



# **Impact of Liquid Bioformulations on Mulberry Leaf Quality and Physiological Traits**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author IN conceived and designed the study, conducted the experiments, and analyzed the data, performed the statistical analysis and contributed to data interpretation. Author SR contributed to the design of the study, provided essential materials, and assisted with data interpretation. Author PLD assisted with laboratory work, collected the data, and prepared the manuscript. Authors MHA and KS drafted and revised the manuscript, provided critical feedback, and approved the final version of the manuscript for submission. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The present study aimed to assess the impact of a liquid organic growth promoter comprising Orgafol, plant growth regulators (PGRs), and beneficial microbial inoculants (*Azospirillum*, *Phosphobacteria*, and VAM) on key physiological and biochemical traits of *Morus indica* cv. V1, with the objective of improving mulberry leaf quality for sustainable sericulture.

**Study Design:** The research was conducted as a factorial experiment in a randomized complete block design (RCBD) under controlled greenhouse conditions.

**Place and Duration of Study:** Department of Sericulture, Forest College & Research Institute, Tamil Nadu Agricultural University (TNAU), Mettupalayam, India; conducted over a growth period of 65 days.

**Methodology:** Ten treatment combinations were formulated using Orgafol in combination with various microbial inoculants and PGRs. These were applied at four graded concentrations (5, 10, 15, and 20 ml/plant) to potted mulberry plants. The study evaluated leaf moisture content (LMC), moisture retention capacity (MRC), total chlorophyll content, and soluble protein using standard biochemical and physiological methods. Statistical analysis was performed using OPSTAT and SPSS software at the 5% significance level.

**Results:** Treatment T9 (Orgafol + NAA + *Azospirillum* + *Phosphobacteria*) consistently recorded the highest values: LMC (78.48%), MRC (80.79%), chlorophyll (4.43 mg g<sup>-1</sup>), and protein content (23.33%). All measured traits showed a dose-dependent improvement. Differences between treatments were statistically significant ( $p < 0.05$ ).

**Conclusion:** The integrated use of liquid bioformulations significantly improved mulberry leaf physiological and biochemical traits. These inputs serve as effective, sustainable alternatives to synthetic fertilizers and support eco-friendly sericulture practices.

**Keywords:** *Morus indica*; liquid biofertilizer; Plant growth regulators (PGRs); leaf quality; *Azospirillum*; chlorophyll content; soluble protein; sustainable sericulture.

## 1. INTRODUCTION

Mulberry (*Morus indica* L.) is a deep-rooted, fast-growing perennial crop cultivated extensively across diverse climatic regions. Its foliage serves as the sole food source for the silkworm (*Bombyx mori* L.), making mulberry leaf productivity a cornerstone of sericulture. The intensive and continuous harvesting of leaves up to five times per year places significant pressure on soil nutrient reserves, necessitating efficient nutrient management strategies. Traditionally, chemical fertilizers have been used to maintain yield levels; however, their rising costs and limited availability have driven the need for more sustainable alternatives (Narayanaswamy et al., 2006).

Organic sources of nutrients, particularly when integrated with beneficial microbes, have shown potential to improve soil fertility and crop productivity while reducing dependency on synthetic inputs. Organic amendments enhance soil structure, moisture retention, and microbial activity, all of which are critical for the long-term sustainability of mulberry plantations (Kerenhapet et al., 2007). The use of microbial inoculants such as *Azospirillum*, phosphate-

solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF) has been widely reported to facilitate nutrient mobilization and promote plant growth through biological nitrogen fixation, phosphate solubilization, and enhanced nutrient uptake (Marwaha, 1995; Selvaraj et al., 1996; Lucy et al., 2004; Sundara Rao, W. V. B., & Sinha, 1963). PGPRs (plant growth-promoting rhizobacteria) are also known to synthesize key phytohormones such as auxins, gibberellins, and cytokinins, which modulate physiological and biochemical functions in plants (Glick, 2012).

Numerous studies have highlighted the synergistic effect of combining microbial inoculants with organic manures on mulberry growth and leaf quality (Rashmi et al., 2009). In fact, dual inoculations involving VAM and bacterial biofertilizers have consistently resulted in enhanced crop performance (Pawar, 1993; Sansammaet al., 1998; Sumana and Bagyaraj, 2002), including in mulberry, where reduced doses of chemical fertilizers were required to achieve similar or superior results (Umakanth and Bagyaraj, 1998; Ram et al., 2013; Kashyap et al., 2004).

Liquid bioformulations offer a practical and efficient means of delivering these beneficial

microbes to crops. Unlike carrier-based formulations, liquid inoculants maintain high cell viability, enable easy application, and ensure uniform distribution in the rhizosphere. However, limited research has explored the application of multi-strain liquid biofertilizers in mulberry systems.

In this context, the present study was undertaken to develop a liquid organic growth promoter incorporating Orgafol and selected microbial inoculants (*Azospirillum*, PSB, and AMF), and to evaluate its effect on key leaf quality parameters of *Morus indica* cv. V1 under controlled greenhouse conditions. The study aims to offer a sustainable and efficient alternative to chemical fertilizers by leveraging microbial consortia to enhance nutrient availability, physiological performance, and ultimately, the quality of mulberry foliage.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Microbial Inoculants

#### 2.1.1 *Azospirillum* spp.

Sterilized root segments of *Morus* spp. (mulberry) were cultured on semi-solid nitrogen-free bromothymol blue (Nfb) medium and incubated at 33 °C for 2–8 days. Colonies showing subsurface, milky-white halo growth were isolated and purified through repeated streaking. These isolates, initially smooth and grey-white, developed a wrinkled texture over time. Pure cultures were preserved at 4 °C (Dobereiner et al., 1976).

#### 2.1.2 Phosphate-Solubilizing Bacteria (PSB)

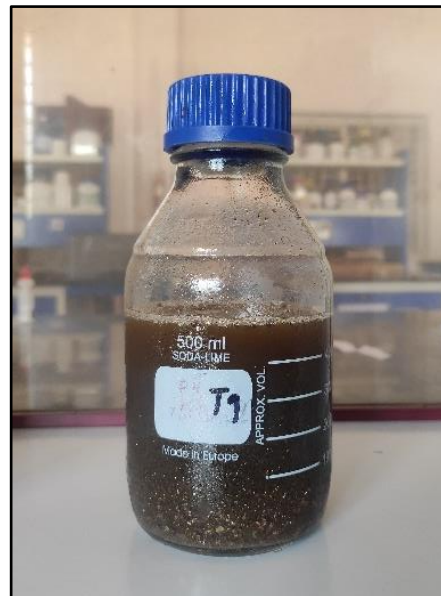
Soil collected from an undisturbed field at the Forest College & Research Institute, Mettupalayam was serially diluted and plated onto Pikovskaya's agar using pour and streak methods. After incubation at 28 ± 2 °C for four days, colonies forming clear halos were selected as PSB isolates (Sundaro and Sinha, 1963).

#### 2.1.3 Arbuscular Mycorrhizal Fungi (AMF)

AMF spores were extracted from rhizosphere soil of mulberry using wet-sieving and decanting (Gerdemann and Nicolson, 1963, Panwar, 1993). Trap cultures were developed with onion seedlings in a funnel system. The substrate, along with colonized onion roots, served as the inoculum.

### 2.2 Formulation of Liquid Growth-Promoter

The isolated microbes were mass-multiplied in a nutrient broth comprising yeast extract (20 g/L), beef extract (20 g/L), peptone (20 g/L), bone meal (20 g/L), and agar (1 g/L). Separately, an emulsion was made by melting 50 g of beeswax in 250 mL boiling water and mixing it with 2 g borax. This emulsion was added to the microbial broth at a concentration of 100 mL/L (Fig.1).



**Fig.1. Bioformulation prepared from *Azospirillum*, phosphobacteria and VAM cultures**

### 2.3 Experimental Setup

The trial was conducted in a naturally lit greenhouse at the Department of Sericulture, Forest College & Research Institute, TNAU, Mettupalayam (11.20 °N, 76.56 °E; 320 m AMSL). V1 variety mulberry cuttings were planted in pots and maintained at ambient greenhouse conditions (31–42 °C and ~68% RH).

### 2.4 Treatment Details

The study involved 10 treatment combinations integrating Orgafol with different microbial inoculants. The formulated liquid biofertilizer were applied at five graded dosages per plant: 5 ml (P1), 10 ml (P2), 15 ml (P3), and 20 ml (P4), as detailed in Table 1.

## 2.5 Data Collection and Analysis

Leaf quality assessments were carried out between 60 and 65 days after planting, focusing on fully matured leaves. Leaf moisture content and moisture retention capacity, measured six hours post-harvest, were evaluated according to the procedure described by Tikader and Kamble (2008). The total chlorophyll content was quantified following the method of Hiscox and Israelstam (1979). For protein analysis, the collected leaf samples were oven-dried, and total soluble protein content was determined using the Lowry et al. (1951) method.

**Table 1. Summary of the experimental treatments**

Treatment No.	Treatment Compositions
T1 (Control)	Orgafol
T2	Orgafol + NAA
T3	Orgafol + <i>Azospirillum</i>
T4	Orgafol + Phosphobacteria
T5	Orgafol + VAM
T6	Orgafol + NAA + <i>Azospirillum</i>
T7	Orgafol + NAA + Phosphobacteria
T8	Orgafol + NAA + VAM
T9	Orgafol + NAA + <i>Azospirillum</i> + Phosphobacteria
T10	Orgafol + <i>Azospirillum</i> + VAM

## 2.6 Experimental design and Statistical Analysis

The trial followed a factorial arrangement in a randomized complete block design (RCBD) with four replications, examining the interaction between pellet type and dosage on mulberry growth and productivity. The data collected was analyzed using OPSTAT and SPSS 23 software at 5% probability level.

## 3. RESULTS AND DISCUSSION

### 3.1 Total Chlorophyll Content

The data presented in Table 2 reveal a significant influence of different treatment combinations and concentrations on the chlorophyll content of mulberry leaves. Among all treatments, T9 consistently recorded the highest chlorophyll levels across all concentrations, reaching a peak value of 4.43 mg g<sup>-1</sup> at 20 ml/plant. This was closely followed by T10, which showed 4.12 mg

g<sup>-1</sup>, suggesting a strong synergistic effect between beneficial microorganisms and plant growth regulators. An increasing trend was observed with higher concentrations, indicating a clear dose-responsive enhancement in chlorophyll content from 5 ml to 20 ml per plant. Statistical analysis confirmed that the differences among the treatments and concentrations were significant at the 5% level (P = .05).

These results demonstrate that the combination of Orgafol with NAA and a consortium of beneficial microorganisms, particularly *Azospirillum* and Phosphobacteria, are highly effective in improving leaf chlorophyll content in mulberry. Similar enhancements in chlorophyll levels due to the combined use of soil-applied *Azospirillum*, *Rhizobium*, and effective microorganisms at 25, 40, and 60 days after pruning (DAPR) have been reported (Vinoj, 2008). Additionally, increased chlorophyll content following foliar application of EM in mulberry was also observed (Gnanaselvi, 2007).

These findings align with earlier studies by Singh et al. (1991), Das et al. (1994), and Ramarethinamet al. (2005), who also reported improved chlorophyll synthesis with biofertilizer application. Panneerselvam et al. (1997) attributed similar results to enhanced intercellular CO<sub>2</sub> concentration and increased photosynthetic activity, leading to efficient carbohydrate utilization. Ahmed et al. (2017) also supported this view, linking elevated chlorophyll content with higher photosynthetic output. Furthermore, Sujathamma and Dandin (2000) suggested that chlorophyll content serves as a reliable indicator of photosynthetic efficiency in mulberry. This is further supported by the observations of Patil et al. (1998), Watson (1952), and Raj and Tripathy (1999), who emphasized that higher chlorophyll levels are closely associated with greater photosynthetic performance in plants.

### 3.2 Total Protein Content

A significant and positive influence of biofertilizers on the soluble protein content of mulberry leaves was observed (Table 3). The results clearly demonstrate that both the type of treatment and the level of application had a notable effect on protein synthesis. Among the treatments, T9 consistently showed the highest protein content at all concentrations, with a peak value of 23.33% at 20 ml/plant. This was closely followed by T6 and T10, suggesting that the combination of biofertilizers with plant growth

regulators significantly enhances protein accumulation in mulberry foliage. In contrast, the control treatment T1 showed the lowest protein levels across all concentrations, with values ranging from 12.10 % at 5 ml/plant to 15.10 % at

20 ml/plant. This highlights the limited effect of Orgafol without the addition of microbial or hormonal components. Statistical analysis confirmed that these differences were significant at the 5% level ( $P = .05$ ).

**Table 2. Effect of different biofertilizer treatments on the total chlorophyll content (mg g<sup>-1</sup>) of mulberry leaves**

Treatments	Concentrations			
	5 ml/ plant	10 ml/ plant	15 ml/ plant	20 ml/ plant
T1 (Orgafol) (Control)	1.50± 0.046 <sup>h</sup>	1.95± 0.064 <sup>j</sup>	2.20± 0.050 <sup>h</sup>	2.39± 0.058 <sup>g</sup>
T2 (Orgafol + NAA)	1.75± 0.036 <sup>g</sup>	2.20± 0.076 <sup>i</sup>	2.50± 0.076 <sup>g</sup>	2.71± 0.067 <sup>f</sup>
T3 (Orgafol + <i>Azospirillum</i> )	2.10± 0.084 <sup>d</sup>	2.60± 0.081 <sup>e</sup>	2.95± 0.085 <sup>d</sup>	3.11± 0.086 <sup>d</sup>
T4 (Orgafol + Phosphobacteria)	1.90± 0.060 <sup>f</sup>	2.40± 0.089 <sup>g</sup>	2.60± 0.080 <sup>f</sup>	2.67± 0.062 <sup>f</sup>
T5 (Orgafol + VAM)	1.85± 0.038 <sup>g</sup>	2.35± 0.085 <sup>h</sup>	2.55± 0.073 <sup>g</sup>	2.65± 0.082 <sup>f</sup>
T6 (Orgafol + NAA + <i>Azospirillum</i> )	2.50± 0.057 <sup>c</sup>	3.00± 0.095 <sup>c</sup>	3.40± 0.060 <sup>c</sup>	3.58± 0.079 <sup>c</sup>
T7 (Orgafol + NAA + Phosphobacteria)	2.05± 0.051 <sup>de</sup>	2.65± 0.068 <sup>d</sup>	2.95± 0.078 <sup>d</sup>	3.09± 0.084 <sup>de</sup>
T8 (Orgafol + NAA + VAM)	2.00± 0.046 <sup>e</sup>	2.55± 0.074 <sup>f</sup>	2.85± 0.055 <sup>e</sup>	3.03± 0.084 <sup>e</sup>
T9 (Orgafol + NAA + <i>Azospirillum</i> + Phosphobacteria)	3.00± 0.111 <sup>a</sup>	3.75± 0.083 <sup>a</sup>	4.10± 0.080 <sup>a</sup>	4.43± 0.094 <sup>a</sup>
T10 (Orgafol + <i>Azospirillum</i> + VAM)	2.85± 0.080 <sup>b</sup>	3.50± 0.080 <sup>b</sup>	3.90± 0.064 <sup>b</sup>	4.12± 0.109 <sup>b</sup>
Mean	2.15	2.70	3.00	3.18

\*The average values obtained from three replications and the results were expressed as Mean ± S.E. Means in the similar row with different letters are statistically significant at  $P < 0.05$  and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant

**Table 3. Effect of different biofertilizer treatments on the total protein content (%) of mulberry leaves**

Treatments	Concentrations			
	5 ml/ plant	10 ml/ plant	15 ml/ plant	20 ml/ plant
T1 (Orgafol) (Control)	12.10± 0.265 <sup>h</sup>	14.30± 0.321 <sup>i</sup>	15.00± 0.404 <sup>h</sup>	15.10± 0.173 <sup>g</sup>
T2 (Orgafol + NAA)	13.50± 0.208 <sup>g</sup>	15.70± 0.265 <sup>h</sup>	16.90± 0.404 <sup>g</sup>	16.87± 0.267 <sup>f</sup>
T3 (Orgafol + <i>Azospirillum</i> )	14.70± 0.265 <sup>de</sup>	18.00± 0.416 <sup>d</sup>	19.80± 0.656 <sup>d</sup>	19.57± 0.418 <sup>d</sup>
T4 (Orgafol + Phosphobacteria)	14.90± 0.379 <sup>cd</sup>	18.60± 0.436 <sup>c</sup>	20.30± 0.513 <sup>c</sup>	20.10± 0.451 <sup>c</sup>
T5 (Orgafol + VAM)	15.00± 0.321 <sup>c</sup>	17.03± 0.318 <sup>fg</sup>	17.80± 0.436 <sup>f</sup>	18.23± 0.285 <sup>e</sup>
T6 (Orgafol + NAA + <i>Azospirillum</i> )	17.10± 0.379 <sup>b</sup>	20.00± 0.379 <sup>b</sup>	21.90± 0.656 <sup>b</sup>	22.10± 0.513 <sup>b</sup>
T7 (Orgafol + NAA + Phosphobacteria)	14.50± 0.265 <sup>e</sup>	16.80± 0.300 <sup>g</sup>	17.07± 0.406 <sup>g</sup>	17.23± 0.260 <sup>f</sup>
T8 (Orgafol + NAA + VAM)	13.90± 0.265 <sup>f</sup>	17.20± 0.265 <sup>f</sup>	18.47± 0.353 <sup>e</sup>	18.40± 0.361 <sup>e</sup>
T9 (Orgafol + NAA + <i>Azospirillum</i> + Phosphobacteria)	18.30± 0.416 <sup>a</sup>	20.93± 0.348 <sup>a</sup>	22.70± 0.600 <sup>a</sup>	23.33± 0.353 <sup>a</sup>
T10 (Orgafol + <i>Azospirillum</i> + VAM)	14.70± 0.265 <sup>de</sup>	17.60± 0.265 <sup>e</sup>	18.80± 0.379 <sup>e</sup>	19.23± 0.233 <sup>d</sup>
Mean	14.87	17.62	18.88	19.02

\*The average values obtained from three replications and the results were expressed as Mean ± S.E. Means in the similar row with different letters are statistically significant at  $P < 0.05$  and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant.

**Table 4. Effect of different biofertilizer treatments on the leaf moisture content (%) of mulberry leaves**

Treatments	Concentrations			
	5 ml/ plant	10 ml/ plant	15 ml/ plant	20 ml/ plant
T1 (Orgafol) (Control)	70.80± 0.318 <sup>j</sup>	72.40± 0.318 <sup>j</sup>	73.00± 0.318 <sup>j</sup>	73.16± 0.318 <sup>j</sup>
T2 (Orgafol + NAA)	71.20± 0.317 <sup>i</sup>	72.70± 0.317 <sup>i</sup>	73.60± 0.318 <sup>i</sup>	73.45± 0.317 <sup>h</sup>
T3 (Orgafol + <i>Azospirillum</i> )	74.00± 0.318 <sup>e</sup>	76.00± 0.370 <sup>d</sup>	76.90± 0.370 <sup>d</sup>	76.91± 0.371 <sup>c</sup>
T4 (Orgafol + Phosphobacteria)	72.50± 0.318 <sup>h</sup>	74.90± 0.318 <sup>h</sup>	75.60± 0.371 <sup>g</sup>	75.50± 0.370 <sup>g</sup>
T5 (Orgafol + VAM)	73.60± 0.318 <sup>g</sup>	75.10± 0.371 <sup>g</sup>	75.50± 0.370 <sup>h</sup>	75.90± 0.370 <sup>f</sup>
T6 (Orgafol + NAA + <i>Azospirillum</i> )	75.20± 0.370 <sup>b</sup>	77.00± 0.370 <sup>c</sup>	77.60± 0.371 <sup>c</sup>	77.85± 0.371 <sup>b</sup>
T7 (Orgafol + NAA + Phosphobacteria)	73.80± 0.318 <sup>f</sup>	75.60± 0.371 <sup>f</sup>	76.30± 0.371 <sup>f</sup>	76.34± 0.370 <sup>e</sup>
T8 (Orgafol + NAA + VAM)	74.30± 0.318 <sup>d</sup>	75.90± 0.370 <sup>e</sup>	76.60± 0.371 <sup>e</sup>	76.48± 0.370 <sup>d</sup>
T9 (Orgafol + NAA + <i>Azospirillum</i> + Phosphobacteria)	76.00± 0.370 <sup>a</sup>	77.90± 0.370 <sup>a</sup>	78.40± 0.370 <sup>a</sup>	78.48± 0.370 <sup>a</sup>
T10 (Orgafol + <i>Azospirillum</i> + VAM)	75.00± 0.318 <sup>c</sup>	77.10± 0.371 <sup>b</sup>	77.70± 0.370 <sup>b</sup>	77.90± 0.370 <sup>b</sup>
Mean	73.64	75.46	76.12	76.20

\*The average values obtained from three replications and the results were expressed as Mean ± S.E. Means in the similar row with different letters are statistically significant at  $P < 0.05$  and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant

**Table 5. Effect of different biofertilizer treatments on the moisture retention capacity (%) of mulberry leaves**

Treatments	Concentrations			
	5 ml/ plant	10 ml/ plant	15 ml/ plant	20 ml/ plant
T1 (Orgafol) (Control)	75.27± 0.375 <sup>i</sup>	76.80± 0.500 <sup>e</sup>	77.40± 0.458 <sup>g</sup>	77.69± 0.393 <sup>i</sup>
T2 (Orgafol + NAA)	75.70± 0.378 <sup>h</sup>	77.10± 0.436 <sup>e</sup>	78.00± 0.458 <sup>f</sup>	77.98± 0.353 <sup>h</sup>
T3 (Orgafol + <i>Azospirillum</i> )	77.00± 0.379 <sup>de</sup>	79.03± 0.549 <sup>c</sup>	79.70± 0.500 <sup>c</sup>	79.89± 0.375 <sup>c</sup>
T4 (Orgafol + Phosphobacteria)	76.00± 0.379 <sup>g</sup>	77.93± 0.521 <sup>d</sup>	78.30± 0.436 <sup>ef</sup>	78.21± 0.360 <sup>g</sup>
T5 (Orgafol + VAM)	76.50± 0.361 <sup>f</sup>	78.10± 0.461 <sup>d</sup>	78.43± 0.441 <sup>e</sup>	78.68± 0.339 <sup>f</sup>
T6 (Orgafol + NAA + <i>Azospirillum</i> )	77.50± 0.458 <sup>b</sup>	79.50± 0.551 <sup>b</sup>	80.20± 0.462 <sup>b</sup>	80.21± 0.453 <sup>b</sup>
T7 (Orgafol + NAA + Phosphobacteria)	76.90± 0.346 <sup>e</sup>	78.77± 0.498 <sup>c</sup>	79.10± 0.436 <sup>d</sup>	79.14± 0.318 <sup>e</sup>
T8 (Orgafol + NAA + VAM)	77.20± 0.378 <sup>cd</sup>	79.00± 0.520 <sup>c</sup>	79.33± 0.433 <sup>d</sup>	79.45± 0.348 <sup>d</sup>
T9 (Orgafol + NAA + <i>Azospirillum</i> + Phosphobacteria)	78.00± 0.379 <sup>a</sup>	80.00± 0.520 <sup>a</sup>	80.60± 0.436 <sup>a</sup>	80.79± 0.322 <sup>a</sup>
T10 (Orgafol + <i>Azospirillum</i> + VAM)	77.40± 0.378 <sup>bc</sup>	79.60± 0.557 <sup>b</sup>	80.03± 0.434 <sup>b</sup>	80.14± 0.318 <sup>b</sup>
Mean	76.75	78.60	79.12	79.23

\*The average values obtained from three replications and the results were expressed as Mean ± S.E. Means in the similar row with different letters are statistically significant at  $P < 0.05$  and analysed by Duncan's multiple range test. Number followed by the same alphabet in the row denotes statistically not significant

The observed enhancement in protein content is likely attributed to improved nitrogen fixation, better nutrient assimilation, and elevated metabolic activity resulting from the synergistic interactions between plant growth regulators and beneficial microbes. Similar findings have been reported by Mary et al. (2015), who noted increased soluble protein levels in plants

following micronutrient and biofertilizer applications. Chakraborty et al. (2008) also observed a comparable increase in protein content when poultry manure was used in conjunction with biofertilizers. The combined application of nutrients and microbial inoculants not only enhances the activity of RuBP carboxylase (an essential enzyme in

photosynthesis) but also leads to increased sugar and soluble protein levels in mulberry leaves. These improvements are crucial for meeting the nutritional demands of both young and mature silkworms. Moreover, the increase in total soluble protein content indicates a marked improvement in the biochemical composition and nutritional quality of mulberry leaves. According to Madhubabu et al. (1992), such increases may be directly related to microbial nitrogen fixation, which boosts nitrogen availability in the soil and thereby elevates protein synthesis in plant tissues.

### 3.3 Leaf Moisture Content and Moisture Retention Capacity

In mulberry, leaf moisture content (LMC) and moisture retention capacity (MRC) are critical physiological traits that directly influence the nutritional quality and palatability of leaves for silkworm consumption. The present study revealed that these parameters were significantly affected by the application of different combinations of Orgafol, plant growth regulators, and biofertilizers applied at varying concentrations. A consistent, dose-dependent increase in both LMC and MRC was observed with the progressive rise in application rate from 5 ml to 20 ml per plant.

Among the treatments, T9 demonstrated the most notable improvement in both traits, with LMC ranging from 76.00% to 78.48% (Table 4), and MRC increasing from 78.00% to 80.79% across the tested concentrations (Table 5). Treatments T6 and T10 also recorded significantly higher values compared to the control, particularly at the higher doses. In contrast, the control treatment (T1) consistently showed the lowest values, with LMC between 70.80% and 73.16%, and MRC from 75.27% to 77.69%. The overall mean values across all treatments showed an upward trend, with LMC increasing from 73.64% to 76.20%, and MRC from 76.75% to 79.23%, further confirming a positive correlation between treatment concentration and physiological response.

Statistical analysis indicated that the integrated application of NAA, *Azospirillum*, and Phosphobacteria was significantly more effective ( $P = .05$ ) in enhancing LMC and MRC compared to single treatments or untreated controls. These results emphasize the potential of integrated

nutrient management in improving the physiological vigor, moisture regulation, and stress resilience of mulberry plants.

As reported by Sujathamma and Dandin (2000), leaves with elevated moisture parameters are indicative of superior leaf quality. The enhanced moisture levels observed in this study may be attributed to the action of microbial inoculants, which likely improved soil moisture availability in the rhizosphere, thereby supporting optimal water absorption, growth, and metabolic activity in mulberry plants.

### 4. CONCLUSION

The present study demonstrates that the combined application of Orgafol, NAA, and selected microbial inoculants (*Azospirillum*, Phosphobacteria, and VAM) significantly improves key physiological and biochemical traits of mulberry (*Morus indica*). Among all treatments, T9 consistently recorded the highest values for total chlorophyll content, soluble protein, leaf moisture content, and moisture retention capacity, indicating a strong synergistic effect of integrated nutrient management strategies. A dose-dependent response was observed across all treatments, with optimal results at 20 ml/plant, highlighting the importance of appropriate application rates. Enhanced LMC and MRC reflect better water balance and tissue hydration, contributing to leaf palatability and improved silkworm nutrition. Additionally, increased chlorophyll and protein levels suggest improved photosynthetic efficiency and metabolic vigor in treated plants. Statistical significance ( $P = 0.05$ ) across parameters validates the efficacy of microbial consortia in promoting plant growth and leaf quality. These findings emphasize the potential of liquid bioformulations as eco-friendly and cost-effective alternatives to chemical fertilizers in sustainable sericulture. The adoption of such integrated inputs could reduce fertilizer dependency, improve leaf yield and quality, and contribute to the long-term productivity of mulberry plantations.

### 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 this manuscript.

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## COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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