



RESEARCH ARTICLE

Optimisation of auxin concentration and exposure duration for enhanced adventitious rooting and survival in *Nerium oleander* L. stem cuttings

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Abstract

Nerium oleander L. is an important ornamental and medicinal shrub widely propagated through stem cuttings; however, successful rooting and survival are often limited by endogenous physiological constraints. Optimisation of auxin concentration and exposure duration is therefore critical for enhancing adventitious root formation and establishment. The present study evaluated the effects of different concentrations and dipping durations of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) on sprouting, rooting, root quality and survival of semi-hardwood stem cuttings of *N. oleander*. The experiment was conducted under controlled polyhouse conditions using a completely randomised design with 15 treatments, including IBA and NAA at 60, 80 and 100 ppm for 12 and 24 hr, along with water-soaked and untreated controls. Observations on days to sprouting, days to rooting, rooting percentage, root length, root number, root diameter and survival percentage were recorded and statistically analysed. Auxin-treated cuttings significantly outperformed controls for all parameters studied. Among the treatments, IBA at 100 ppm for 24 hr resulted in the earliest sprouting (22.1 days) and rooting (26.9 days), highest rooting percentage (98.2 %), superior root length (18.6 cm) and maximum survival (93.7 %). Although NAA treatments enhanced root number, they were comparatively less effective in producing a vigorous and stable root system. The results demonstrate that both auxin concentration and exposure duration play a decisive role in adventitious rooting. Based on overall performance, IBA at 100 ppm for 24 hr is recommended as an optimal protocol for efficient and commercial propagation of *N. oleander*.

Keywords: adventitious rooting; auxins; indole-3-butyric acid (IBA); naphthaleneacetic acid (NAA); *Nerium oleander*; propagation efficiency; root morphology; stem cuttings

Introduction

Nerium oleander L. (Apocynaceae) is an ornamental shrub often grown throughout India. It is reported to have originated from the Mediterranean and has aesthetic and certain medicinal properties (1). The major plants in *Nerium* genus include *N. oleander* and *N. indicum* (2). The plant has multiple names in different Indian languages including Sanskrit name as Karavirah and Hindi name as Kaner, suggesting its cultural importance (1).

It's a wide-spreading evergreen shrub which has large glossy green leaves that emerge in threes. The flowers are in pink, rose and white colors and have both ornamental and fragrance values that they offer for landscaping and bank beautification (1). Furthermore, the shrub's long bloom time provides for a year-round show, adding to its attractiveness in both ornamental and utilitarian applications. In addition to its decorative purpose, *N.oleander* is described for its poisonous and potential medicinal properties, as well as several parts of the plant possess pharmacological characteristics; although caution is

recommended because it is a toxic agent (2).

Although all parts of the plant are toxic, they also contain compounds like oleandrin that have been investigated because of their potential treatments on selected diseases (1). Roots and leaves of the plant have been used in folk medicine to tackle several health issues such as respiratory or skin infections, thus evidencing the dual employment of this plant as ornamental and medicinal (2). In the process of landscaping and traditional medicine, *N. oleander* has a variety of practical functions.

The most employed propagation method of *N. oleander* in India is through cuttings because seed size, seed setting and germination of seeds are very less and found to be successful through cuttings (2). Nevertheless, percentage of rooted cuttings could be increased with the use of rooting hormones. It has been reported that auxins such as Indole-3-butyric acid (IBA) and Naphthaleneacetic acid (NAA) are critical regulators for inducing adventitious rooting of palm seedlings in various studies (3, 4). Results have shown roots and rooting percentage of the cuttings treated with different concentrations of IBA and NAA were significantly higher than in untreated cutting, which is supposed to survive better after transplantation (5, 6).

The number of nodes present on cuttings, in combination with suitable treatment with an auxin are associated with improved rootability in earlier studies (2). In addition, it has been reported that high levels of IBA and NAA can increase rooting yet retard growth of initial shoots, indicating that the dosage should be carefully controlled (7). This finding suggests that it may become important in the future to investigate the ideal concentrations for these PGRs, given effect on root-length, cuttings (4).

To develop commercial applications of *N. oleander*, additional studies on the precise concentrations and timing usages of these growth regulators for better propagation techniques are promising (2, 3). Thus, this will investigate how different concentrations of IBA and NAA influence the rooting of *N. oleander* stem cuttings, valuable findings that can aid in guiding future horticultural practices.

Materials and Methods

This experiment was conducted at the Division of Floriculture and Landscape Architecture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar Campus, Srinagar, India. All procedures were carried out inside a controlled plant propagation polyhouse. The planting material of *N. oleander* was collected from the university field, where healthy, uniform semi-hardwood shoots were selected. Cuttings of approximately 15 cm length and pencil thickness were prepared early in the morning, tied into bundles and immediately transported to the propagation unit for treatment.

Since IBA and NAA are poorly soluble in water, stock solutions of both hormones were prepared using ethyl alcohol. For each hormone, 1 g of IBA and 1 g of NAA were separately dissolved in 50 mL of ethyl alcohol and distilled water was added to each solution to make the final volume 1 L, resulting in 1000 ppm stock solutions. Working solutions of 60, 80 and 100 ppm were obtained by serial dilution of the stock with distilled water; for example, 60 mL of stock solution was diluted to a final volume of 1 L to prepare a 60 ppm solution and similar procedures were followed for the 80 and 100

ppm concentrations for both hormones. The cuttings were grouped into bundles and the basal 2–3 cm portion was dipped in the respective solutions as per treatment requirements. Hormonal treatments consisted of 3 concentrations (60, 80 and 100 ppm) and 2 dipping durations (12 and 24 hr) for both IBA and NAA, resulting in 12 hormone treatments. Three additional controls were included: water dip for 12 hr, water dip for 24 hr and an untreated control. After completion of the respective dipping durations, the treated and control cuttings were planted in propagation beds filled with 2:1 ratio riversand and vermicompost, with irrigation applied whenever required to maintain adequate moisture.

The experiment comprised 15 treatments (Table 1) laid out in a CRD with 3 replications. The treatments included IBA 60, 80 and 100 ppm for 12 and 24 hr; NAA 60, 80 and 100 ppm for 12 and 24 hr; a 12 hr water dip; a 24 hr water dip; and an untreated control. Observations were recorded throughout the rooting and establishment phases. Day wise monitoring was conducted to determine the time required for sprouting and the number of days from planting to the stage when at least 50 % of the cuttings showed visible sprout emergence was recorded. Root initiation was monitored on 1–2 sprouted cuttings per treatment and since root emergence typically occurred 3–4 days after sprouting, the days required for rooting were calculated from planting to the first visible appearance of roots. As all sprouted cuttings successfully produced roots, the rooting percentage for each treatment was calculated

Table 1. Details of treatments used in the experiment

Treatment	Description
T1	IBA 60 ppm – 12 hr
T2	IBA 80 ppm – 12 hr
T3	IBA 100 ppm – 12 hr
T4	IBA 60 ppm – 24 hr
T5	IBA 80 ppm – 24 hr
T6	IBA 100 ppm – 24 hr
T7	NAA 60 ppm – 12 hr
T8	NAA 80 ppm – 12 hr
T9	NAA 100 ppm – 12 hr
T10	NAA 60 ppm – 24 hr
T11	NAA 80 ppm – 24 hr
T12	NAA 100 ppm – 24 hr
T13	Water dip – 12 hr
T14	Water dip – 24 hr
T15	Untreated control

based on the number of sprouted cuttings relative to the total cuttings planted.

To assess root development, 3 cuttings per treatment were carefully uprooted after 3 months. Root length was measured by selecting 5 representative roots per cutting 2 longest, 2 medium-sized and one shortest and the mean root length was calculated. The total number of roots per cutting was counted for all 3 sampled cuttings and averaged to determine the root number for each treatment. Root size was assessed by measuring root diameter at 5 different points on each sample using a digital Vernier calliper and the mean diameter was used for statistical analysis. Survival percentage was recorded 90 days after planting by counting the number of rooted cuttings that remained alive and survival was expressed as a percentage of the cuttings that initially produced roots.

Results and Discussion

The results revealed a significant influence of plant growth regulator

Table 2. Effect of indole-3-butyric acid and naphthaleneacetic acid treatments on sprouting, rooting and survival parameters of *Nerium* cuttings

Trt No.	Treatment	Days for sprouting	Days for rooting	Rooting (%)	Root length (cm)	Root number	Root size (mm)	Survival %
1	IBA 60 ppm for 12 hr	24.8	27.9	93.8	16.7	43.5	0.89	92.8
2	IBA 80 ppm for 12 hr	23.2	26.1	92.4	14.1	50.2	1.01	88.6
3	IBA 100 ppm for 12 hr	21.6	26.5	89.5	13.6	64.1	0.86	90.3
4	IBA 60 ppm for 24 hr	25.3	28.8	97.9	11.5	54.8	1.13	87.2
5	IBA 80 ppm for 24 hr	24.2	27.3	92.8	12.8	46.7	1.02	83.5
6	IBA 100 ppm for 24 hr	22.1	26.9	98.2	18.6	39.4	0.84	93.7
7	NAA 60 ppm for 12 hr	26.4	29.6	88.1	11.1	55.6	0.90	84.8
8	NAA 80 ppm for 12 hr	25.1	28.7	91.2	10.3	73.2	1.06	87.1
9	NAA 100 ppm for 12 hr	24.8	29.0	92.0	11.9	58.3	1.09	82.0
10	NAA 60 ppm for 24 hr	27.0	30.3	93.3	12.4	42.1	0.92	89.9
11	NAA 80 ppm for 24 hr	25.7	28.1	89.7	13.1	55.0	0.81	87.5
12	NAA 100 ppm for 24 hr	26.2	29.8	88.8	13.3	72.6	0.94	83.1
13	Water soaking 12 hr	29.6	32.7	80.4	14.6	47.2	0.77	61.8
14	Water soaking 24 hr	31.1	33.8	82.3	13.4	48.9	0.72	60.4
15	No treatment (control)	33.2	36.2	63.7	9.2	33.6	0.65	55.1
C.D.		0.422	0.405	0.416	0.415	0.416	0.405	0.437
SE(m)		0.145	0.140	0.143	0.143	0.143	0.140	0.151
SE(d)		0.205	0.197	0.203	0.202	0.202	0.198	0.213
CV		0.611	0.564	0.547	0.593	0.573	0.596	0.693

treatments on sprouting, rooting and survival parameters (Table 2). This results clearly demonstrate a unique pattern through which IBA proved to be superior than NAA on various parameters supporting earlier findings on the efficiency of auxins as mediators of rooting characteristics (Fig. 1). In particular, maximum root quality was registered from the treatments with 100 ppm of IBA for 24 hr, which significantly reduced the mean days for sprouting (22.1 days) and rooting (26.9 days) (8, 9). The rapid adventitious root initiation and improved physiological responses may be due to the ability of IBA to promote cell dedifferentiation and meristematic activity and the stability of IBA compared to NAA, which has been documented in hardwood and semi-hardwood cutting (10, 11).

Treatment with IBA 100 ppm for 24 hr not only resulted in the earliest sprouting and rooting, but also showed the greatest percentage of rooted cuttings (98.2 %) and good survival after rooting (93.7 %), emphasising the potential of this treatment as physiological in effective root initiation and establishment at post-rooting (Fig. 2). Compared to results for root elongation, rooting evaluations demonstrated a significant treatment time $\times$ auxin interaction on root proliferation rates and were consistent with

reports on the action of IBA on sustained cell elongation (12, 13). In contrast, NAA treatments showed intermediate rooting percentages but shorter roots and lower survival rates as compared to IBA, indicating the superiority of IBA with respect to a vigorous root system (14, 15) (Fig. 3).

Findings from this study showed that exogenous applications of auxin were important in breaking an endogenous limitation in semi-hardwood cuttings and indicated that this process is dependent on optimising both the concentration and duration of auxin (16) (Fig. 4). Trait coefficient of variation was within normal ranges and confirms precision of measurement and experimental design reliability that supports the overall reproducibility of our analyses. The calculated standard errors and critical differences at a 5 % significance level confirm that IBA-based treatments outperform NAA and non-auxin controls in maximising rooting efficiency and subsequent stability of cuttings (17).

In the end, it is indicated that treatment of 100 ppm of IBA in 24 hr is the best treatment in making the roots easier, quality root and survival (Fig. 5). This corroborating evidence is consistent with the significant literature highlighting the power of IBA in numerous

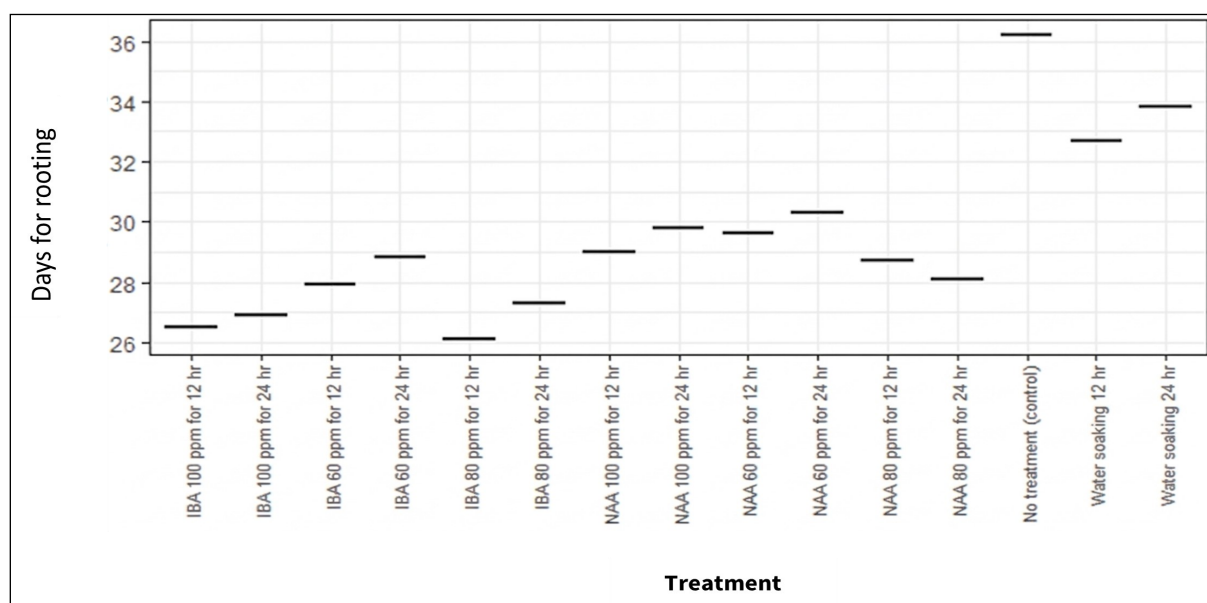


Fig. 1. Boxplot showing the effect of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) treatments on days required for rooting in *Nerium oleander* stem cuttings.

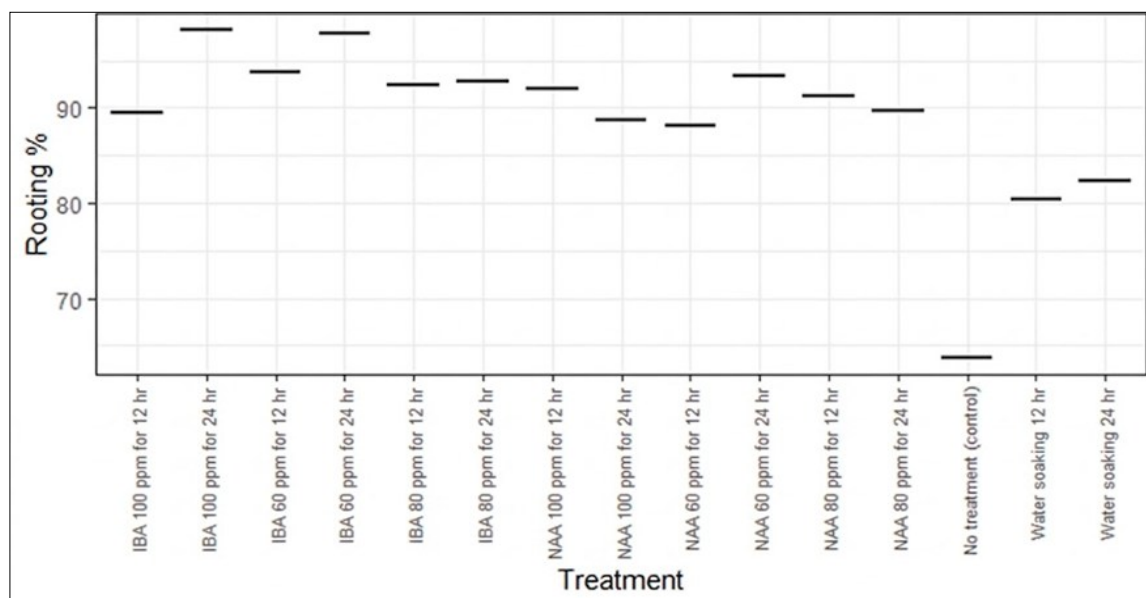


Fig. 2. Boxplot showing the effect of indole-3-butyric acid and naphthaleneacetic acid treatments on days rooting percentage (%) in *Nerium oleander* stem cuttings

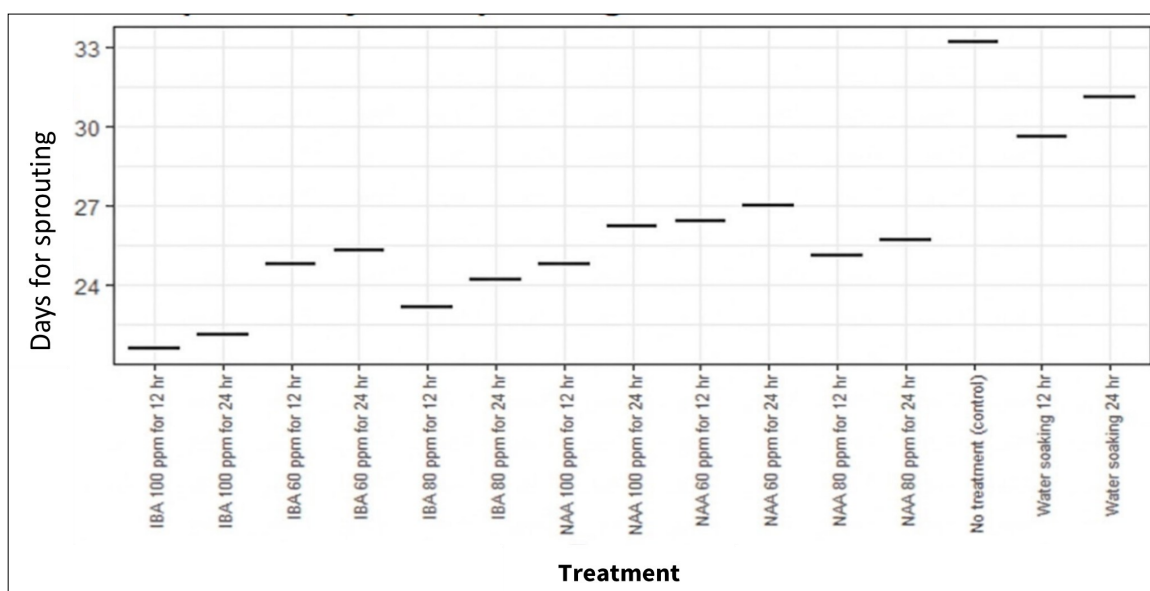


Fig. 3. Boxplot showing the effect of indole-3-butyric acid and naphthaleneacetic acid treatments on days for sprouting in *Nerium oleander* stem cuttings.

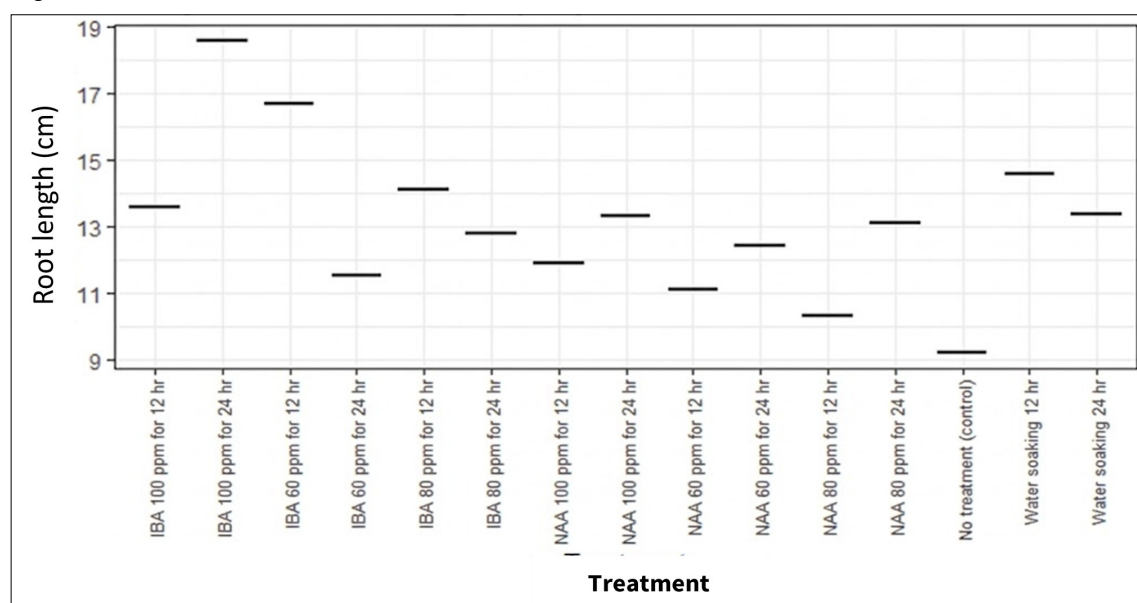


Fig. 4. Boxplot showing the effect of indole-3-butyric acid and naphthaleneacetic acid treatments on root length (cm) in *Nerium oleander* stem cuttings.

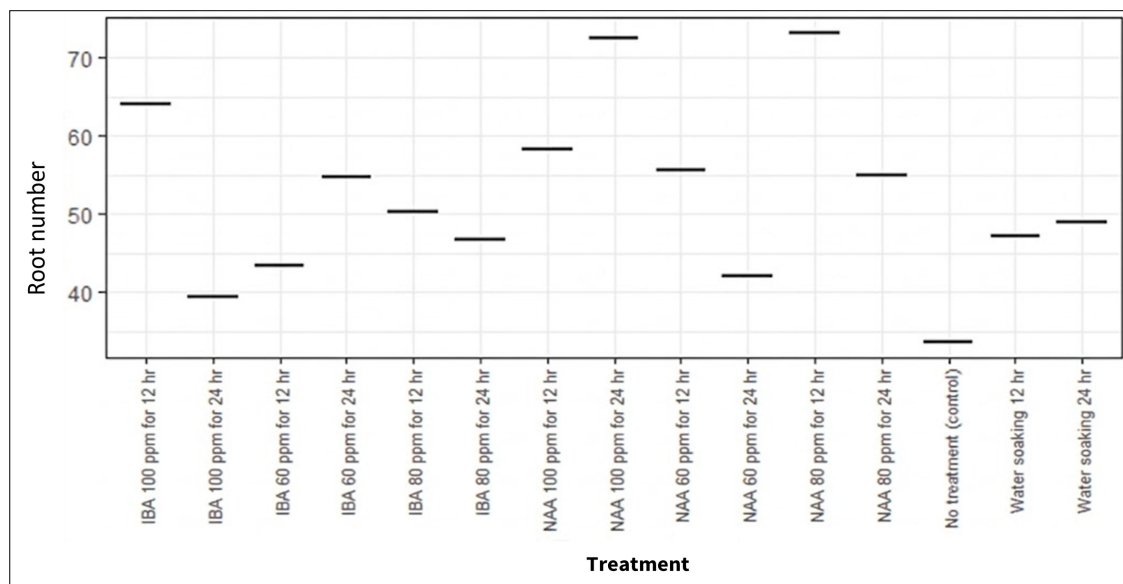


Fig. 5. Boxplot showing the effect of indole-3-butyric acid and naphthaleneacetic acid treatments on number of roots in *Nerium oleander* stem cuttings.

horticultural contexts, indicative of progress towards benchmarks for informing commercial propagation practice (7, 18, 19).

Ethical issues: None

Conclusion

The study confirmed that application of auxins markedly improved sprouting, rooting and survival of *Nerium oleander* stem cuttings compared with water-treated and untreated controls. Among the treatments evaluated, indole-3-butyric acid (IBA) at 100 ppm for 24 hr was the most effective, producing earlier sprouting and rooting, the highest rooting percentage, superior root length and maximum survival. Although naphthaleneacetic acid (NAA) treatments enhanced root number, they were less effective than IBA and in developing a vigorous and stable root system. The results clearly indicate that both auxin concentration and duration of treatment are critical determinants of successful rooting. Hence, IBA 100 ppm for 24 hr is recommended as an optimal protocol for efficient and commercial propagation of *N. oleander*.

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Authors' contributions

PM, GH conceptualised the study and performed the formal analysis. PM, GH, RJ, NR, SKV and BR worked together in structuring the methodology, supervised and validated the study. ACV, CNRS, CD and RP contributed to manuscript editing, summarisation and revision of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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