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A Comprehensive Study on the Impact of IBA-infused Rooting Powder on Rooting and Shoot Development in *Citrus karna* Hardwood Cuttings

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ABSTRACT

Background: Within the expansive realm of citrus, rootstocks play a pivotal role in bolstering crop adaptability against the backdrop of both biotic and abiotic stresses. In the face of the contemporary climate change era, these rootstocks have emerged as indispensable tools, pivotal in countering the challenges posed by shifting environmental dynamics. The investigation explores the impact of Indole-3-butyric acid (IBA)-infused rooting powder on the root induction and shoot behaviour of *Citrus karna* hardwood cuttings.

Methods: The experiment was conducted from August- November 2023 in the 50 per cent shade in the green 50 per cent shade in the green coloured shade net area with gat the School of Agriculture research farm at Lovely Professional University (LPU) in Phagwara, Punjab employed a randomized block design (RBD) comprising nine different treatments.

Result: The impact of Indole-3-butyric acid (IBA)-infused rooting powder with Zinc Sulphate on *Citrus karna* hardwood cuttings. The T_9 treatment, consisting of 0.15% IBA and 0.50% Zinc Sulphate, significantly outperforms other treatments. T_9 achieved a high rooting percentage (92.6%), substantial root and shoot growth and overall improved plant vigour. The combination of IBA and Zinc enhances root induction, promoting robust root systems and vigorous shoot development. This research underscores the effectiveness of the IBA-Zinc combination in propagating *Citrus karna* cuttings, offering valuable insights for horticultural practices.

Key words: Auxin, Iba-zinc, Indole-3-butyric acid, Propagation, Talcum, Vegetative.

INTRODUCTION

Citrus, a quintessential cornerstone of global agriculture stands as a vital source of sustenance and economic prosperity for diverse populations. Within the expansive realm of citrus, rootstocks play a pivotal role in bolstering crop adaptability against the backdrop of both biotic and abiotic stresses. In the face of the contemporary climate change era, these rootstocks have emerged as indispensable tools, pivotal in countering the challenges posed by shifting environmental dynamics. As the world grapples with these transformations, the significance of citrus rootstocks amplifies, ensuring the resilience and productivity of citrus crops (Gupta *et al.,* 2024). Among the array of rootstocks, Citrus *karna* Raf (Family - *Rutaceae*) wild species found in India. Locally named ″Karna (Kharna Khatta, Karna Nimbu, Khatta Nimbu). It counters two primary problems of cracking and sun-scalding (Singh *et al.,* 2021). *Citrus karna* commands attention for its exceptional compatibility with a diverse array of citrus varieties and produces vigorous trees of pineapple, Jaffa, Mosambi, Valencia and Hamlin Oranges (Bowman *et al.,* 2023). The leaves of Mosambi plants budded on the ‘Karna Khatta’ rootstock had higher levels of macronutrients (N, P and K) and micronutrients (Zn and Fe) than those from plants on other rootstocks (Mishra and Dash, 2019). The extensive root system of ″Karna Khatta″ facilitates the efficient absorption of nutrients from the soil and their transport to the scion. As a result, when “Mosambi” is budded on ″Karna Khatta,″ the resulting fruits tend to be larger in both weight and volume (Hayat *et al.,* 2022).

The intricate interaction between rootstock and scion lies at the crux of citrus cultivation success, particularly

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within the context of evolving environmental conditions. Efficiently propagating *Citrus karna* rootstock through hardwood cuttings not only holds promise in preserving its desirable traits but also in maintaining genetic uniformity (Rao *et al.,* 2021). Nonetheless, the journey to establish hardwood cuttings as self-sufficient entities necessitates the establishment of robust root systems and subsequent shoot growth-an intricate process laden with complexities and hurdles (Casales *et al.,* 2018). Within the realm of plant physiology, auxins, a class of plant hormones, wield considerable influence over processes such as root initiation and growth. Indole-3-butyric acid (IBA), a specific auxin variant, has garnered prominence for its capacity to bolster root development in diverse plant species. Traditionally, auxins like IBA found application in liquid form. However, the liquid medium's inherent limitations hindered optimal efficiency. Consistent renewal of auxin solutions and the prospect of cell sap exudation from immersed cutting ends posed challenges that beckoned innovative solutions in auxin application methods. As a response to these challenges, our research embarks on an exploration involving the application of IBA-containing rooting powder, utilizing talcum powder as the delivery medium. By embracing this innovative approach, we aim to transcend the confines of traditional liquid auxin application. The utilization of talcum powder provides a controlled and efficient avenue for IBA delivery, mitigating the need for frequent solution refreshment and diminishing the risk of cell sap exudation. This pioneering methodology bears the potential to revolutionize the propagation of *Citrus karna* hardwood cuttings, simplifying the root induction process and heightening shoot development.

Central to our study is the investigation into the effects of IBA-containing rooting powder on the dual aspects of root induction and shooting behaviour in *Citrus karna* hardwood cuttings (Yulianti *et al.,* 2021). By meticulously analysing the response of *Citrus karna* rootstock to this innovative application technique, we aspire to unlock insights that hold the potential to reshape propagation practices for this indispensable rootstock species (Mirza *et al.,* 2023). In the subsequent sections, we unveil the methodologies harnessed for our study, lay out the results and acquired data and delve into a comprehensive discourse on the broader implications of our findings, situated within the contexts of citrus propagation and horticulture. By bridging the gap between scientific inquiry and pragmatic application, our research strives not only to enrich our understanding of the intricate processes governing propagation but also to present viable solutions to the challenges inherent in conventional auxin application methods. Ultimately, our aim to elevate the propagation practices of *Citrus karna*, thereby fostering resilience and sustainability within the sphere of citrus cultivation amid the dynamic panorama of global climate change.

MATERIALS AND METHODS

Experimental site

The experiment was conducted from August to November 2023 in the 50 per cent shade in the green coloured shade net area at the School of Agriculture research farm at Lovely Professional University (LPU) in Phagwara, Punjab. Phagwara is located in the Kapurthala district of Punjab, at 31.25°N latitude and 75.70°E longitude and the research site is 249 meters above sea level.

Experimental design

The study employed a completely randomized design (CRD) comprising nine different treatments tabulated in Table 1.

Statistical analysis

The data collected was analysed for ANOVA using the OPSTAT software. Nine different treatments with 3 replications were utilized in total. Specifically, seven treatments (T_3 to T_9) were prepared in powder form with talcum powder as the carrier agent. In this study, healthy hardwood cuttings were taken from the 5-year-old mother plant. The base of each cutting was moistened and the recommended amount of rooting hormone was placed in a container. The base of each cutting was then coated with this rooting powder mixture before being inserted into the soil. Each replication consisted of 10 cuttings, resulting in 30 cuttings per treatment and 270 hardwood stem cuttings for the entire investigation. The bases of the cuttings were wounded to promote early root development. After planting the cuttings 5 to 10 cm below the soil surface in each bed, they were uniformly irrigated. Observations related to rooting parameters were recorded, including rooting percentage, number of roots, root length (cm), root diameter (girth), in mm, root volume (cm^3), number of sprouts, time to root, time to first bud sprout, fresh root weight (g) and dry root weight (g), Leaf area (cm^2). These observations were made at 90 days and the data were analysed. The study was conducted using the completely randomized design (CRD) statistical method.

Procedure for recording observations

The rooting percentage was calculated by dividing the number of cuttings that successfully rooted by the total number of cuttings. Roots were manually counted for each

Table 1: Treatments used under investigation.

	Treatments
T_1	Control
T_2	Indole-3-Butyric acid 500 ppm (Solution)
T_3	Talcum powder
T_4	Indole-3-Butyric acid 500ppm
T_5	Indole-3-Butyric acid 1000ppm
T_6	Indole-3-Butyric acid 1500 ppm
T_7	Indole-3-Butyric acid 500 ppm + $ZnSO_4$ 500 ppm
T_8	Indole-3-Butyric acid 1000 ppm + $ZnSO_4$ 500 ppm
T_9	Indole-3-Butyric acid 1500 ppm + $ZnSO_4$ 500 ppm

cutting and their length was measured from the base to the tip of the longest root using a ruler. Root girth was measured with a digital vernier caliper and root volume was determined through the water displacement method. The number of sprouts per cutting was visually counted.

The time to root was recorded as the number of days from planting until the first visible roots appeared. Observations were conducted daily, carefully inspecting the base of the cuttings without disturbing the rooting medium to maintain precision and consistency. Similarly, the time to the first bud sprout was noted as the days taken from planting until the first bud emerged. Fresh root weight was measured immediately after harvesting using an electronic balance and dry root weight was determined by oven-drying the roots at 70°C until a consistent weight was achieved. Leaf area was quantified using a leaf area meter for accurate measurements.

RESULTS AND DISCUSSION

The findings derived from the present study are outlined below.

Effect on rooting percentage (%)

The rooting percentage at 90 DAP (Table 2) varied between 44.2% and 92.6%. The highest rooting percentage was observed in treatment T_9 at 92.6%, followed by treatment T_6 at 85.1%. Conversely, the lowest rooting percentage was found in the untreated control group (T_1) at 44.2%.

Number of roots

The number of roots per cutting at 90 DAP (Table 2) ranged from 6.15 to 24.22. The highest number of roots was observed in treatment T_9, with 24.22 roots, followed by treatment T_6 with 21.28 roots. The untreated control group (T_1) had the fewest roots, with a count of 6.15.

Root length (cm)

At 90 DAP, root lengths per cutting (Table 2) varied from 9.8 cm to 16.8 cm. The longest roots were found in treatment T_9 at 16.8 cm, followed by T_6 with 15.5 cm and T_8 with 15.4 cm. The shortest roots were in the untreated control group (T_1) at 9.8 cm.

Root diameter (mm)

At 90 DAP, the root diameter per cutting (Table 2) varied from 0.54 mm to 1.47 mm. The thickest roots were observed in treatment T_9 at 1.47 mm, followed by T_6 at 1.35 mm. The untreated control group (T_1) had the thinnest roots at 0.54 mm.

Volume of roots (cm3)

At 90 DAP, the root volume per cutting (Table 2) ranged from 1.12 cm^3 to 2.35 cm^3. The largest root volume was found in treatment T_9 at 2.35 cm^3. The smallest root volume was observed in the untreated control group (T_1) at 1.12 cm^3.

Number of sprouts

At 90 DAP, the count of sprouts per cutting (Table 3) spanned from 1.12 to 4.68. Notably, the most abundant sprouting occurred in treatment T_9, with a count of 4.68. Conversely, the lowest number of sprouts per cutting was observed in the untreated control group (T_1), registering at 1.12.

Effect on the time taken for first bud sprout and rooting (In days)

A review of Table 3 reveals that the various concentrations of IBA did not significantly influence the time taken for first rooting and shooting. However, rooting first occurred in T_7, 22 days after planting, while the initial bud sprout was observed at 18 days post-planting in treatment T_6. The onset of first rooting and sprouting was delayed, recorded at 27 days for rooting and 23 days for sprouting in T_1 (Control). Data were collected every 5 to 7 days in each treatment. The treatments showed no discernible impact on the timing of rooting or sprouting over the course of root emergence.

Effect on fresh root weight at 90 days after planting

The data in Table 3 showed significant variation among the treatments, ranging from 1.11 g to 3.21 g. On the 90th day post-planting, treatment T9 exhibited the highest fresh root weight per cutting at 3.21 g, followed by T_6 at 2.78 g,

Table 2: Rooting percentage (%), number of roots, root length (cm), root diameter (mm), volume of roots (cm^3) at 90 DAP.

	Treatments	Rooting percentage (%)	Number of roots	Root length (cm)	Root diameter (mm)	Volume of roots (cm^3)
T_1	Control	44.2f	6.15e	9.8d	0.54d	1.12d
T_2	Indole-3-Butyric acid 500 ppm (Solution)	49.1e	17.28c	14.3bc	0.90c	1.38cd
T_3	Talcum powder	48.8e	13.22d	12.9c	0.84cd	1.32d
T_4	Indole-3-Butyric acid 500 ppm	62.1d	18.12c	14.5bc	1.07bc	1.51cd
T_5	Indole-3-Butyric acid 1000 ppm	75.1c	20.21b	15.1abc	1.19ab	1.98abc
T_6	Indole-3-Butyric acid 1500 ppm	85.1b	21.28b	15.5ab	1.35ab	2.32ab
T_7	Indole-3-Butyric acid 500 ppm +$ZnSO_4$ 500 ppm	62.4d	18.15c	14.6abc	1.18bc	1.73bcd
T_8	Indole-3-Butyric acid 1000 ppm + $ZnSO_4$ 500 ppm	83.5b	20.81b	15.4ab	1.28ab	2.31ab
T_9	Indole-3-Butyric acid 1500 ppm + $ZnSO_4$ 500 ppm	92.6a	24.22a	16.8a	1.47a	2.35a
SE	0.58	0.20	0.60	0.04	0.16	
CV	4.5	3.39	13.98	12.38	12.80	
CD	1.75	0.60	1.80	0.14	0.37	

Table 3: Number of sprouts, days to first bud sprout, days to first rooting, fresh weight of roots (g), dry weight of roots (g), leaf area (cm^2) at 90 DAP.

	Treatments	Number of sprouts	Days to first bud sprout	Days to first rooting	Fresh weight of roots (g)	Dry weight of roots (g)	Leaf area (cm^2)
T_1:	Control	1.12^{f}	23^{a}	27^{a}	1.11^{c}	0.17^{b}	3.1^{c}
T_2:	Indole-3-Butyric acid 500 ppm (Solution)	1.62^{def}	22^{ab}	23^{cd}	1.32^{c}	0.18^{b}	3.8^{bc}
T_3:	Talcum Powder	1.28^{ef}	23^{a}	24^{bcd}	1.21^{c}	0.17^{b}	3.13^{c}
T_4:	Indole-3-Butyric acid 500ppm	1.72^{de}	21^{ab}	26^{ab}	2.24^{b}	0.33^{ab}	4.1^{abc}
T_5:	Indole-3-Butyric acid 1000ppm	2.62^{c}	20^{bc}	25^{abc}	2.46^{b}	0.42^{a}	4.87^{abc}
T_6:	Indole-3-Butyric acid 1500 ppm	3.32^{b}	18^{c}	24^{bcd}	2.78^{ab}	0.45^{a}	5.63^{ab}
T_7:	Indole-3-Butyric acid 500 ppm +$ZnSO_4$ 500 ppm	2.02^{d}	22^{ab}	22^{d}	2.45^{b}	0.35^{ab}	4.27^{abc}
T_8:	Indole-3-Butyric acid 1000 ppm + $ZnSO_4$ 500 ppm	2.76^{bc}	20^{bc}	25^{abc}	2.73^{ab}	0.43^{a}	5.4^{ab}
T_9:	Indole-3-Butyric acid 1500 ppm + $ZnSO_4$ 500 ppm	4.68^{a}	21^{ab}	24^{bcd}	3.21^{a}	0.48^{a}	6.03^{a}
SE	0.01	0.54	0.51	0.04	0.03	0.36	
CV	1.64	7.70	6.14	5.32	22.03	6.48	
CD	0.04	0.62	1.54	0.01	0.09	0.82	

which was comparable to T_8 at 2.73 g. Conversely, the untreated control group (T_1) displayed the lowest fresh root mass, measuring only 1.11 g. Complementary findings were reported by Tanwar *et al.* (2020) in Pomegranate and Natarajan *et al.* (2023) in Guava, who found that 2000 ppm concentration gave the highest fresh root mass values.

Dry weight of roots at 90 days after planting

The data presented in Table 3 regarding the dry weight of roots per cutting varied significantly among treatments, ranging from 0.17 g to 0.48 g. On the 90th day after planting, treatment T_9 exhibited the highest dry weight per cutting at 0.48 g, which was on par with T_6 at 0.45 g, T_8 at 0.43 g, T_5 at 0.42 g, followed by T_7 at 0.35 g, which was comparable to T_4 at 0.33 g. Conversely, the untreated control group (T_1) displayed the lowest dry weight of roots, measuring only 0.17 g. Coinciding results have been documented by Natarajan *et al.* (2023) in Guava; Guchhait *et al.* (2024) in citrus noted that the most favourable result was attained by using 2000 ppm of IBA.

Leaf area (cm^2)

The leaf area per cutting showed substantial variation among the treatments in the results presented in Table 3, ranging from 3.1 cm² to 6.03 cm². The highest leaf area was recorded in treatment T_9 at 6.03 cm², followed by T_6 at 5.63 cm², which was comparable to T_8 at 5.4 cm². In contrast, the untreated control group (T_1) exhibited the smallest leaf area at 3.1 cm². Consistent findings have been unveiled by Hawramee *et al.* (2019) in *Morus*; Ghangale *et al.* (2021) in *Vitis*.

The inclusion of a comprehensive discussion in paper explores the effects of IBA-infused rooting powder in conjunction with Zinc on the root induction and shooting behavior of hardwood cuttings of *Citrus karna*. The inclusion of Zinc Sulfate in the treatment enhances root induction and growth in hardwood cuttings of *Citrus karna* by providing essential sulfur, which plays a crucial role in synthesizing amino acids and proteins, improving enzyme activity and facilitating nutrient assimilation, thereby promoting overall plant health and development. The results of the study unequivocally demonstrate that the utilization of IBA combined with zinc has a substantial positive impact on multiple facets of hardwood cutting propagation in *Citrus karna*. Notably, the rooting percentage has been significantly elevated under this treatment, signifying a remarkable improvement in the overall success rate of root induction. The synergistic action of IBA and Zinc appears to stimulate the physiological processes involved in root development, leading to more efficient root initiation and subsequent establishment (Sourati *et al.,* 2022). The proliferation in the quantity of roots in the treated cuttings is a noteworthy observation. This could be attributed to the dual action of IBA and Zinc, which likely promote cell division and elongation in the cambium, facilitating the development of a higher number of root primordia (Elizalde *et al.,* 2022) and (Kondhare *et al.,* 2021). Moreover, the elongation of root length and enhancement of root diameter are indicative of improved root system quality. Additionally, auxin promotes cell division, leading to the growth and differentiation of cambial initials into root primordia. It also facilitates the movement and allocation of food reserves to the sites of root formation and initiation, encouraging the development of numerous roots and enhancing their length and diameter (Lynch *et al.,* 2021) and (El-Banna *et al.,* 2023).

The combined treatment seems to foster a robust root system, which is crucial for nutrient uptake and overall plant health (Butova *et al.,* 2023). The expansion of root volume, as evidenced results, highlights the positive impact of IBA-Zinc combination on root system architecture (Alexandre *et al.,* 2023). The treated cuttings display a greater volume of roots, which can lead to improved water and nutrient absorption, ultimately contributing to better growth and establishment of the propagated plants. The shorter duration required for root initiation in the treated cuttings compared to the control group signifies the role of IBA and Zinc in expediting the initiation of root primordia, demonstrating their promotive effect on early root development (Sao and Verma, 2021) and (Rehana *et al.,* 2023). The increase in both fresh and dry weights of roots further reinforces the positive impact of the IBA-Zinc treatment on root development (Sun *et al.,* 2023) and (Adiba *et al.,* 2022). This weight gain can be attributed to enhanced cell division and elongation processes facilitated by the growth regulators (Sujin *et al.,* 2020). Moreover, the substantial rise in the number of sprouts and leaf area in the treated group signifies the efficacy of IBA-Zinc combination in promoting shoot development (Rehana *et al.,* 2023). This observation suggests that the treated cuttings are endowed with increased vigor and potential for future growth (Izadi *et al.,* 2022). This expedited bud initiation could be attributed to the accelerated hormonal response triggered by IBA and Zinc, promoting shoot meristem activation and subsequent bud development (Mishra *et al.,* 2022) and (wang *et al.,* 2022). This section of paper will unveil the manifold ways in which the appropriate application of IBA with Zinc has substantively improved various parameters related to root induction and shooting behaviour of hardwood cuttings in *Citrus karna.* IBA rooting powder with talc as a carrier agent offers distinct advantages over liquid IBA solutions for promoting robust plant root development.

The incorporation of talc enables controlled and sustained hormone release, fostering gradual absorption by plant tissues, minimizing hormone overexposure and ensuring healthier root growth (Abha Manohar *et al.,* 2022) and (Ferreira Neto *et al.,* 2022). Talc preserves moisture and aiding successful root establishment (Itabana *et al.,* 2023). The powder's precise application allows accurate dosing, minimizing waste and optimizing hormone use. Talc's protective barrier reduces contamination risk, creating a favourable environment for root growth (McLeod *et al.,* 2022). The powder's extended shelf life enhances convenience and cost-effectiveness. IBA liquid formulations come with several drawbacks that make them less favourable than IBA talc rooting powder. One notable concern is the challenge of controlling hormone dosage during application. This overexposure can have detrimental effects, hindering the rooting process and negatively affecting overall plant health. Furthermore, the liquid nature of IBA solutions presents an increased susceptibility to disease contamination (Kohler *et al.,* 2022). The liquid medium provides an ideal environment for pathogens to thrive,

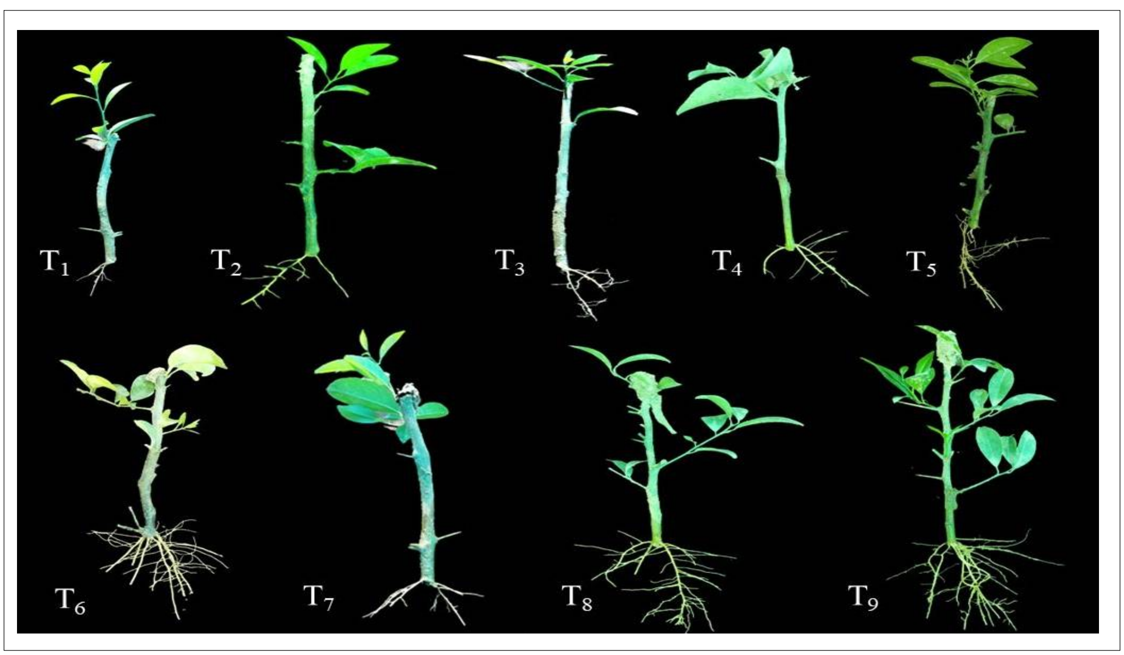

Fig 1: Figure representing various treatments.

increasing the likelihood of disease transmission to the cuttings (Singh, 2022). Prolonged immersion of cuttings in liquid IBA solutions can lead to excessive uptake of the hormone, causing imbalances that hinder the rooting process and overall plant health. This is particularly problematic as the hormone dosage is harder to regulate with liquid applications. Additionally, liquid IBA solutions tend to have shorter shelf lives compared to talc-based powder. Factors like light and heat exposure can lead to a decline in effectiveness over time, potentially rendering the solution less potent before it is fully used. The mechanisms by which these growth regulators interact to enhance rooting percentage, root characteristics, shoot development and other relevant traits warrant careful analysis and interpretation. This discussion not only validates the importance of findings but also offers valuable insights for future research and practical applications in the field of horticulture.

CONCLUSION

This study examined the impact of Indole-3-butyric acid (IBA)-infused rooting powder with Zinc Sulphate on *Citrus karna* hardwood cuttings. The T_9 treatment, consisting of 0.15% IBA and 0.50% Zinc Sulphate, significantly outperforms other treatments. T_9 achieved a high rooting percentage (92.6%), substantial root and shoot growth and overall improved plant vigor. The combination of IBA and Zinc enhances root induction, promoting robust root systems and vigorous shoot development. The superior performance of T_9 in rooting percentage, number of roots, root length, root diameter, root volume, fresh and dry root weights, number of sprouts and leaf area demonstrates its efficacy. The combined treatment fosters efficient root initiation and establishment by stimulating cell division and elongation. Additionally, the powder form of IBA, with talc as a carrier, offers advantages over liquid formulations by providing controlled hormone release, reducing contamination risks and ensuring healthier root growth. This research underscores the effectiveness of the IBA-Zinc combination in propagating *Citrus karna* cuttings, offering valuable insights for horticultural practices.

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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

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

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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