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Effect of Indole-3-Butyric Acid and A-Naphthaleneacetic Acid on Rooting of Bottle Brush [*Callistemon lanceolatus* (Sm.) Sweet] Cuttings

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ABSTRACT

Background: *Callistemon lanceolatus* (bottle brush) is a popular woody ornamental tree valued for its bright inflorescences and adaptability to urban landscapes. However, propagation through cuttings is limited by poor rooting and delayed establishment. This study evaluated the effect of cutting type and auxin concentration on root and shoot development in *C. lanceolatus* to enhance clonal propagation efficiency.

Methods: The experiment was conducted (April 2024 - May 2025) at VISTAS, Chennai, using a factorial completely randomized design (FCRD). Hardwood, semi-hardwood and cuttings formed in the previous season (CFPS) were treated with IBA (1000, 1500, 2000 mg L^{-1}) and NAA (500, 1000, 1500 mg L^{-1}), along with a control. Cuttings were planted in sterilized sand under mist chamber conditions (28-32°C; 80-90% RH). Data on rooting percentage, number of roots, root length and shoot growth were analysed statistically.

Result: Significant variations were observed among treatments. CFPS treated with 1000 mg L^{-1} IBA showed the highest rooting (88.5%), maximum roots (15.6), root length (23.9 cm) and shoot length (20.8 cm), outperforming all other combinations. IBA proved more effective than NAA and moderate concentrations were superior to higher levels. The study concludes that CFPS with 1000 mg L^{-1} IBA provides optimum rooting and sprouting, offering a simple and reliable method for vegetative propagation of *Callistemon lanceolatus*.

Key words: Auxin, *Callistemon lanceolatus,* Clonal propagation, IBA, Rooting efficiency.

INTRODUCTION

Ornamental plants, particularly flowering trees, play a vital role in enhancing urban landscapes by offering both aesthetic value and ecological benefits (Anahita *et al.,* 2023). Their contribution to the visual appeal and structural interest of gardens and parks is well established (Wallace *et al.,* 2014). Despite their importance, the majority of ornamental tree species are commercially propagated through seeds, a method that often results in prolonged juvenile phases, excessive vegetative growth and delayed flowering factors that ultimately reduce reproductive efficiency and commercial viability (De, 2017). Vegetative propagation has emerged as a preferred alternative, as it shortens the juvenile period and accelerates the onset of flowering, thereby improving overall propagation efficiency (Liu *et al.,* 2022). *Callistemon lanceolatus* (Sm.) Sweet (commonly known as bottlebrush), a visually striking member of the Myrtaceae family, is extensively cultivated in tropical and subtropical regions of Asia for its ornamental attributes, including pendulous branches and vibrant cylindrical flower spikes (Sowndhararajan *et al.,* 2021). Although propagation by seeds introduces significant genetic variability, making it unsuitable for maintaining true-to-type plants in commercial production (Hartmann *et al.,* 2002). Moreover, the species exhibits inherently moderate rooting potential in stem cuttings, which poses a major challenge for effective mass multiplication through vegetative propagation (Kanimozhi *et al*., 2025). It has been demonstrated that the limiting factors for the rooting of cuttings in tree species are the age, physiological status of the stock plants (*i.e.* the season of cutting collection) and the genotype peculiarity of the donor plant (Parthiban *et al*., 2026; Hartmann *et al.,* 2002). Environmental conditions

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during rooting also exert a major effect on the rooting (Hartmann *et al.,* 2002). Successful rooting in such species generally needs the application of specific auxin treatments and precise control of environmental conditions (Vielba *et al.,* 2020). Auxins have been widely recognized for their role in enhancing root initiation and development across various ornamental species (Sosnowski *et al.,* 2023). Although stem cutting propagation is a commonly employed method, its effectiveness is often limited by the low regenerative capacity observed in many ornamental taxa (Sanz *et al.,* 2025; Kumaresan *et al.,* 2023). The success of cutting-based propagation is influenced by multiple factors, including cutting type, internal physiological state and external environmental conditions (Bannoud and Bellini 2021; Patalasingh *et al.,* 2024). Inadequate rooting frequently leads to propagation failures and economic losses (Kentelky *et al.,* 2021, Feyisa 2023). Auxins, play a pivotal role in promoting radicle development and accelerating adventitious root formation (Kashyap *et al.,* 2021, Monder *et al.,* 2021). Indole-3-butyric acid (IBA) and á-naphthaleneacetic acid (NAA) have been shown to significantly improve rooting frequency, enhance the development of root primordia and promote uniform root distribution (Sourati *et al.,* 2022, Sekhukhune and Maila 2024). They act by enhancing endogenous auxin activity, which is critical for regulating root development and other physiological processes (Hassan *et al.,* 2024). The application of exogenous auxins has demonstrated considerable success in improving vegetative propagation outcomes in various commercially important ornamental species (Kumaresan *et al.,* 2026). In addition to root development, auxins play essential roles throughout the plant life cycle, including embryogenesis, organogenesis and reproductive development (Hu *et al.,* 2021). Their capacity to stimulate rooting in difficult-to-propagate species has been well documented (Gao *et al.,* 2024; Muhamed *et al.,* 2024), with NAA showing particular efficacy in enhancing root formation and plant establishment in soil (Zishan *et al.,* 2024). Due to the inherently low rooting potential of *Callistemon lanceolatus* stem cuttings, the application of auxins offers a promising approach to enhance rooting efficiency and support large-scale vegetative propagation of this economically important ornamental species. The aim of this study is to evaluate the effects of different auxins, their concentrations and type of cuttings on the rooting efficiency of *Callistemon lanceolatus*.

MATERIALS AND METHODS

The research was carried out at the Department of Horticulture, School of Agriculture, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India (12^{o}582 N, 80^{o}092 E; elevation 26/ m AMSL), from April 2024 to May 2025. The study area has a tropical climate with average annual temperatures ranging from 25^{o}C to 31^{o}C, with summer peaks reaching up to 38^{o}C. The region receives an average annual rainfall of 1,150/ mm and the relative humidity varies between 80% - 90%. Cuttings were collected from 15-year-old healthy, disease-free donor plants. Five uniform mother plants were selected to minimize genetic and physiological variability. All donor plants were maintained under similar cultural conditions and cuttings were collected from non-flowering, well-exposed shoots during early morning hours to ensure physiological uniformity and minimize moisture stress. The cuttings were collected in May, during the early rainy season. The hardwood cuttings were obtained from fully lignified shoots of the previous season. Semi-hardwood cuttings are collected from the current season’s growth, once the shoots are partially lignified. The cuttings formed in previous season (CFPS), collected from the terminal growth. Cuttings were collected and prepared to a uniform size of 12 cm in length, each containing 3 - 4 nodes. All type of cuttings were prepared by making a transverse cut at the top and a slanting cut at the base. Prior to auxin treatment, the cuttings were dipped in a 2% copper oxychloride solution for 2 - 3 min to prevent fungal infection. The study followed a factorial completely randomized design (FCRD) and each treatment were use 10 cuttings with three replications. The cuttings were treated with IBA at 1000, 1500 and 2000 mg L^{-1} and NAA at 500, 1000 and

A) General view of the planted cuttings. B) Rooted cuttings after treatment with 1000 mg L^{-1} IBA.

Fig 1: A) General view of the planted cuttings and B) rooted cuttings.

1500 mg L^{-1}, along with a control treatment without auxin application. These concentrations were selected based on previously reported effective ranges for woody plant species. The auxin was first dissolved in a small amount (50%) of alcohol, then diluted with distilled water to the desired concentrations. The basal ends of the cuttings were then immersed in their respective auxin treatments, air-dried for 10 minutes and subsequently planted vertically in polyethylene bags (15 cm × 10 cm) filled with sterilized river sand. These bags were placed in a mist chamber maintained at 28-32°C with 80 - 90% relative air humidity to promote rooting. Moisture levels, fungal infection and overall plant health were monitored (Fig 1A).

Data collected included percentage of rooted cuttings, number of roots, root length (cm), shoot length (cm) and number of leaves. The observations on the rooting were recorded at 30, 60 and 90 days after planting. Measurements were taken from 15 randomly selected cuttings per treatment. The recorded data were analysed using two-way analysis of variance (ANOVA) as per the method described by Steel *et al.* (1997). The significance of treatment effects was tested using the F-test and mean comparisons were performed using Tukey's Honest Significant Difference (HSD) test at the 5% probability level. All statistical analyses were conducted using SAS version 9.0 software.

RESULTS AND DISCUSSION

Effect of auxins and cutting types on rooting percentage

Rooting percentage varied significantly among cutting types and auxin concentrations (Table 1). The highest rooting (88.5%) was recorded in cuttings formed in the previous season (CFPS) treated with 1000 mg L^{-1} IBA, whereas the control recorded the lowest (51.8%). Similar findings were reported by Prakash *et al.* (2018) in *Psidium guajava*, where

Table 1: Effect of cutting type and auxins on rooted cuttings and days taken for rooting of *Callistemon lanuolatus*.

Treatments	Rooted cuttings (%)			Days taken for rooting		
	Hardwood cutting	Semi-hardwood cutting	CFPS	Hardwood cutting	Semi-hardwood cutting	CFPS
Control	11.5±1.0e	20.0±0.9d	51.8±0.6e	32.0±0.3a	32.0±0.4a	32.2±0.6a
1000 mg l^{-1} IBA	65.0±0.9a	67.1±0.9a	88.5±0.7a	31.0±0.5a	29.0±0.9ab	28.6±0.7ab
1500 mg l^{-1} IBA	57.6±0.8ab	64.0±0.8a	82.1±0.8b	30.0±0.8a	29.4±0.9ab	30.0±0.7a
2000 mg l^{-1} IBA	32.0±0.7c	42.0±1.0b	80.5±0.5b	29.5±0.9ab	29.0±0.7ab	31.5±0.7a
500 mg l^{-1} NAA	21.2±0.5d	24.6±0.9d	65.7±0.9d	31.0±0.4a	31.7±0.7a	29.4±0.6ab
1000 mg l^{-1} NAA	24.6±0.7d	32.3±0.7c	70.6±0.7c	31.0±0.4a	31.5±0.7a	29.0±0.7ab
1500 mg l^{-1} NAA	30.8±0.6c	35.2±0.5c	77.0±0.5c	30.0±0.6a	30.0±0.6a	31.0±0.8a
Cutting (C)	1.0		0.7	0.3		0.9
Growth regulator (T)	0.9		1.1	0.6		1.2
Interaction (C × T)	1.1		2.0	0.9		2.0

Means±standard deviation within a column followed by the same letter are not significantly different according Tukey's multiple range test at p≤0.05.

Legend: CFPS - Cuttings formed in the previous season.

Table 2: Effect of cutting type and auxins on number and length of roots in the *Callistemon lanuolatus*.

Treatments	Number of roots			Length of root (cm)		
	Hardwood cutting	Semi-hardwood cutting	CFPS	Hardwood cutting	Semi-hardwood cutting	CFPS
Control	5.4±0.4d	6.5±0.7c	8.6±0.5d	8.0±0.4e	9.7±0.5e	11.0±0.5d
1000 mg l^{-1} IBA	11.2±1.3ab	14.0±1.2a	15.6±1.3a	18.7±0.8ab	19.2±0.8ab	23.9±0.8a
1500 mg l^{-1} IBA	11.5±0.8ab	13.5±1.0ab	14.5±1.0a	19.0±1.0ab	21.5±0.8a	20.8±0.9ab
2000 mg l^{-1} IBA	12.0±0.9a	13.1±1.0ab	14.0±0.9b	20.5±1.2a	20.0±1.2ab	20.0±0.9ab
500 mg l^{-1} NAA	10.3±0.7c	12.0±0.9b	12.3±0.4c	13.5±0.6d	12.5±0.6d	19.0±0.5c
1000 mg l^{-1} NAA	10.9±0.7b	13.0±0.8ab	13.0±0.8b	17.7±0.6c	15.4±0.7c	20.1±0.7ab
1500 mg l^{-1} NAA	10.5±0.6b	12.4±0.6b	12.8±0.5b	15.8±0.5c	14.0±0.6c	19.7±0.7ab
Cutting (C)	0.5		1.0	0.5		1.4
Growth regulator (T)	0.8		1.7	1.3		1.2
Interaction (C × T)	1.5		3.1	1.8		1.9

Means ± standard deviation within a column followed by the same letter are not significantly different according Tukey's multiple range test at p≤0.05.

Legend: CFPS – Cuttings formed in the previous season.

softwood cuttings exhibited superior rooting due to higher meristematic activity, carbohydrate reserves and endogenous auxin levels (Kaur and Singh, 2022).

The superiority of CFPS over hardwood and semi-hardwood cuttings in the present study can be attributed to their physiologically active tissues and greater moisture retention, facilitating better hormonal absorption and cell differentiation. IBA at 1000 mg L^{-1} proved more effective than NAA, possibly due to its higher stability and slower degradation in plant tissues (Sekhukhune *et al.,* 2024). Goa *et al.* (2024) also observed that moderate IBA concentrations enhance plantlet establishment and root vigour in ornamental species.

Rooting time, root number and root length

Rooting initiated earliest (after 28.6 days) in CFPS treated with 1000 mg L^{-1} IBA. These cuttings also produced the maximum number of roots (15.6) and longest root length (23.9 cm), while untreated controls had only 8.6 roots with 11.0 cm length (Table 2; Fig 1B). The improvement in rooting attributes under IBA treatment may be due to its role in promoting cell division, nutrient mobilization and formation of root primordia (Roychoudhry *et al.,* 2023; Emile *et al.,* 2019). IBA likely enhances accumulation of soluble sugars, proteins and phenolics at the basal region, which are crucial for root initiation and elongation (Razvi *et al.,* 2015; Sourati *et al.,* 2022).

Although NAA treatments (1000 and 1500 mg L^{-1}) also improved rooting over the control (up to 77.0%), their effects were comparatively lower than IBA. This may be due to differences in tissue sensitivity and auxin transport efficiency (Chang *et al*., 2023; Abu-Zahra *et al*., 2026).

The superior response of CFPS to IBA may stem from higher metabolic activity and cambial regeneration capacity, leading to quicker vascular connection formation. These findings are in agreement with Sekhukhune *et al.* (2024) in *Actinidia deliciosa*, who demonstrated that IBA facilitates auxin accumulation in rooting zones, improving vascular connections. In contrast, the relatively weaker response of NAA-treated cuttings could be attributed to its rapid

Table 3: Effect of cutting type and auxins on number and length of shoot in the *Callistemon lanuolatus.*

Treatments	Number of shoots			Length of shoot (cm)		
	Hardwood cutting	Semi-hardwood cutting	CFPS	Hardwood cutting	Semi-hardwood cutting	CFPS
Control	1.0±0.7d	2.0±0.4a	2.2±0.5a	5.0±0.9d	7.2±0.9c	8.8±0.7d
1000 mg l^{-1} IBA	3.7±0.6ab	4.1±0.4a	5.4±0.9a	14.5±0.9a	17.5±0.9a	20.8±1.1a
1500 mg l^{-1} IBA	3.3±0.8ab	4.0±0.2a	4.3±0.9a	13.2±1.0ab	15.6±1.1ab	16.0±1.4ab
2000 mg l^{-l} IBA	3.2±0.5a	3.8±0.4a	4.0±0.8a	12.3±1.3ab	13.7±1.2b	14.0±1.3b
500 mg l^{-1} NAA	2.0±0.8c	2.0±0.2a	2.3±0.6a	11.9±0.9b	13.6±0.9b	12.0±0.8c
1000 mg l^{-1} NAA	3.0±0.9b	2.4±0.2a	3.0±0.5a	11.3±0.6c	13.2±0.7b	12.5±0.7c
1500 mg l^{-1} NAA	3.0±0.4b	3.0±0.6a	3.5±0.7a	12.0±0.8b	13.5±0.8b	13.5±0.9b
Cutting (C)	0.06		0.1	0.5		1.0
Growth regulator (T)	NS		NS	0.8		1.7
Interaction (C × T)	NS		NS	1.5		3.1

Means ± standard deviation within a column followed by the same letter are not significantly different according Tukey's multiple range test at $p \leq 0.05$.

Legend: CFPS – Cuttings formed in the previous season.

Table 4: Effect of cutting type and auxins on number of leaves on cuttings of *Callistemon lanuolatus*.

Treatments	Hardwood cutting	Semi-hardwood cutting	CFPS
Control	10.0±0.9c	13.0±0.6c	15.9±0.7d
1000 mg l^{-1} IBA	20.0±1.3a	30.2±1.3a	35.7±1.3a
1500 mg l^{-1} IBA	17.5±1.1ab	25.0±1.2ab	32.0±1.2a
2000 mg l^{-1} IBA	15.0±1.1ab	22.3±1.2ab	29.0±1.0b
500 mg l^{-1} NAA	10.9±0.9c	20.0±0.9b	20.5±0.6c
1000 mg l^{-1} NAA	12.0±0.8b	22.4±0.7ab	25.3±0.8b
1500 mg l^{-1} NAA	13.0±0.9ab	25.0±0.9ab	28.6±0.9b
Cutting (C)	1.3		1.5
Growth regulator (T)	1.4		1.7
Interaction (C × T)	1.2		1.3

Means ± standard deviation within a column followed by the same letter are not significantly different according Tukey's multiple range test at $p \leq 0.05$.

Legend: CFPS – Cuttings formed in the previous season.

conjugation and reduced physiological stability. Such variation among auxin types has also been documented in *Platycladus orientalis* (Chang *et al.,* 2023) and *Guava* (Prakash *et al.,* 2018).

Effect of auxins on shoot growth parameters

IBA treatments not only improved rooting but also enhanced shoot number, shoot length and leaf production (Tables 3 and 4). The CFPS treated with 1000 mg L^{-1} IBA recorded the highest number of shoots (5.4), longest shoots (20.8 cm) and maximum leaves (35.7), representing a substantial increase over the control (2.2 shoots, 8.8 cm length and 15.9 leaves). NAA also promoted shoot initiation, though less effectively, with the best performance at 1500 mg L^{-1} (3.5 shoots and 13.5 cm shoot length).

Enhanced shoot proliferation under IBA treatment may be attributed to its stimulatory effect on cell elongation, vascular differentiation and enzymatic activities such as peroxidase and polyphenol oxidase, which are vital for shoot growth (Karim and Muhammad, 2020; Hassan and Bernard, 2024). The higher number of leaves in CFPS + IBA treatment indicates improved root–shoot signaling and nutrient absorption. Kleynhans *et al.* (2017) reported similar trends, attributing enhanced leaf formation to increased root vigor and water uptake.

CONCLUSION

The study demonstrated that both auxin type and cutting maturity significantly influenced rooting and sprouting in *Callistemon lanceolatus*. Cuttings formed in the previous season (CFPS) possess higher physiological maturity, which favors enhanced callus formation and subsequent adventitious root initiation due to better tissue differentiation, accumulated carbohydrate reserves and balanced endogenous hormone levels. Treated with 1000 mg L^{-1} IBA showed the highest rooting percentage, root length, shoot growth and leaf production. This indicates that physiologically younger cuttings combined with an optimal IBA concentration enhance propagation success. IBA proved more effective than NAA in stimulating root initiation and shoot development. The findings demonstrate the effectiveness of IBA-treated cuttings formed in the previous season (CFPS) in enhancing the vegetative propagation response of Callistemon lanceolatus.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

No animals were used in this research study.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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