caused hindrance to the infestation by

nematodes, thereby affecting the quality and yield attributes of mulberry (Narasimhamurthy, 2010)^[15].

Table 1: Effect of different bioagents used against RKN on mulberry leaf moisture content, moisture retention percentage, total chlorophyll and total carbohydrates

Treatments	Leaf moisture content (%)			Moisture retention capacity (%)			Total chlorophyll (mg/g)			Total carbohydrates (%)		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T ₁	65.88	67.08	70.59	88.95	97.13	97.98	0.062	0.104	0.158	0.672	1.218	1.869
T ₂	64.08	66.02	68.68	90.91	97.44	97.41	0.062	0.114	0.162	0.679	1.246	1.960
T ₃	64.84	69.16	70.98	89.21	97.83	98.32	0.063	0.116	0.170	0.686	1.281	1.967
T ₄	63.53	64.74	69.18	88.38	96.81	97.24	0.062	0.102	0.155	0.687	1.176	1.841
T ₅	63.81	64.29	69.52	89.69	97.05	97.26	0.062	0.112	0.159	0.672	1.190	1.806
T ₆	66.32	70.58	71.91	90.06	97.92	98.50	0.062	0.123	0.176	0.679	1.498	2.184
T ₇	64.94	63.10	69.45	90.33	96.40	97.13	0.062	0.101	0.137	0.679	1.127	1.757
T ₈	66.03	66.84	68.92	90.48	96.39	96.93	0.062	0.099	0.132	0.672	1.113	1.631
T ₉	65.77	64.84	66.55	90.57	90.81	90.08	0.062	0.062	0.062	0.672	0.616	0.602
F-test	NS	NS	NS	NS	*	*	NS	*	*	NS	*	*
SEm ±	-	-	-	-	1.07	0.95	-	0.004	0.006	-	0.096	0.097
CD _{0.05}	-	-	-	-	3.14	2.79	-	0.012	0.019	-	0.280	0.282

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

Table 2: Effect of different bioagents used against RKN on total protein, crude protein, crude fibre and ash content in mulberry leaves

Treatments	Total protein (%)			Crude protein (%)			Crude fibre (%)			Ash (%)		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T ₁	14.52	21.80	24.07	4.45	7.39	11.92	4.22	5.89	7.27	2.75	6.50	9.25
T ₂	14.57	23.22	25.30	4.37	7.28	11.65	4.22	5.71	7.24	3.00	6.75	9.00
T ₃	14.75	24.10	27.65	4.43	8.46	13.03	4.21	6.07	8.23	3.25	7.75	9.50
T ₄	14.22	19.50	23.12	4.39	6.45	9.14	4.21	5.18	6.82	2.75	5.25	8.00
T ₅	14.30	21.00	23.82	4.34	6.84	10.93	4.22	5.57	7.15	3.00	6.25	8.50
T ₆	14.55	27.15	28.60	4.33	7.93	12.31	4.22	6.34	8.47	2.75	8.00	10.25
T ₇	14.12	19.25	19.50	4.34	6.54	10.43	4.21	5.29	6.96	2.75	5.50	8.00
T ₈	14.60	18.25	16.77	4.37	5.64	6.03	4.22	5.10	5.85	3.25	4.50	5.00
T ₉	14.15	13.35	14.70	4.41	4.06	3.84	4.21	4.22	4.09	3.00	3.00	2.75
F-test	NS	*	*	NS	*	*	NS	*	*	NS	*	*
SEm ±	-	2.12	1.40	-	0.18	0.15	-	0.04	0.03	-	0.71	1.01
CD _{0.05}	-	6.21	4.10	-	0.54	0.46	-	0.12	0.09	-	2.08	2.97

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

Table 3: Effect of different bioagents used against RKN on the primary nutrient content (N, P and K) in the mulberry leaves

Treatments	Nitrogen (%)			Phosphorous (%)			Potassium (%)		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T ₁	0.71	1.18	1.91	2.193	2.425	2.927	0.155	0.194	0.266
T ₂	0.70	1.16	1.86	2.190	2.409	2.915	0.155	0.190	0.262
T ₃	0.71	1.35	2.08	2.194	2.436	2.941	0.156	0.231	0.302
T ₄	0.70	1.02	1.46	2.192	2.391	2.901	0.155	0.172	0.253
T ₅	0.69	1.09	1.75	2.190	2.464	2.987	0.155	0.181	0.259
T ₆	0.69	1.27	1.97	2.192	2.454	2.957	0.155	0.233	0.323
T ₇	0.69	1.05	1.67	2.193	2.336	2.875	0.155	0.170	0.220
T ₈	0.70	0.90	0.96	2.191	2.262	2.444	0.155	0.161	0.189
T ₉	0.70	0.65	0.62	2.193	2.206	2.192	0.156	0.153	0.147
F-test	NS	*	*	NS	*	*	NS	*	*
SEm ±	-	0.03	0.02	-	0.007	0.016	-	0.007	0.006
CD _{0.05}	-	0.08	0.07	-	0.022	0.049	-	0.022	0.019

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

Table 4: Effect of different bioagents used against RKN on the secondary nutrient content (Ca, Mg and S) in the mulberry leaves

Treatments	Calcium (%)			Magnesium (%)			Sulphur (%)		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T ₁	1.960	2.060	2.932	0.125	0.155	0.180	0.085	0.109	0.169
T ₂	1.952	2.032	2.862	0.115	0.177	0.192	0.083	0.107	0.157
T ₃	1.972	2.085	2.940	0.105	0.217	0.225	0.082	0.118	0.179
T ₄	1.975	1.997	2.515	0.092	0.125	0.145	0.083	0.096	0.138
T ₅	1.995	2.042	2.710	0.100	0.122	0.175	0.083	0.100	0.146
T ₆	1.952	2.230	3.072	0.107	0.262	0.282	0.083	0.134	0.185
T ₇	1.967	1.942	2.160	0.105	0.122	0.137	0.084	0.091	0.134
T ₈	1.955	1.985	2.042	0.110	0.117	0.127	0.085	0.088	0.106
T ₉	1.977	1.915	1.835	0.120	0.107	0.085	0.084	0.069	0.066
F-test	NS	*	*	NS	*	*	NS	*	*
SEm ±	-	0.056	0.075	-	0.034	0.028	-	0.006	0.005
CD 0.05	-	0.166	0.218	-	0.099	0.082	-	0.018	0.016

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

Table 5: Effect of different bioagents used against RKN on the micronutrient content in the mulberry leaves

Treatments	Zinc (ppm)			Manganese (ppm)			Copper (ppm)			Iron (ppm)		
	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT	Initial	60 DAT	120 DAT
T ₁	16.55	17.50	22.45	22.05	28.40	49.95	18.35	23.80	32.30	195.25	204.80	226.05
T ₂	16.40	17.00	21.15	22.00	27.80	49.15	18.40	23.20	31.30	195.85	205.10	225.70
T ₃	16.50	18.40	23.10	22.15	30.70	51.70	18.60	24.70	36.40	194.45	205.95	228.35
T ₄	16.65	16.85	20.00	22.10	26.65	47.05	18.35	21.60	27.75	195.40	204.10	220.60
T ₅	16.90	17.15	20.65	22.25	27.95	48.65	18.40	22.40	30.75	195.60	204.05	224.85
T ₆	16.70	18.90	23.75	22.15	31.10	52.55	18.50	25.00	37.75	195.40	208.75	232.40
T ₇	16.35	16.80	19.90	22.15	25.90	45.85	18.55	21.30	24.80	195.55	201.20	219.35
T ₈	16.80	16.90	17.75	22.05	24.90	27.25	18.70	20.10	22.45	195.47	201.05	209.70
T ₉	16.50	14.80	13.95	22.05	22.00	20.70	18.65	18.30	17.75	195.30	192.55	191.25
F-test	NS	*	*	NS	*	*	NS	*	*	NS	*	*
SEm ±	-	0.47	0.53	-	0.79	0.77	-	0.65	0.82	-	1.21	2.28
CD 0.05	-	1.37	1.56	-	2.33	2.25	-	1.92	2.41	-	3.52	6.66

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

Conclusion

This study demonstrates the efficacy of bioagents in enhancing the biochemical and elemental composition of mulberry foliage. The application of bioagents significantly augmented the leaf moisture content, moisture retention capacity, total chlorophyll content, carbohydrate, protein, crude protein, crude fibre, ash and elemental (N, P, K, Ca, Mg, S, Zn, Mn, Cu and Fe) content in mulberry leaves, compared to the untreated control. These findings suggest that bioagents can be a viable, eco-friendly alternative for improving the nutritional quality of mulberry leaves alongside RKN management, thereby potentially enhancing the productivity and sustainability of sericulture. Further research is warranted to explore the large-scale applicability of bioagents in mulberry cultivation and their impact on silkworm growth, development and cocoon production.

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Conflict of Interest

None

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