

Effect of Different Dosages of Indole-3-Butryic Acid and Naphthalene Acetic Acid Application on Growth Parameters of *Morus Alba* Mulberry

Karthick Mani Bharathi B¹, Susikaran S²*, Vasanth V¹, Rubeesh Kumar B³

¹Research Scholar, Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam.

^{2*}Assistant Professor, Directorate of Open and Distance Learning, Tamil Nadu Agricultural University, Coimbatore.
³Department of Economics, Bharathiyar University, Coimbatore.

*Corresponding author: - Dr. S. Susikaran

*Assistant Professor, Directorate of Open and Distance Learning, Tamil Nadu Agricultural University. Coimbatore -641 003, Email: susi.agri@gmail.com

Abstract

Plant growth regulators such as Indole-3-butyric acid (IBA) and α -naphthalene acetic acid (NAA) play a significant role in enhancing the rooting and vegetative growth of *Morus alba* (mulberry). This study evaluates the effects of different concentrations of IBA (1000, 2000, 3000, 4000 and 5000 ppm) and NAA (1000, 2000, 3000, 4000 and 5000 ppm) on key growth parameters, including shoot length, number of roots per plant, root length and number of leaves per plant at 90 days after planting (DAP). The experiment was conducted using a completely randomized design (CRD) with three replications, and data significance was assessed through Analysis of Variance (ANOVA). Results indicated that both IBA and NAA significantly influenced the growth attributes of *Morus alba*, with the optimal concentration being 1000–2000 ppm. IBA@1000ppm produced the highest shoot length (17.45 cm), root number (11.42 no's), number of leaves (8 no's) and root length (18.37 cm), while NAA@2000ppm exhibited the highest shoot length (16.37 cm), root number (10.50 no's), number of leaves (7.75 no's) and root length (17.50 cm). Higher concentrations (\geq 3000 ppm) resulted in growth inhibition due to possible auxin toxicity. These findings emphasize the importance of selecting appropriate auxin levels to optimize mulberry propagation. Future studies should investigate the combined effects of IBA and NAA for enhanced plant growth and productivity.

Keywords: Apical buds, Growth characters, IBA, Mini clones, Morus alba, Mulberry, NAA.

Introduction

Morus alba, commonly known as white mulberry, is a fast-growing and deciduous tree species widely cultivated for its economic, agricultural and medicinal importance (Datta *et al.*, 2000; Singh *et al.*, 2013; Kiruthika *et al.*, 2020). It plays a crucial role in the sericulture industry as the primary food source for silkworms (*Bombyx mori* L.) thereby making its propagation and growth optimization a significant area of research. Additionally, *Morus alba* has applications in agroforestry, soil conservation and traditional medicine where its leaves, bark and fruits exhibit antioxidant, antimicrobial and anti-inflammatory properties (Kalyoncu *et al.*, 2009; Hawramee *et al.*, 2019). The plant's adaptability to diverse climatic conditions and its ability to thrive in varied soil types make it an ideal candidate for large-scale cultivation (Wahab *et al.*, 2001; Sabarish *et al.*, 2017; Singh, 2018).

The successful establishment and propagation of *Morus alba* depend significantly on its ability to develop strong root and shoot systems (Vijayan *et al.*, 1997; Prakash *et al.*, 2017). However, natural rooting and vegetative propagation are often slow and inconsistent thereby necessitating the use of plant growth regulators (PGRs) to enhance plant development (Hartmann *et al.*, 1990; Galavi *et al.*, 2013; Mithilasri *et al.*, 2021). Among these regulators, auxins such as Indole-3-butyric acid (IBA) and α -naphthalene acetic acid (NAA) have been widely used to promote root initiation, elongation and overall plant vigour (Singh *et al.*, 2011; Bharathi *et al.*, 2022). These synthetic auxins mimic natural plant hormones thus regulating various physiological processes including cell division, elongation and differentiation. Their application in different concentrations has been extensively studied in various plant species but their specific impact on *Morus alba* requires further exploration (Ghatnatti *et al.*, 1997; Packialakshmi and Sudhagar, 2019).

The effect of auxins on plant growth is concentration-dependent. Low to moderate doses generally enhance root and shoot development while excessive concentrations may lead to growth inhibition due to hormonal imbalance (Parthiban *et al.*, 1999; Kumar, 2011; Sabarish *et al.*, 2017). The ability of auxins to stimulate adventitious root formation is particularly crucial for vegetative propagation, where cuttings require efficient rooting for successful establishment (Hartmann *et al.*, 2002; Singh *et al.*, 2014). IBA is known for its effectiveness in promoting root elongation and increasing the number of roots per plant whereas NAA is often used to stimulate root initiation and improve shoot growth (Ullah *et al.*, 2005; Rani *et al.*, 2018). The combined or individual application of these auxins at varying concentrations can significantly impact the growth attributes of *Morus alba* thereby influencing factors such as shoot length, root number, root length and leaf production (Kumar, 2011; Kiruthika *et al.*, 2020; Bharathi *et al.*, 2022).

The present study aims to investigate the effects of different concentrations of IBA and NAA on the growth parameters of Morus alba seedlings at 90 days after planting (DAP) (Riaz et al., 2007; Bharathi et al., 2022). The research focuses on determining the optimal auxin concentration that maximizes plant growth without inducing phytotoxicity (Singh et al., 2008; Sabarish et al., 2017). By evaluating key growth attributes including shoot length, root number, root length and leaf production, this study seeks to provide insights into the most effective PGR treatment for enhancing Morus alba propagation (Rupert, 1974; Habibi et al., 2010; Baroudi et al., 2017). The findings are expected to benefit the sericulture industry by improving mulberry plantation success rates, ultimately enhancing silk production. Additionally, optimized mulberry growth can contribute to agroforestry and environmental conservation efforts by providing a sustainable source of biomass, soil stabilization and carbon sequestration (Koyunchu and Senel, 2003; Parthiban and Seenivasan, 2017). In summary, the application of plant growth regulators such as IBA and NAA has the potential to significantly enhance the vegetative propagation of Morus alba (Ismail et al., 2007; Pallavi et al., 2018). However, determining the appropriate dosage is critical to avoid inhibitory effects and ensure optimal plant growth (Leakey et al., 1982; Husen et al., 2015). This study aims to bridge the knowledge gap by identifying the most effective concentrations of IBA and NAA for improving the shoot and root development of Morus alba. The results will contribute to the scientific understanding of auxin-mediated growth regulation and support efforts to enhance mulberry cultivation for commercial and environmental applications.

Materials and method

Experimental site and design

The experiment was conducted in a controlled environment at Clonal propagation complex, FCRI, Mettupalayam. Thus ensuring uniform conditions for optimal plant growth. A completely randomized design (CRD) was employed with three replications per treatment to minimize experimental variability. The study focused on evaluating the effects of Indole-3butyric acid (IBA) and α -naphthalene acetic acid (NAA) at different concentrations on the growth attributes of *Morus alba*.

Plant material preparation and Treatment application

Healthy and disease-free stem cuttings of *Morus alba* were selected for the study. The cuttings each measuring approximately 15 cm in length with 3–4 active buds were collected from mature and well-established mother plants. These cuttings were surface sterilized using a 0.1% HgCl₂ solution or systemic fungicide for 2–3 minutes followed by thorough rinsing with distilled water to remove any chemical residues. The stem cuttings were treated with different concentrations of IBA and NAA (Kiruthika *et al.*, 2020). The auxins were prepared in distilled water at concentrations of 1000, 2000, 3000, 4000 and 5000 ppm. The cuttings were dipped in their respective auxin solutions for 10 minutes to ensure proper absorption before being planted in the prepared soil medium. The experiment included the following treatments: IBA and NAA Treatments: 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, 5000 ppm and control (distilled water) (Bharathi *et al.*, 2022).

Soil and growth conditions

The treated cuttings were planted in polybags filled with a well-aerated mixture of soil, sand and organic compost in a 1:1:1 ratio. The soil mixture was sterilized before planting to eliminate any potential pathogens. The experiment was conducted under greenhouse conditions with controlled temperature ($25-30^{\circ}$ C), relative humidity ($60-70^{\circ}$), and light exposure (12-hour photoperiod). Regular irrigation was maintained to keep the soil moisture at optimal levels. Weeds were manually removed and plants were monitored for any signs of disease or pest infestations (Sabarish *et al.*, 2017).

Growth parameters measured

Observations were recorded at 90 days after planting (DAP) to assess the impact of IBA and NAA on various growth parameters. The following attributes were measured:

- **1.** Survival per cent (%): Proportion of plants that remain alive after a specific period following planting.
- 2. Shoot length (cm): The length of the longest shoot per plant was measured using a measuring tape.
- **3.** Number of roots per plant: The total number of roots per cutting was counted manually after carefully uprooting the plants.
- 4. Root length (cm): The length of the longest root was measured using a measuring scale.
- 5. Number of leaves per plant: The total number of leaves per cutting was counted to assess vegetative growth.

Statistical analysis

The collected data were subjected to Analysis of Variance (ANOVA) following the standard methodology of Panse and Sukhatme (1978) to determine the statistical significance of differences among treatments. The mean values were compared using the Least Significant Difference (LSD) test at a 5% probability level (P \leq 0.05). Standard error of the difference (SEd) and critical difference (CD) values were calculated to assess treatment variations. Each treatment was replicated four times. The randomized layout ensured uniform distribution of environmental factors across all treatments. The validity of the results was ensured by maintaining consistent experimental conditions and using a sufficient number of samples to minimize variability.

Results and discussion Effect of different dosages of IBA and NAA application on growth parameters of *Morus alba* Survival per cent (%)

At 1000 ppm, IBA exhibits the highest survival rate (86.31%) followed by NAA (78.25%). As the concentration increases to 2000 ppm, the survivability percentage declines slightly with IBA at 83.50% and increase slightly with NAA at 82.06%, showing a closer performance between the two treatments (Sabarish *et al.*, 2017). Highest survival found in IBA (1000 ppm) and NAA (2000 ppm) as shown in Figure 1. A further increase in concentration to 3000 ppm results in a continued decline (IBA: 81.87%, NAA: 77.43%) (Kiruthika *et al.*, 2020). The trend persists at 4000 ppm, with survivability dropping to 76.31% (IBA) and 71.50% (NAA) and at 5000 ppm, the lowest recorded values are observed (IBA: 71.93%, NAA: 67.87%) (Figure 1). The control plants, which received no treatment with auxins showed zero per cent survivability thus indicating that exogenous application of plant growth regulators is essential for successful establishment (Husen *et al.*, 2015; Mithilasri *et al.*, 2021). These results suggest that both IBA and NAA play a crucial role in enhancing the survivability of *Morus alba* cuttings with IBA being more effective than NAA at all concentrations. However, excessive concentrations (>1000 ppm) lead to a gradual decline in effectiveness, likely due to phytotoxic effects or hormonal imbalance. The optimal concentration appears to be 1000 ppm, where maximum survivability is achieved (Bharathi *et al.*, 2022). The findings highlight the importance of selecting appropriate hormone concentrations to promote successful rooting and establishment of mulberry cuttings.

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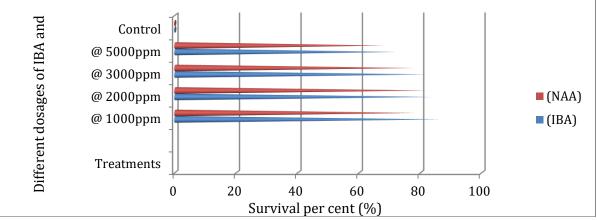


Figure 1: Effect of different dosages of IBA and NAA on survival per cent (%) of Morus alba mulberry

Shoot Length (cm)

Shoot length is an essential parameter that reflects the vegetative vigour of a plant. The highest shoot length was observed at IBA@1000ppm (17.45 cm) thus indicating that a lower concentration of IBA effectively promotes elongation (Baroudi *et al.*, 2017; Sabarish *et al.*, 2017). As the concentration increased, the shoot length decreased with the lowest recorded at IBA@4000ppm (13.42 cm) and IBA@5000ppm (13.70 cm) as shown in Table 1. The control plants which did not receive any IBA treatment showed no shoot growth thereby confirming the necessity of exogenous auxin for shoot initiation. The observed decline in shoot length at higher concentrations suggests possible auxin toxicity or hormonal imbalance which may inhibit cell division and elongation (Singh *et al.*, 2013; Packialakhsmi and Sudhagar, 2019).

The maximum shoot length was observed at NAA@2000ppm (16.37 cm) thus demonstrating that moderate auxin concentrations effectively promote shoot elongation. However, as the concentration increased beyond 2000ppm, shoot length declined with the lowest recorded at NAA@5000ppm (9.55 cm) as shown in Table 2. The control plants exhibited no shoot growth, reinforcing the necessity of exogenous auxins for shoot development (Vijayan *et al.*, 1997; Prakash *et al.*, 2017; Mithilasri *et al.*, 2021). The decreasing trend at higher concentrations may be attributed to auxin toxicity which can suppress shoot elongation by disrupting hormonal balance (Husen *et al.*, 2015; Parthiban and Seenivasan, 2017).

Table	1: Effect of different	dosages IBA	on growth	attributes	of Morus	alba mulberry	

Treatments	On 90DAP						
(IBA)	Shoot length (cm)	No of roots/plant (no's)	Root length (cm)	No of leaves/plant (no's)			
IBA@1000ppm	17.45 (4.18)	11.42 (3.38)	18.37 (4.29)	8.0 (2.83)			
IBA@ 2000ppm	15.35 (3.92)	9.37 (3.06)	16.42 (4.05)	7.0 (2.65)			
IBA@3000ppm	14.37 (3.80)	8.50 (2.92)	15.20 (3.90)	7.0 (2.65)			
IBA@ 4000ppm	13.42 (3.66)	6.45 (2.54)	15.60 (3.95)	6.75 (2.60)			
IBA@ 5000ppm	13.70 (3.70)	5.50 (2.35)	13.37 (3.66)	6.50 (2.55)			
Control	0(0)	0(0)	0(0)	0(0)			
Mean	12.38	6.87	13.16	5.88			
SEd	2.55	1.62	2.71	1.19			
CD (.05%)	5.35**	3.40**	5.70**	2.51**			

Significant @ *P*=0.05 level. Each value is the mean of four replications

Values in brackets are square root transformed values

Number of roots per plant (no's)

Root proliferation is a critical factor for plant establishment and nutrient uptake. The maximum number of roots per plant was observed at IBA@1000ppm (11.42 roots per plant) (Table 1) followed by a gradual decrease with increasing IBA concentrations. At IBA@5000ppm, the number of roots per plant dropped significantly to 5.50 no's (Habibi *et al.*, 2010; Galavi *et al.*, 2013). This pattern suggests that lower concentrations of IBA enhance root induction by promoting cell division and elongation while higher concentrations may exert inhibitory effects due to excessive auxin accumulation thereby leading to hormonal imbalance and potential root suppression (Ghatnatti *et al.*, 1997; Riaz *et al.*, 2007; Singh *et al.*, 2014).

The highest number of roots per plant was observed at NAA@2000ppm (10.50 roots), followed by NAA@1000ppm (9.35 no's). A steady decline was noted as NAA concentration increased beyond @2000ppm with the lowest number at NAA@5000ppm (5.35 no's) (Table 2) (Hartmann *et al.*, 2002; Sabarish *et al.*, 2017). This trend suggests that while lower concentrations of NAA enhance root proliferation by stimulating cell division and elongation, excessive auxin levels may exert inhibitory effects thereby potentially disrupting natural root development and causing hormonal imbalances (Parthiban *et al.*, 1999; Bharathi *et al.*, 2022).

Root Length (cm)

Root length is an essential indicator of root system development thus affecting water and nutrient absorption. The longest root length was recorded at IBA@1000ppm (18.37 cm) while the shortest was at IBA@5000ppm (13.37 cm) as shown in Table 1 (Singh *et al.*, 2011; Rani *et al.*, 2018; Mithilasri *et al.*, 2021). A decline in root length with increasing IBA concentrations suggests that while moderate auxin levels promote root elongation, excessive IBA can interfere with normal root development. Root elongation is crucial for plant stability and resource uptake with the observed trend confirms that optimal auxin concentration is necessary for balanced growth (Kalyoncu *et al.*, 2009; Bharathi *et al.*, 2022). The longest root length was observed at NAA@2000ppm (17.50 cm) while the shortest was recorded at NAA@5000ppm (9.65 cm) as shown in Table 2 (Ismail *et al.*, 2007; Hawramee *et al.*, 2019). Similar to the trends observed in shoot length and root number, root length followed a bell-shaped response to increasing NAA concentrations. Moderate doses (1000–2000 ppm) promoted root elongation while higher concentrations likely induced auxin toxicity thereby limiting cell expansion and elongation (Kumar, 2011; Pallavi *et al.*, 2018).

	On 90DAP					
Treatments	Shoot length	No of roots/plant	Root length	No of leaves/plant		
(NAA)	(cm)	(no's)	(cm)	(no's)		
NAA@ 1000ppm	14.05 (3.75)	9.35 (3.06)	15.27 (3.91)	7.25 (2.69)		
NAA@ 2000ppm	16.37 (4.05)	10.50 (3.24)	17.50 (4.18)	7.75 (2.78)		
NAA@ 3000ppm	13.25 (3.64)	8.45 (2.91)	14.50 (3.81)	6.25 (2.50)		
NAA@ 4000ppm	11.20 (3.35)	5.50 (2.35)	12.47 (3.53)	6.50 (2.55)		
NAA@ 5000ppm	9.55 (3.09)	5.35 (2.31)	9.65 (3.11)	5.75 (2.40)		
Control	0 (0)	0 (0)	0 (0)	0 (0)		
Mean	10.07	5.96	10.82	5.25		
SEd	2.52	1.62	2.73	1.24		
CD (.05%)	5.30**	3.40**	5.74**	2.59**		

Table 2: Effect of different dosages NAA on growth attributes of *Morus alba* mulberry

Significant @ *P*=0.05 level. Each value is the mean of four replications Values in brackets are square root transformed values

Number of leaves per plant (no's)

Leaf development is directly linked to photosynthetic capacity and overall plant biomass. The highest number of leaves per plant was observed at IBA@1000ppm (8 leaves per plant) with a gradual decrease as IBA concentration increased. At IBA@5000ppm, only 6.50 leaves per plant were recorded (Table 1) (Singh *et al.*, 2018; Mithilasri *et al.*, 2021; Bharathi *et al.*, 2022). This decline may be attributed to the reduced shoot growth and root development at higher auxin levels which may indirectly limit the plant's ability to generate new leaves (Wahab *et al.*, 2001; Sabarish *et al.*, 2017; Hawramee *et al.*, 2019).

The highest number of leaves per plant was found at NAA@2000ppm (7.75 leaves), followed by NAA@1000ppm (7.25 leaves). A decreasing trend was noted as NAA concentration increased with the lowest number of leaves at NAA@5000ppm (5.75 leaves) (Table 2) (Parthiban *et al.*, 1999; Bharathi *et al.*, 2020). This decline at higher concentrations may result from reduced shoot growth and root development thus affecting overall plant health and limiting leaf production (Husen *et al.*, 2015; Parthiban and Seenivasan, 2017). The experiment clearly denotes that the effects are highly significant at P=0.05 thus confirming that the variation in growth parameters is not due to random chance but a direct consequence of IBA and NAA application.

Conclusion

The application of different dosages of Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) significantly influenced the growth parameters of Morus alba (mulberry). Findings from this study indicate that auxin supplementation enhances root and shoot development thus contributing to improved overall plant vigour. Among the tested concentrations, optimal dosages of IBA (2000 ppm) and NAA (2000 ppm) resulted in maximum shoot length, leaf number, root length and biomass accumulation thereby suggesting their potential role in promoting vegetative propagation and establishment of healthy plants. Higher concentrations of IBA were particularly effective in enhancing root initiation and elongation while NAA contributed to shoot proliferation and leaf expansion. However, excessive auxin application demonstrated inhibitory effects thus leading to growth suppression and morphological abnormalities thereby indicating the importance of precise dosage determination. The combined application of IBA and NAA at moderate levels exhibited synergistic effects thereby promoting balanced root and shoot growth which is critical for the successful propagation of mulberry. These results underline the significance of auxin-based growth regulation in commercial mulberry cultivation particularly in sericulture, mulberry for paper cultivation and afforestation programs. Future studies should focus on long-term effects, interactions with other phytohormones and field-level validations to optimize application protocols. Additionally, molecular studies exploring gene expression changes in response to auxin treatments could provide further insights into the mechanisms governing mulberry growth regulation. Overall, the findings confirm that strategic use of IBA and NAA enhances the propagation potential of Morus alba thus contributing to sustainable mulberry production and improved agricultural practices.

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