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# Impact of different concentrations of rooting hormones and substrates on nutritional quality of mini clonal leaves of Mulberry (*Morus indica*) at nursery level

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**Abstract**
An experiment was conducted to analyze the nutritional quality of mini clonal leaves treated with different concentrations of rooting hormones such as IBA (1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, 5000 ppm) and NAA (1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, 5000 ppm) and different types of rooting medium (soil, soil+ sand+ FYM, soil+ coir pith, soil+ sand+ vermicompost, soil+ coir pith+ FYM) where mulberry mini cuttings grown. For this study V1 variety of mulberry was taken and this study was carried out in suitable environmental conditions using mini clonal technology. Biochemical parameters namely moisture content, total protein, carbohydrate, crude protein, nitrogen, phosphorus, potassium were recorded on 90 DAP. Among all treatments IBA @ 3000 ppm recorded maximum in all biochemical constituents.

**Keywords:** V1 (Victory 1), Mini clonal technology, IBA, NAA

## Introduction
The family Moraceae includes the mulberry (*Morus* sp.). The mulberry tree is a deciduous woody perennial with a deep root system that grows quickly. It has lobes that range from one to five, and its petiole is whole or alternating. It has a catkin-style inflorescence, meaning that unisexual blooms are held on a dangling or drooping peduncle. On the other hand, triploids are also commonly grown due of their high leaf quality, rapid development, and flexibility. Mulberry could be commercially significant in developing Asian countries where sericulture makes up a sizable portion of the economy. Mulberry is the only sole food for *Bombyx mori* L., so the viability of sericulture depends largely on the availability of high-quality mulberry leaves. Mulberry fruits have been used to meet human needs in the form of fresh fruit or a variety of confections, including wine, ice cream, pulp, juice, paste, jam, and marmalade, as well as frozen sweets (Vijayan *et al.*, 1998) [36]. Mulberry leaf production performance depends on several factors, such as variety, climate, and cultivation techniques like strong roots, quick growth, high yield, as well as soil quality and plant protection measures (Babu *et al.* 2019) [2]. Mulberry is one of the most important tree crops in terms of economic importance, and man's interest in mulberry cultivation dates back to the rise of civilization and his desire for high-quality silk, which led him to search for the material. Approximately 70% of the silk produced by silkworms is directly derived from the protein of mulberry leaves, as the quantity and quality of mulberry leaves affect cocoon harvest (Datta, 2000) [7].

The secret to a superior silkworm cocoon crop is leaf quality. It is a known truth that mulberry varieties vary in leaf quality, which causes the variations in silkworm rearing performances (Bharathi *et al.*, 2022) [3]. The moisture content of mulberry leaves was used to determine their quality. Varieties, cultivation methods, preservation strategies, leaf age, and leaf position all had a significant impact (Bongale *et al.*, 1997) [4]. Higher moisture content in mulberry leaves facilitates nutritional absorption, digestion, and assimilation, all of which have an immediate impact on silkworm development and growth (Doss *et al.*, 2007) [8]. Silkworms prefer mulberry leaves that are higher in water, total sugar, soluble carbohydrates, and lower in minerals. The nutritional needs of silkworm larvae change according to how mature the leaves they are given.

The nutritional needs of silkworm larvae change according to how mature the leaves they are given. Due to their ease of digestion, Chawki silkworms require leaves with a high moisture level, whereas late-age silkworms prefer mature leaves with lower moisture content because they have the strength to digest. However, an excessive amount of mature leaves lacks necessary biochemical components, and their moisture content makes them unfit for feeding silkworms (Krishnaswami *et al.,* 1971) [18]. Keeping in view, it is an important to assess the nutritional values of miniclonal leaves grown in desired rooting medium with suitable climatic conditions.

Rooting hormones can accelerate the development of root primordia and promote adventitious root growth, leading to a noticeable improvement in rooting rate of mulberry cuttings. Most researchers believe that exogenous hormones control endogenous hormone expression and the distribution of nutrients in cuttings (Kiruthika *et al.,* 2020) [39]. The ratio of endogenous hormone levels during plant cuttings is closely related to the adventitious root development process. (Chen *et al.*,2023, Huang *et al*., 2022) [6]. IAA has the ability to regulate the development of callus tissue and promote cell division in the cambium. IAA has a short duration of action, is very unstable, easily breaks down in water, and is readily eliminated by plant metabolism (Jiang *et al.*,2021) [13]. Protoxylem pole-adjacent pericycle cells were activated by exogenous IAA to form a lateral root primordium.

IBA and IAA are widely employed in root induction of mulberry cuttings and IBA application has significant economic impacts (Pooja *et al.,* 2022 and Chen *et al.,* 2023) [23, 6]. The use of IBA exogenously has the potential to accelerate the translocation and transfer of carbohydrates from the leaf to the incision site of the shoot or air layering, hence fostering root formation and seedling development (Stefancic *et al*., 2005, Husen *et al.,* 2017, Khandaker *et al*.,2022) [31, 12, 17]. Many researchers have documented how NAA stimulates plant stem cuttings to root, and one of the important properties of NAA is known to be the promotion of adventitious root formation. Development of adventitious root includes the process of re differentiation during which preset cells deviate from their morphogenetic course to act as mother cells for the root primordia (Yan *et al.,* 2014) [38].

## Materials and Methods

The ruling mulberry variety V1 was taken as source plant material. The apical cutting of V1 was collected from main field in early morning to avoid desiccation. Later the cuttings were trimmed to 7-8 cm length and kept in cool place. Desired apical cuttings of *Morus* were dipped in various concentrations of rooting hormone *viz*., IBA ($T_1$-1000 ppm, $T_2$- 2000 ppm, $T_3$- 3000 ppm, $T_4$- 4000ppm, $T_5$- 5000 ppm) and NAA ($T_1$-1000 ppm, $T_2$- 2000 ppm, $T_3$-3000 ppm, $T_4$- 4000 ppm, $T_5$- 5000 ppm). The dipped planting materials were place in different rooting medium *viz*., soil, soil+ sand+ FYM, soil+ coir pith, soil+ sand+ vermicompost, soil+ coir pith+ FYM. These plants were kept inside the low cost polytunnel under shade net at a temperature of 25-35 °C and relative humidity of 75 to 80 per cent with intermittent spraying ofof water using rose cans and thereafter maintained in a mist chamber. After 90 days miniclonal mulberry leaf samples were collected and shade dried for analysis. The dried leaves were powdered using mixer grinder and stored in air tight container. The studies were conducted in Completely Randomized Design (CRD) with four replications. And also the rooting medium properties *viz.,* water holding capacity, soil organic nitrogen, available nitrogen, available phosphorus and available potassium were determined by various methods. The samples were collected, air dried and then grounded with wooden pestle and mortar, mixed thoroughly and sieved through 2 mm sieve. The trials were carried out in the Agroforestry Department Laboratory of the Forest College and Research Institute located in Mettupalayam, Coimbatore District, Tamil Nadu.

**Methodology used for properties of rooting medium**

| | | |
|---|---|---|
| Water holding capacity | Keen-Raczkowski box method | (Gupta and Dakshinamoorthy, 1980) [9] |
| Soil organic carbon | Wet chromic acid digestion | (Walkley and Black, 1934) [37] |
| Available nitrogen | Alkaline permanganate method | (Subbiah and Asija, 1956) [32] |
| Available phosphorus | Bray 1 method | (Bray and Kurtz, 1945) [5] |
| Available potassium | Neutral normal NH4OAc, using Flame photometry | (Stanford and English, 1949) |

### Miniclonal leaves assessment

### Estimation of moisture content

By weighing the fresh and dried leaves, the moisture content of mulberry leaves was ascertained and expressed as a percentage (Sujathamma and Dandin, 2000) [33]. After gathering the leaves from the mulberry plant and wiping them with a muslin cloth to get rid of any dust, the fresh weight was promptly noted. The leaves were then dried in a hot air oven for 48 hours at 80°C until a constant weight was achieved; at that time, the dry weight was recorded. The following formula was used to determine the moisture content, and in order to obtain precise results, the procedure was repeated.

$$Leaf\ Moisture\ content\ (\%) = \frac{Fresh\ weight\ of\ leaves - Dry\ \ weight\ of\ leaves}{Fresh\ weight\ of\ leaves} X100$$

### Estimation of total protein

Folin-phenol reagent (Lowry *et al*., 1951) [20] was used to measure the total proteins from 0.5 g of dried leaf samples that were homogenized using 80% ethanol with a pestle and mortar. The supernatant was discarded, and the sediment was transferred to 10 ml of 10% trichloroacetic acid (TCA) for 30 minutes to precipitate proteins, and centrifuged at 5000 rpm for 10 minutes. The sediment was then washed with 5% TCA to remove interfering amino acids and phenols. The precipitate-containing sediment was dissolved in 1N NaOH by letting it sit in a hot water bath for 30 minutes, and the extracted proteins in 1N NaOH were diluted ten times with distilled water. Bovine Serum Albumin (BSA) was used as the standard to calculate the protein content of 1 ml of protein and 5 ml of alkaline copper reagent added to it. The mixture was then allowed to stand at room temperature for 10 minutes before being mixed with 0.5 ml of folin-phenol reagent. Finally, absorbance was measured in a UV visible spectrophotometer at 660 nm.

### Estimation of carbohydrate using Anthrone method (mg/g)

Carbohydrate was determined by anthrone method (Hedge and Hofreiter, 1962) [11].

$$Amount\ of\ carbohydrate = \frac{mg\ \ of\ \ glu\cos e}{Volume\ of\ \ test\ sample\ \ (100mg)} \times 100$$

### Estimation of crude protein

The amount of crude protein in the sample was calculated by multiplying the per cent of nitrogen content with factor 6.25.

**Macronutrient analysis**

**Nitrogen**

Total nitrogen was estimated by using Kjeldahl's method. In digestion 0.5 g of dried sample and 10 ml of di acid were added into test tube and kept for overnight. 10 ml of di acid extract were pipetted out and transferred into distillation flask. Then 25 ml of 2% boric acid with double indicator were kept at the delivery end of the distillation unit. 10 ml of 40% NaOH were added to the distillation flask. After the dilution, moist red litmus paper was used to measure the distillation. Absence of blue color indicated that ammonia has been distilled. Then the delivery end was detached and washed with distilled water. Furthermore, 0.02 N H2SO4 was used to titrate it.

The end color changed from greenish blue to wine red. Amount of nitrogen present was the sample is calculated using the formula,

$$N(\%) = \frac{0.00028 \times X \times 100 \times 100 \times 100}{10 \times 1 \times 100 - x}$$

x – Moisture content of the leaf

**Phosphorus**

Estimation of total phosphorus by Vanadomolybdate yellow color method. 5 ml of tri acid extract was pipetted out into 25 ml volumetric flask and 5 ml of Barton's reagent was added and make up to the volume with distilled water. After 30 minutes the color development was measured with photoelectric colorimeter at 470 nm.

**Potassium**

5 ml of triacid extract was added into 25 ml volumetric flask and the solution was neutralized with ammonium hydroxide. Distilled water was added into the volume. The amount of potassium was measured using flame photometer.

**Results and Discussion**

Results revealed that significant variations were observed in available nitrogen content with respect to different rooting media. It varied between 95.20 to 122.80 kg/ha. Among five rooting mediums M5 (soil: coir pith: FYM) recorded significantly higher nitrogen content (122.80 kg/ha) followed by M2 (116.30 kg/ha) and M1 (107.11 kg/ha), M3 (105.02 kg/ha) were on par with each other. (Table 1). Potassium is essential for several biochemical processes in mulberries, including the growth and yield of foliage, as well as the enhancement of leaf quality (Shankar *et al*., 1995) [29]. The highest amount of phosphorus content recorded in M1 (85.80 kg/ha) which means red loamy soils have more amount of phosphorus. Potassium content also high in M4 (405 kg/ha) medium followed by M5(392 kg/ha), M2 (390 kg/ha), M3 (388 kg/ha). Organic carbon content significantly varied due to different rooting media. It varied between 0.16 to 0.22 per cent. The maximum organic carbon content was registered in M5(soil: coir pith: FYM) (0.22%) followed by M3 (0.20%), M1 and M4 (0.17%) which were on par with each other and the minimum organic carbon content was registered in M2 (0.16%). This is due to presence of farm yard manure in the mixture media which has more organic carbon content. These results were similar to the findings of Ramesh kumar *et al*., (2016) [26]. And also water holding capacity percent recorded highest in soil: coir pith: FYM medium M5 (133.84%) because of presence of coir pith (Table 1). Because of the pore space in the coir pith, the bulk density of the soil decreased and its ability to hold water and maintain organic carbon levels increased. (Prakash *et al*., 2021) [24] and also Sivakumar *et al*., (2000) [30] were proved that the coir pith act as best medium for mulberry cultivation.

**Table 1:** Properties of rooting medium

| Rooting medium | Available Nitrogen (Kg/ha) | Available Phosphorus (Kg/ha) | Available Potassium (Kg/ha) | Organic Carbon (%) | Water holding capacity (%) |
|---|---|---|---|---|---|
| M1-Soil | 107.11 | 85.80 | 375 | 0.17 | 108.41 |
| M2-Soil+ Sand+ FYM | 116.30 | 50.30 | 390 | 0.16 | 112.30 |
| M3-Soil+ Coir pith | 105.02 | 79.10* | 388 | 0.20 | 119.82* |
| M4-Soil+ Sand+ Vermicompost | 95.20 | 60.40 | 405* | 0.17 | 118.70 |
| M5-Soil+ Coir pith+ FYM | 122.80* | 75.60* | 392* | 0.22* | 133.84* |
| SE(d) | 1.24 | 0.88 | 5.74 | 0.002 | 1.55 |
| CD @ 0.05% | 2.65 | 1.88 | 12.25 | 0.005 | 3.32 |

The mulberry varieties with high leaf moisture content increases the growth and development of silkworms. Higher moisture content is considered to improve the amount of intake and digestibility of silkworms because moisture acts as an olfactory and gustatory stimulant. The nutritional quality of the leaves is crucial to the rearing of silkworms (Ueda and Suzuki, 1967) [34] and the nutritional parameters has fluctuation from the date of sprouting till maturity (Angotra *et al*., (2021) [1]. Tender leaves contains most of the biochemical constituents in higher concentration followed by medium and coarse leaves (Tajamul., 2023).

According to this finding, the optimal concentration of IBA for all the features under study is 3000 ppm. The outcome aligned with Partheeban *et al*., (2022) [21], which showed that IBA concentrations of 3000 ppm increased the viability of mango cuttings and may be utilized as a rooting hormone for promoting roots and bud growth. IBA at 3000 ppm treated leaves showed 79.23% of moisture content which is on par to control leaves (80.00%). Similarly results showed NAA @ 4000 ppm treated plants recorded maximum moisture content as 77.38% (Fig. 1). This higher moisture content of leaves maybe due to growth of plants in soil: coir pith: FYM which has maximum water holding capacity (133.84%) and also presence of farm yard manure in rooting medium which plays vital role in growth of plants. These results are concomitant with Ramamoorthy *et al*., (2018) [27], Shah *et al*., (2019) [28] who recorded Vishala variety has maximum moisture percent (76.83%) compared to all other varieties.

Protein is an essential source for silkworm rearing. Jyothi *et al*., (2014) [14] found that V1 is the best one containing highest total proteins and other biochemical properties. Chawki silkworm demands for higher amount of proteins instead of late age worms. In this study significant amount of protein (27.11 mg/g) was recorded in IBA @ 3000 ppm treated leaves (Fig. 2). These

results were supported with Karthick mani Bharathi *et al.*, (2024) [3] who has done work on protein content (26.04 mg/g), moisture content (77.61%) of mini clonal leaves. Mulberry leaf carbohydrates are vital component of silkworm development, particularly in the later stages of the larval stage. In mature leaf stages carbohydrates increase with decrease of protein content. The amount of carbohydrates in mulberry leaves varies greatly depending on their surroundings.

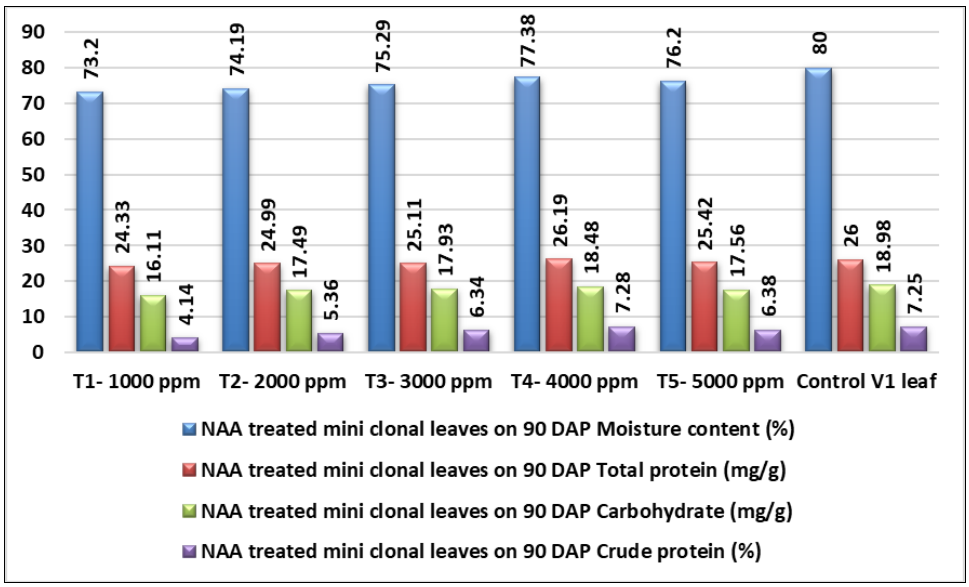


**Fig 1:** Effect of NAA on proximate composition of mini clonal mulberry leaves

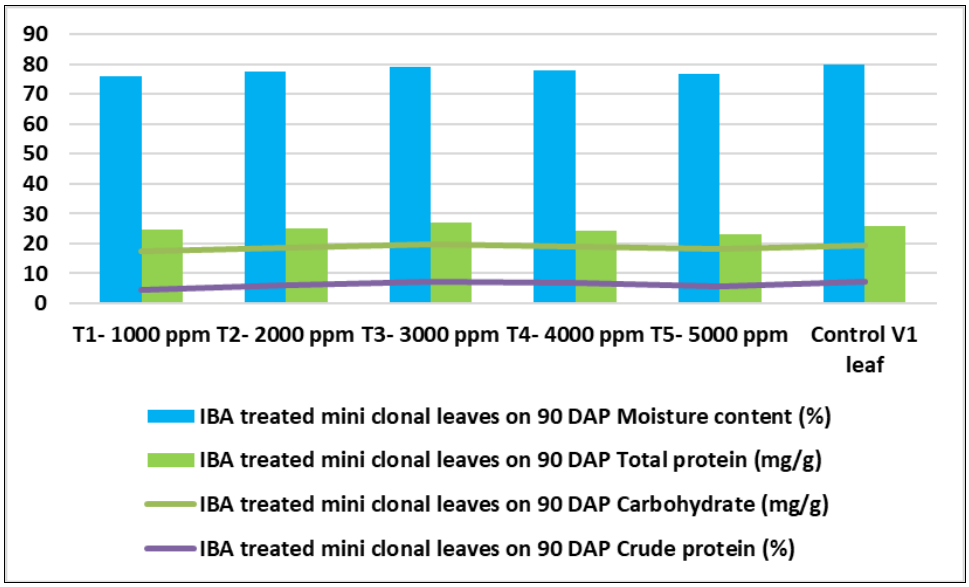


**Fig 2**: Effect of IBA on proximate composition of mini clonal mulberry leaves

Results revealed in this study carbohydrate content of IBA @ 3000 ppm treated leaves showed 19.83 mg/g (Fig. 2) which is on par with control (18.98 mg/g). Similarly, NAA @ 4000 ppm (18.48 mg/g) treated mulberry leaves were lower to control and IBA treatment. Crude protein content of IBA @ 3000 ppm (7.39%) treated leaves showed maximum compared to control (7.25%) and NAA @ 4000 ppm (7.28%). These results are supported by Umesha and sannappa (2014) [35] who worked in biochemical and mineral constituents of mulberry variety V1 with different supplementation of organic manures and Kumari raj *et al.*, (2009) [19] who were registered protein content in mulberry genotypes lies between 6.38% to 10.73%. Hadimani *et al.*, (2019) [10] recorded maximum in total soluble proteins, carbohydrates, starch and total soluble sugars in V1 followed by S-36 and lower in TG leaves.

Nitrogen content of mini clonal leaves treated with IBA @ 3000 ppm (3.93%) recorded maximum compared to control leaf (3.86%) and NAA @ 4000 ppm (3.76%). Similarly, phosphorus and potassium content of IBA @ 3000 ppm (0.34%) and NAA @ 4000 (0.32%) claimed more than control treatment (0.38% and 1.81%). Mulberry leaves NPK content rises when organic manures like FYM are added to the soil because they promote nitrogen uptake. This illustrated how the addition of organic manure encouraged the development of beneficial microorganisms in growth media that work in concern in a way. This increased NPK content of mini clonal leaves may be due to presence of increased amount of available nitrogen and organic carbon of soil: coir pith: FYM medium. This study was supported by Ram *et al.* (2017) [25] who studied the interaction effect of FYM and Potassium humate on the growth of *Morus alba* variety.

**Table 2:** Effect of IBA and NAA on major nutrients of mini clonal mulberry leaves

| | IBA on 90 DAP | | | NAA on 90 DAP | | |
|---|---|---|---|---|---|---|
| | **Nitrogen (%)** | **Phosphorus (%)** | **Potassium (%)** | **Nitrogen (%)** | **Phosphorus (%)** | **Potassium (%)** |
| $T_1$- 1000 ppm | 2.81 | 0.27 | 1.42 | 2.31 | 0.21 | 1.33 |
| $T_2$- 2000 ppm | 3.04 | 0.29 | 1.49 | 2.93 | 0.28 | 1.39 |
| $T_3$- 3000 ppm | 3.93* | 0.34 | 1.79* | 3.4 | 0.27 | 1.54 |
| $T_4$- 4000 ppm | 2.65 | 0.26 | 1.42 | 3.76* | 0.32* | 1.66* |
| $T_5$- 5000 ppm | 2.44 | 0.25 | 1.31 | 3.22 | 0.26 | 1.43 |
| Control V1 leaf | 3.86 | 0.38* | 1.81* | 3.86* | 0.38* | 1.81* |
| SE(d) | 0.26 | 0.02 | 0.09 | 0.23 | 0.02 | 0.07 |
| CD | 0.54 | 0.04 | 0.18 | 0.49 | 0.05 | 0.16 |

## Conclusion

The findings of the present study suggest that IBA @ 3000 ppm treated mini cloned mulberry leaves planted in soil: coir pith: FYM rooting medium attained maximum in moisture content (79.23%) total protein (27.11 mg/g), carbohydrate (19.83 mg/g), crude protein (7.39%), and major nutrient composition such as Nitrogen (3.93%), Phosphorus (0.34%), Potassium (1.79%) compared to mulberry nursery leaves. Nutritional quality of mulberry leaf directly impact on silk production. Silkworm requires more number of leaves with higher nutrient composition. Hence this study, investigated the essential requirement of nutrients present in mini clonal growing mulberry leaves with desired rooting hormone and rooting medium.

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