**Original Research Article**

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

**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⁻¹), 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 (Kerenhap*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). 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; Sansamma et 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). 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.

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

|  |  |
| --- | --- |
| **Treatment No.** | **Treatment Compositions** |
|
| **T1 (Control)** | Orgafol |
| **T2** | Orgafol + NAA |
| **T3** | Orgafol + *Azospirillum* |
| **T4** | Orgafol + Phosphobacteria |
| **T5** | Orgafol + VAM |
| **T6** | Orgafol + NAA + *Azospirillum* |
| **T7** | Orgafol + NAA + Phosphobacteria |
| **T8** | Orgafol + NAA + VAM |
| **T9** | Orgafol + NAA + *Azospirillum* + Phosphobacteria |
| **T10** | Orgafol + *Azospirillum* + VAM |

**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.

**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.

1. **RESULTS AND DISCUSSION**
	1. **Total Chlorophyll Content**

The data presented in Table 2reveal 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⁻¹** at 20 ml/plant. This was closely followed by T10, which showed **4.12 mg g⁻¹,** 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 Ramarethinam*et al.* (2005), who also reported improved chlorophyll synthesis with biofertilizer application. Panneerselvam *et al.* (1997) attributed similar results to enhanced intercellular CO₂ 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.

**Table2. Effect of different biofertilizer treatments on the total chlorophyll content (mg g-1) of mulberry leaves**

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

* 1. **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).

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.

**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± 0.265h | 14.30± 0.321i | 15.00± 0.404h | 15.10± 0.173g |
| **T2 (Orgafol + NAA)** | 13.50± 0.208g | 15.70± 0.265h | 16.90± 0.404g | 16.87± 0.267f |
| **T3 (Orgafol + *Azospirillum*)** | 14.70± 0.265de | 18.00± 0.416d | 19.80± 0.656d | 19.57± 0.418d |
| **T4 (Orgafol + Phosphobacteria)** | 14.90± 0.379cd | 18.60± 0.436c | 20.30± 0.513c | 20.10± 0.451c |
| **T5 (Orgafol + VAM)** | 15.00± 0.321c | 17.03± 0.318fg | 17.80± 0.436f | 18.23± 0.285e |
| **T6 (Orgafol + NAA + *Azospirillum*)** | 17.10± 0.379b | 20.00± 0.379b | 21.90± 0.656b | 22.10± 0.513b |
| **T7 (Orgafol + NAA + Phosphobacteria)** | 14.50± 0.265e | 16.80± 0.300g | 17.07± 0.406g | 17.23± 0.260f |
| **T8 (Orgafol + NAA + VAM)** | 13.90±0.265f | 17.20± 0.265f | 18.47± 0.353e | 18.40± 0.361e |
| **T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)** | 18.30± 0.416a | 20.93± 0.348a | 22.70± 0.600a | 23.33±0.353a |
| **T10 (Orgafol + *Azospirillum* + VAM)** | 14.70± 0.265de | 17.60± 0.265e | 18.80±0.379e | 19.23±0.233d |
| **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.

* 1. **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.

**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± 0.318j | 72.40± 0.318j | 73.00± 0.318j | 73.16± 0.318i |
| **T2 (Orgafol + NAA)** | 71.20± 0.317i | 72.70± 0.317i | 73.60± 0.318i | 73.45± 0.317h |
| **T3 (Orgafol + *Azospirillum*)** | 74.00± 0.318e | 76.00± 0.370d | 76.90± 0.370d | 76.91± 0.371c |
| **T4 (Orgafol + Phosphobacteria)** | 72.50± 0.318h | 74.90± 0.318h | 75.60± 0.371g | 75.50± 0.370g |
| **T5 (Orgafol + VAM)** | 73.60± 0.318g | 75.10± 0.371g | 75.50± 0.370h | 75.90± 0.370f |
| **T6 (Orgafol + NAA + *Azospirillum*)** | 75.20± 0.370b | 77.00± 0.370c | 77.60± 0.371c | 77.85± 0.371b |
| **T7 (Orgafol + NAA + Phosphobacteria)** | 73.80± 0.318f | 75.60± 0.371f | 76.30± 0.371f | 76.34± 0.370e |
| **T8 (Orgafol + NAA + VAM)** | 74.30±0.318d | 75.90± 0.370e | 76.60± 0.371e | 76.48± 0.370d |
| **T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)** | 76.00± 0.370a | 77.90± 0.370a | 78.40± 0.370a | 78.48± 0.370a |
| **T10 (Orgafol + *Azospirillum* + VAM)** | 75.00± 0.318c | 77.10± 0.371b | 77.70±0.370b | 77.90± 0.370b |
| **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± 0.375i | 76.80± 0.500e | 77.40± 0.458g | 77.69± 0.393i |
| **T2 (Orgafol + NAA)** | 75.70± 0.378h | 77.10± 0.436e | 78.00± 0.458f | 77.98± 0.353h |
| **T3 (Orgafol + *Azospirillum*)** | 77.00± 0.379de | 79.03± 0.549c | 79.70± 0.500c | 79.89± 0.375c |
| **T4 (Orgafol + Phosphobacteria)** | 76.00± 0.379g | 77.93± 0.521d | 78.30± 0.436ef | 78.21± 0.360g |
| **T5 (Orgafol + VAM)** | 76.50± 0.361f | 78.10± 0.461d | 78.43± 0.441e | 78.68± 0.339f |
| **T6 (Orgafol + NAA + *Azospirillum*)** | 77.50± 0.458b | 79.50± 0.551b | 80.20± 0.462b | 80.21± 0.453b |
| **T7 (Orgafol + NAA + Phosphobacteria)** | 76.90± 0.346e | 78.77± 0.498c | 79.10± 0.436d | 79.14± 0.318e |
| **T8 (Orgafol + NAA + VAM)** | 77.20±0.378cd | 79.00± 0.520c | 79.33± 0.433d | 79.45± 0.348d |
| **T9 (Orgafol + NAA + *Azospirillum* + Phosphobacteria)** | 78.00± 0.379a | 80.00± 0.520a | 80.60± 0.436a | 80.79± 0.322a |
| **T10 (Orgafol + *Azospirillum* + VAM)** | 77.40± 0.378bc | 79.60± 0.557b | 80.03±0.434b | 80.14± 0.318b |
| **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.

**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 **liquidbioformulations** 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.

**COMPETING INTERESTS DISCLAIMER:**

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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