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RESEARCH ARTICLE

Influence of IBA concentrations, zinc synergy and carrier agents on rooting and shooting attributes of rough lemon rootstock cuttings

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Abstract

Adequate indole-3-butyric acid (IBA) concentration enhances cutting success rates and promotes uniform root growth, resulting in healthy plants with the desired traits and higher yields. This research investigated the effects of different IBA concentrations (2000 ppm and 4000 ppm) on the rooting and shooting characteristics of rough lemon cuttings. The present study employed a random block design with nineteen treatments, each with three replicates. Furthermore, primary root count, secondary root count, root diameter, root length, fresh root mass, dry root mass root volume, rooting, root-to-shoot ratio, shoot diameter, shoot length, shoots number, leaves number, sprouting percentage, survival percentage, fresh shoot mass, dry shoot mass and leaf area were studied. The present study also employed a comparative analysis of carrier agents, i.e., talc and activated charcoal, in the application of IBA for rough lemon cutting propagation. The research explored the synergistic effects of zinc and IBA on the rooting and shooting aspects of rough lemon stem cuttings. The 2000 ppm IBA performed better than 4000 ppm in the rooting and shooting parameters of the rough lemon. The application of (activated charcoal powder + 2000 ppm IBA + 0.50 % $ZnSO_4$) concentration had better effect on various shooting parameters of rough lemon cuttings. Cuttings treated with activated charcoal as a carrier agent for IBA demonstrated superior performance. Zinc at concentrations of 0.25 % and 0.50 % with IBA (2000 ppm and 4000 ppm) demonstrated superior performance as compared to IBA alone (2000 ppm and 4000 ppm) in various morphological attributes of rough lemon. This study highlights the optimization of horticultural practices for the successful propagation and cultivation of rough lemon plants.

Keywords: activated charcoal; auxin; *Citrus jambhiri*; synergism; talc powder; zinc

Introduction

Citrus fruits, known for their nutritional value, have gained popularity in tropical and subtropical regions, with the rough lemon (*Citrus jambhiri*) being a sought-after variety due to its role as a rootstock. Its sour taste makes it popular in many dishes and drinks (1). Rough lemon demonstrates exceptional resistance to salinity, alkalinity, poor drainage and it is known for its drought tolerance as well, making it highly suitable for cultivation in diverse soil types and various environmental conditions (2). The most popular method for propagating *Citrus* species has been budding onto seedling rootstocks. Extensive experimentation and trials have been conducted since the early 1950s to evaluate different rootstocks suitable for *Citrus* trees under varying environmental conditions (3). However, the propagation of *C. jambhiri* through cuttings report a lower success rate without proper treatment (1). To enhance the success rate, researchers have investigated the utilization of bioregulators to standardize the concentrations of growth regulators. In light of the intrinsic challenges of rooting hormone requirements, this study aims firstly to ascertain the extent to which specific indole-3-butyric acid (IBA) concentrations, namely 2000 ppm and 4000 ppm, modulate the rooting efficacy of rough lemon cuttings (Fig. 1).

Rooting hormones are often sensitive to environmental factors, such as light, heat and moisture, which can reduce their success. The effectiveness of IBA greatly depends on its delivery method and carrier agent (2). Carrier agents serve as vehicles for delivering rooting hormones to the plant tissues, ensuring their proper absorption and optimal performance (3). Among the various carrier agents available, talc and activated charcoal have gained significant attention due to their unique properties and potential benefits in rooting hormone applications (4). Talc, a naturally occurring mineral composed of magnesium, silicon and oxygen, has been widely used as a carrier agent for rooting hormones in plant propagation (5). It serves as an effective carrier for rooting hormones due to its unique properties that facilitate hormone delivery, protection from degradation and adherence to plant tissues (6). It also acts as a barrier, shielding the hormone from external influences and preserving its potency until it reaches the target tissues. This ensures that the rooting hormone remains active and capable of stimulating root development in the cuttings (7). Studies have shown that talc can improve the performance of auxin and promote rooting in several plant species (8). Activated charcoal, also called activated carbon, is a versatile substance widely used for induction of rooting process (9). It acts as a sponge, absorbing and neutralizing harmful substances that could hinder root growth, promoting a healthier environment for root initiation and development (10). Furthermore, by maintaining appropriate moisture levels and enhancing air circulation, activated charcoal promotes healthy root growth and minimizes the risk of fungal or bacterial infections by providing sterile environment (11). Incorporating activated charcoal with IBA into plant propagation protocols can increase rooting success and contribute to successful plant propagation and cultivation. Hence, this research aims to comprehensively investigate the efficacy of talc and activated charcoal as carrier agent for enhancing the propagation of rough lemon cuttings.

Zinc is an essential micronutrient, plays a crucial role in numerous physiological processes within plants (12). It is a cofactor for enzymes involved in auxin biosynthesis, such as indole-3-pyruvic acid decarboxylase (IPDC) and tryptophan aminotransferase (TAA) (13, 14). Zinc-deficient plants often exhibit reduced auxin levels, leading to compromised root and shoot growth (15). While the individual importance of zinc and auxin in plant development has been extensively studied, their combined role in promoting root and shoot growth of rough lemon cuttings remains a research gap. Therefore, recent studies have unveiled the synergistic relationship between zinc and IBA, wherein zinc availability directly influences the synthesis, transport and signalling of auxin within plant cells. By maintaining an optimal zinc concentration, auxin synthesis and activity can be effectively modulated, resulting in enhancement of cellular elongation, vigorous adventitious root formation and robust shoot development. Therefore, the aim of the present investigation is to determine the significance of zinc supplementation in conjunction with IBA for root and shoot growth in rough lemon cuttings and to analyze the synergistic role of zinc and IBA in promoting root and shoot growth of rough lemon cuttings.

Materials and Methods

Experimental site

The experiment took place between August and November 2022 in the shade net area of the School of Agriculture research farm at Lovely Professional University (LPU) in Phagwara, Punjab, coordinates at 31.25 °N latitude and 75.70 °E longitude. The research site is at an elevation of 249 m above sea level.

Experimental design

The research used a randomized block design (RBD) with 19 treatments and three replications (T_1; Talc powder + 2000 ppm IBA, T_2; Talc powder + 2000 ppm IBA + 0.25 % $ZnSO_4$, T_3; Talc powder + 2000 ppm IBA + 0.50 % $ZnSO_4$, T_4; Talc powder + 4000 ppm IBA, T_5; Talc powder + 4000 ppm IBA + 0.25 % $ZnSO_4$, T_6; Talc powder + 4000 ppm IBA + 0.50 % $ZnSO_4$, T_7; Talc powder, T_8; Talc powder + 0.25 % $ZnSO_4$, T_9; Talc

Fig. 1. Callus induction and emergence of root hairs.

powder + 0.50 % $ZnSO_4$, T_{10}; Activated charcoal powder + 2000 ppm IBA, T_{11}; Activated charcoal powder + 2000 ppm IBA + 0.25 % $ZnSO_4$, T_{12}; Activated charcoal powder + 2000 ppm IBA + 0.50 % $ZnSO_4$, T_{13}; Activated charcoal powder + 4000 ppm IBA, T_{14}; Activated charcoal powder + 4000 ppm IBA + 0.25 % $ZnSO_4$, T_{15}; Activated charcoal powder + 4000 ppm IBA + 0.50 % $ZnSO_4$, T_{16}; Activated charcoal powder, T_{17}; Activated charcoal powder + 0.25 % $ZnSO_4$, T_{18}; Activated charcoal powder + 0.50 % $ZnSO_4$, T_{19}; Control). Cuttings were planted directly into the soil, with an average temperature of 26.98 °C and a relative humidity of 63.69 %, which fostered an environment conducive to successful growth and development. Observations were recorded 120 days after planting (DAP) (Fig. 2).

Preparation of cuttings

To ensure the successful propagation of the rough lemon plant, the selection of the right plant for cutting is essential. Therefore, a thorough assessment of the plant's health, vigour and disease-free status was ascertained prior to selecting the cutting material. Treatment method of cutting was basal application through dry -dip method (16). The selected plant was identified having branches with pencil thickness and 15-20 cm length and containing at least three to four viable buds. To prepare the cuttings, it was crucial that the leaves were removed from the branch to prevent moisture loss and fungal infection, while retaining some for photosynthesis. Afterward, a slanting cut was made at the base of each cutting to increase the surface area of the cutting that would be in contact with the soil. This method enabled the cutting to absorb water and nutrients from the soil more effectively, resulting in faster growth and increased chances of rooting

Fig. 2. T_1; Talc powder + 2000 ppm IBA, T_2; Talc powder + 2000 ppm IBA + 0.25 % $ZnSO_4$, T_3; Talc powder + 2000 ppm IBA + 0.50 % $ZnSO_4$, T_4; Talc powder + 4000 ppm IBA, T_5; Talc powder + 4000 ppm IBA + 0.25 % $ZnSO_4$, T_6; Talc powder + 4000 ppm IBA + 0.50 % $ZnSO_4$, T_7; Talc powder, T_8; Talc powder + 0.25 % $ZnSO_4$, T_9; Talc powder + 0.50 % $ZnSO_4$, T_{10}; Activated charcoal powder + 2000 ppm IBA, T_{11}; Activated charcoal powder + 2000 ppm IBA + 0.25 % $ZnSO_4$, T_{12}; Activated charcoal powder + 2000 ppm IBA + 0.50 % $ZnSO_4$, T_{13}; Activated charcoal powder + 4000 ppm IBA, T_{14}; Activated charcoal powder + 4000 ppm IBA + 0.25 % $ZnSO_4$, T_{15}; Activated charcoal powder + 4000 ppm IBA + 0.50 % $ZnSO_4$, T_{16}; Activated charcoal powder, T_{17}; Activated charcoal powder + 0.25 % $ZnSO_4$, T_{18}; Activated charcoal powder + 0.50 % $ZnSO_4$, T_{19}; Control.

success.

Application of growth regulators and planting of cuttings

Immediately after the preparation of the cuttings, they were immersed in water to preserve moisture until planting, thereafter cutting was inserted in honey as it acts as a good antibacterial agent as well as good adherent with plant growth regulators (PGRs) and effective in controlling microbial contamination in plant tissue culture is supported by several studies (17, 18). Then, the lower end of each cutting was treated with IBA (2000 ppm and 4000 ppm) along with talc powder as carrier agent and zinc (0.25 % $ZnSO_4$, 0.50 % $ZnSO_4$) and IBA (2000 ppm and 4000 ppm) along with activated charcoal powder as carrier agent and zinc (0.25 % $ZnSO_4$, 0.50 % $ZnSO_4$) through a dip. Following this, treated cuttings were inserted into the soil and the surrounding soil was gently pressed to secure the cuttings in place. To ensure sufficient moisture content, the rooting media was watered immediately after planting the cuttings.

Statistical analysis

The statistical analysis was performed using R-Program version 4.3.1 for windows (R software, Auckland, New Zealand). All data collected was submitted to analysis of variance and the means was compared by the Tukey test ($P \leq 0.05$).

Results

Rooting parameters

Primary root count and root diameter

For primary root count, charcoal-based treatments generally outperformed talc-based treatments. The highest count was observed in T_{12} with 16.37 roots, while T_6 recorded the highest among talc treatments at 13.37 roots. The control (T_{19}) had the lowest root count of 5.03. In terms of root diameter, charcoal treatments again exhibited superior results. T_{12} had the highest root diameter at 2.18 mm, while the highest diameter in talc-based treatments was in T_6 (1.8). The control group (T_{19}) showed the smallest diameter of 0.65 mm. The presence of zinc sulphate, especially at 0.50 %, enhanced root count and diameter in both talc and charcoal treatments (Table 1).

Primary root length

At 120 DAP, increasing zinc concentration had a notable impact on root development. In talc-based treatments, T_6 (7.72 cm) showed the highest mean primary root length, followed by T_5 (7.42 cm) with 0.25 % $ZnSO_4$. Similarly, in charcoal-based treatments, T_{12} (9.26 cm) with 0.50 % $ZnSO_4$ outperformed T_{11} (8.91 cm) with 0.25 % $ZnSO_4$, indicating that higher zinc concentration led to better root growth. In terms of the highest primary root length, it ranged from 9.07 cm (T_{19}, control) to 18.3 cm (T_{12}). Charcoal based treatments generally performed better, with T_{12} (18.3 cm) and T_{11} (17.7 cm) outperforming all talc based treatments, where T_6 (15.97 cm) was the best among them (Table 1).

Secondary root length, diameter and count

At 120 DAP, secondary root length ranged from 2.5 cm (T_{19}, control) to 5.13 cm (T_{12}). Charcoal-based treatments generally performed better, with T_{12} (5.13 cm) showing the longest root length, while the best talc-based treatment, T_6, reached 4.37 cm. Regarding zinc concentration, higher levels of $ZnSO_4$ improved root length in both talc and charcoal treatments, with 0.50 % $ZnSO_4$ consistently yielding better results (T_{12}, 5.13 cm; T_6, 4.37 cm) compared to 0.25 %. For secondary root diameter, T_{12} (1.15 mm) had the highest, while the control (T_{19}) had the lowest (0.08 mm). Charcoal based treatments also performed better in this regard. Zinc concentration positively influenced root diameter, with 0.50 % ZnSO4 (T_{12}, 1.15 mm) outperforming 0.25 % (T_{11}, 1.05 mm). In root count, T_{12} (68.7 mm) exhibited the highest count, with T_6 (52.8 mm) being the best among talc based treatments (Table 1).

Fresh root mass and dry root mass

At 120 DAP, fresh root mass ranged from 1.08 g (control, T_{19}) to 2 g (T_{12}). Charcoal based treatments generally outperformed talc-based ones, with T_{12} (2 g) achieving the highest fresh root mass, while the best talc-based treatment, T_6, reached 1.55 g. Zinc concentrations of 0.50 % ($ZnSO_4$) enhanced fresh root mass, particularly in charcoal treatments (T_{12}, 2 g; T_6, 1.55 g). For dry root mass, T_{12} also led with 0.49 g, while the control (T_{19}) had the lowest (0.15 g). Charcoal-based treatments with 0.50 % $ZnSO_4$ (T_{12}, 0.49 g) again performed best, while talc treatments showed moderate increases, with T_6 (0.37 g) as the highest (Table 2).

Root dry mass percentage

For root dry mass percentage, treatments with activated charcoal consistently outperformed talc-based treatments. T_{12} (24.5 %) had the highest root dry mass. Talc treatments, like T_6 (23.77 %) and T_5 (23.68 %), showed moderate success, while the control (T_{19}) exhibited the lowest percentage (14.02 %) (Table 2).

Root volume

The highest root volume was observed in T_{12} (1.59 cm^3), demonstrating the effectiveness of zinc combined with activated charcoal and IBA. Treatments containing zinc consistently outperformed talc based treatments, which resulted in lower root volumes. The control (T_{19}) had the smallest root volume at 0.54 cm^3, emphasizing the positive impact of zinc on root development (Table 2).

Rooting percentage

In terms of rooting percentage, treatments with activated charcoal consistently outperformed those with talc, with T_{12} achieving the highest rooting percentage at 80 %, followed by T_{11} at 77.77 %. In contrast, talc treatments like T_1 and T_2 yielded lower rooting percentages, ranging from 45.55 % to 46.66 % (Table 2).

Root to shoot ratio

Regarding the root-to-shoot ratio, treatment T_{12} also recorded the highest ratio at 0.21, indicating superior balance between root and shoot growth. Other treatments with activated charcoal, such as T_{11} and T_{10}, showed comparable ratios. In comparison, the control group (T_{19}) exhibited the lowest root-to-shoot ratio (0.14), underscoring the beneficial effects of treatments containing activated

Table 1. Primary root count, root diameter, mean primary root length, highest primary root length, secondary root length, secondary root diameter and secondary root count of rough lemon cuttings treated with IBA (2000 ppm and 4000 ppm) and carrier agents (Talc and Activated charcoal) shade net area at 120 DAP

	Treatments	Primary root count	Root diameter (mm)	Mean primary root length (cm)	Highest primary root length (cm)	Secondary root length (cm)	Secondary root diameter (mm)	Secondary root count
T_1	Talc Powder + 2000 ppm IBA	9.7^{g}	1.29^{efg}	6.17^{j}	12.9^{gh}	3.43^{ghij}	0.63^{ef}	36.63^{i}
T_2	Talc Powder + 2000 ppm IBA + 0.25 % $ZnSO_4$	10.53^{f}	1.33^{efg}	6.5^{i}	13.63^{fg}	3.5^{fghij}	0.73^{de}	43.5^{h}
T_3	Talc Powder + 2000 ppm IBA + 0.50 %$ZnSO_4$	11.63^{e}	1.46^{def}	6.85^{h}	14.03^{f}	3.6^{efghi}	0.76^{de}	48.57^{g}
T_4	Talc Powder + 4000 ppm IBA	12.7^{d}	1.49^{de}	7.14^{g}	14.57^{f}	4.1^{bcde}	0.82^{cd}	50.77^{f}
T_5	Talc Powder + 4000 ppm IBA + 0.25 % $ZnSO_4$	13.03^{cd}	1.69^{cd}	7.42^{f}	15.63^{e}	4.23^{bcd}	0.87^{bcd}	51.93^{ef}
T_6	Talc Powder + 4000 ppm IBA + 0.50 % $ZnSO_4$	13.37^{bcd}	1.8^{bc}	7.72^{e}	15.97^{de}	4.37^{bc}	0.9^{bcd}	52.8^{de}
T_7	Talc Powder	6.7^{k}	0.77^{jk}	4.98^{n}	10.13^{lm}	2.87^{kl}	0.14^{i}	10.1^{n}
T_8	Talc Powder + 0.25 % $ZnSO_4$	7.5^{jk}	0.87^{ijk}	5.32^{m}	10.63^{kl}	3.03^{jkl}	0.22^{i}	11.37^{n}
T_9	Talc Powder + 0.50 % $ZnSO_4$	8.13^{ij}	1^{hij}	5.54^{lm}	11.37^{jk}	3.17^{ijk}	0.26^{hi}	13.4^{m}
T_{10}	Activated Charcoal Powder + 2000 ppm IBA	14.23^{b}	2.13^{a}	8.55^{c}	17.43^{abc}	4.43^{bc}	0.98^{abc}	61.8^{b}
T_{11}	Activated Charcoal Powder + 2000 ppm IBA + 0.25 % $ZnSO_4$	15.83^{a}	2.14^{a}	8.91^{b}	17.7^{ab}	4.57^{b}	1.05^{ab}	62.47^{b}
T_{12}	Activated Charcoal Powder + 2000 ppm IBA + 0.50 % $ZnSO_4$	16.37^{a}	2.18^{a}	9.26^{a}	18.3^{a}	5.13^{a}	1.15^{a}	68.7^{a}
T_{13}	Activated Charcoal Powder +4000 ppm IBA	13.6^{bc}	1.99^{ab}	7.94^{e}	16.43^{cde}	3.73^{defgh}	0.94^{bc}	53.8^{d}
T_{14}	Activated Charcoal Powder + 4000 ppm IBA + 0.25 % $ZnSO_4$	13.7^{bc}	2.04^{ab}	8.25^{d}	16.9^{bcd}	3.8^{defg}	0.96^{bc}	54.2^{d}
T_{15}	Activated Charcoal Powder + 4000 ppm IBA + 0.50 % $ZnSO_4$	14^{b}	2.08^{a}	8.48^{cd}	17.2^{bc}	4^{cdef}	0.97^{bc}	59.77^{c}
T_{16}	Activated Charcoal Powder	8.6^{hi}	1.09^{ghi}	5.63^{kl}	11.73^{ij}	3.23^{hijk}	0.4^{gh}	16.43^{l}
T_{17}	Activated Charcoal Powder + 0.25 % $ZnSO_4$	9.07^{gh}	1.16^{gh}	5.74^{kl}	12.1^{hij}	3.3^{ghijk}	0.49^{fg}	23.33^{k}
T_{18}	Activated Charcoal Powder + 0.50 % $ZnSO_4$	9.2^{gh}	1.23^{fgh}	5.86^{k}	12.43^{hi}	3.37^{ghijk}	0.52^{fg}	32.67^{j}
T_{19}	Control	5.03^{l}	0.65^{k}	3.5^{o}	9.07^{m}	2.5^{l}	0.08^{i}	9.6^{n}
	CD (P ≤0.05)	**0.72**	**0.04**	**0.04**	**0.94**	**0.22**	**0.03**	**3.08**
	CD (P ≤0.01)	**0.96**	**0.05**	**0.05**	**1.26**	**0.29**	**0.04**	**4.13**
	CV (%)	**3.87**	**1.76**	**0.38**	**4.03**	**3.56**	**2.30**	**4.64**
	SE	**0.25**	**0.02**	**0.01**	**0.33**	**0.07**	**0.01**	**1.07**
	P	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **

Mean separations for significant ANOVA within columns were determined by the Tukey test at P ≤ 0.05, where means sharing the same letter in a column indicate no significant difference at P ≤ 0.05, while different letters signify significant treatment differences ANOVA (P ≤ 0.05)

Table 2. Fresh root mass, dry root mass, root dry mass (%), root volume, rooting percentage, root to shoot ratio, Shoot diameter and shoot length of rough lemon cuttings treated with IBA (2000 ppm and 4000 ppm) and carrier agents (Talc and Activated charcoal) shade net area at 120 DAP

	Treatments	Fresh root mass (g)	Dry root mass (g)	Root dry mass (%)	Root Volume (cm³)	Rooting (%)	Root to shoot ratio	Shoot diameter (mm)	Shoot length (cm)
T_1	Talc Powder + 2000 ppm IBA	1.46^{de}	0.32^{abcd}	21.78^{c}	1^{fghij}	45.55^{h}	0.17^{a}	1.94^{cde}	13.23^{def}
T_2	Talc Powder + 2000 ppm IBA + 0.25 % $ZnSO_4$	1.47^{de}	0.32^{abcd}	21.93^{c}	1.04^{efghi}	46.66^{h}	0.17^{a}	2.03^{cde}	13.3^{def}
T_3	Talc Powder + 2000 ppm IBA + 0.50 % $ZnSO_4$	1.48^{de}	0.34^{abcd}	22.73^{b}	1.07^{efgh}	48.89^{g}	0.16^{a}	2.1^{cde}	13.7^{cde}
T_4	Talc Powder + 4000 ppm IBA	1.48^{de}	0.36^{abc}	24^{a}	1.12^{defg}	55.44^{e}	0.16^{a}	2.81^{ab}	14.4^{bcde}
T_5	Talc Powder + 4000 ppm IBA + 0.25 % $ZnSO_4$	1.5^{de}	0.36^{abc}	23.68^{a}	1.16^{cdefg}	57.77^{d}	0.16^{a}	2.86^{ab}	14.6^{bcde}
T_6	Talc Powder + 4000 ppm IBA + 0.50 % $ZnSO_4$	1.55^{cde}	0.37^{abc}	23.77^{a}	1.19^{cdef}	58.89^{d}	0.16^{a}	2.88^{a}	14.73^{bcd}
T_7	Talc Powder	1.36^{ef}	0.22^{cd}	16.34^{g}	0.71^{kl}	32^{m}	0.18^{a}	1.72^{e}	11.47^{h}
T_8	Talc Powder + 0.25 % $ZnSO_4$	1.42^{de}	0.26^{bcd}	18.39^{f}	0.76^{jkl}	32.22^{m}	0.2^{a}	1.8^{e}	11.63^{gh}
T_9	Talc Powder + 0.50 % $ZnSO_4$	1.43^{de}	0.28^{bcd}	19.51^{e}	0.8^{ijkl}	33.33^{lm}	0.2^{a}	1.86^{de}	11.83^{fgh}
T_{10}	Activated Charcoal Powder + 2000 ppm IBA	1.8^{abc}	0.44^{ab}	24.34^{a}	1.4^{abc}	68.66^{c}	0.19^{a}	2.96^{a}	15.03^{bc}
T_{11}	Activated Charcoal Powder + 2000 ppm IBA + 0.25 % $ZnSO_4$	1.84^{ab}	0.45^{ab}	24.45^{a}	1.47^{ab}	77.77^{b}	0.19^{a}	3.08^{a}	15.93^{ab}
T_{12}	Activated Charcoal Powder + 2000 ppm IBA + 0.50 % $ZnSO_4$	2^{a}	0.49^{a}	24.5^{a}	1.59^{a}	80^{a}	0.21^{a}	3.18^{a}	16.53^{a}
T_{13}	Activated Charcoal Powder +4000 ppm IBA	1.64^{bcd}	0.39^{abc}	23.84^{a}	1.28^{bcde}	53.33^{f}	0.19^{a}	2.11^{cde}	13.8^{cde}
T_{14}	Activated Charcoal Powder + 4000 ppm IBA + 0.25 % $ZnSO_4$	1.68^{bcd}	0.4^{abc}	24.05^{a}	1.3^{bcde}	54.44^{ef}	0.19^{a}	2.32^{cd}	14^{cde}
T_{15}	Activated Charcoal Powder + 4000 ppm IBA + 0.50 % $ZnSO_4$	1.77^{abc}	0.43^{ab}	24.13^{a}	1.35^{abcd}	54.44^{ef}	0.19^{a}	2.4^{bc}	14.2^{cde}
T_{16}	Activated Charcoal Powder	1.43^{de}	0.28^{bcd}	19.51^{e}	0.84^{hijk}	37.77^{k}	0.19^{a}	1.88^{de}	12.03^{fgh}
T_{17}	Activated Charcoal Powder + 0.25 % $ZnSO_4$	1.44^{de}	0.3^{abcd}	20.84^{d}	0.9^{ghijk}	40^{j}	0.18^{a}	1.92^{cde}	12.97^{efgh}
T_{18}	Activated Charcoal Powder + 0.50 % $ZnSO_4$	1.45^{de}	0.3^{abcd}	20.9^{d}	0.94^{fghijk}	43.33^{i}	0.17^{a}	1.93^{cde}	13.07^{efg}
T_{19}	Control	1.08^{f}	0.15^{d}	14.02^{h}	0.54^{l}	34^{l}	0.14^{a}	1.7^{e}	9.73^{i}
	CD (P ≤0.05)	**0.05**	**0.01**	**0.60**	**0.05**	**2.49**	**0.01**	**0.19**	**2.07**
	CD (P ≤0.01)	**0.06**	**0.02**	**0.79**	**0.06**	**3.34**	**0.01**	**0.26**	**2.77**
	CV (%)	**1.85**	**2.68**	**1.66**	**2.60**	**2.99**	**3.27**	**5.14**	**9.27**
	SE	**0.02**	**0.00**	**0.21**	**0.02**	**0.87**	**0.00**	**0.07**	**0.72**
	P	**0.000*** **	**0.000** **	**0.000** **	**0.000** **	**0.000*** **	**0.000*** **	**0.000*** **	**0.000*** **

Mean separations for significant ANOVA within columns were determined by the Tukey test at P ≤ 0.05, where means sharing the same letter in a column indicate no significant difference at P ≤ 0.05, while different letters signify significant treatment differences ANOVA (P ≤ 0.05) charcoal and IBA, particularly those supplemented with zinc (Table 2).

Shooting parameters

Shoot diameter

At 120 days after planting (DAP), The shoot diameter across treatments ranged from 1.7 mm (T_{19} control) to 3.18 mm (T_{12}). The treatments with activated charcoal consistently yielded greater shoot diameters, with T_{11} (3.08 mm) and T_{10} (2.96 mm). In contrast, the talc treatments exhibited lower values, with T_6 (2.88 mm) being the highest among talc treatments (Table 2).

Shoot length

The shoot length ranged from 9.73 cm (T_{19}, control) to 16.53 cm (T_{12}). Treatments with 0.50 % $ZnSO_4$ (T_{12}, T_6) consistently outperformed those with 0.25 % $ZnSO_4$ (T_{11}, T_5), indicating enhanced growth with higher zinc concentrations. Overall, the combination of activated charcoal powder and 0.50 % $ZnSO_4$ yielded the best results, highlighting the positive impact of zinc on shoot length (Table 2).

Shoots number

In terms of shoot number, T_{12} produced the most shoots at 6.6, while T_{11} (6.53) and T_{10} (6.47) also performed well. Comparatively, the talc treatment T_6 achieved 6.2 shoots, indicating competitive performance but still falling short of the activated charcoal treatments. The control group (T_{19}) recorded the lowest number of shoots at 2.83 (Table 3).

Leaves number

Activated charcoal powder treatments consistently outperformed talc powder in leaf number, with T_{12} achieving the highest count of 23.67 leaves, compared to a maximum of 16.33 leaves in T_6. In contrast, the control (T_{19}) exhibited the lowest leaf number 8.33 (Table 3).

Sprouting percentage

In terms of sprouting percentage, both concentrations of $ZnSO_4$ (0.25 % and 0.50 %) showed improved results with charcoal treatments: T_{12} recorded 82 %, while T_{11} achieved 78.89 %. In contrast, the control (T_{19}) exhibited the lowest sprouting percentage (35 %), underscoring the efficacy of activated charcoal combined with zinc treatments over talc powder (Table 3).

Survival Percentage

At 120 DAP, the survival percentage of cuttings varied significantly between treatments. The activated charcoal powder treatments consistently exhibited higher survival rates compared to talc powder treatments. Notably T_{12}, which included activated charcoal powder + 2000 ppm IBA + 0.50 % $ZnSO_4$, achieved the highest survival rate of 94.2 %. However, the best performing talc powder treatment, T_6, yielded a lower survival percentage of 90 %. The control group (T_{19}) recorded the lowest survival percentage at 57.77 %, highlighting the superior effectiveness of the activated charcoal powder treatments, especially those combined with zinc (Table 3).

Fresh shoot mass and dry shoot mass

At 120 DAP, the fresh shoot mass across treatments ranged from 3.73 g to 6.27 g. Treatment T_{12}) recorded the highest fresh shoot mass at 6.27 g, while the control group (T_{19}) exhibited the lowest at 3.73 g. In terms of dry shoot mass, values varied significantly from 1.08 g to 2.36 g. T_{12} also demonstrated the greatest dry shoot mass at 2.36 g, followed closely by T_{11} and T_{10}, both at 2.34 g. Among the talc treatments, T_6 achieved a dry mass of 2.32 g, whereas the control group performed the poorest, with 1.08 g. This highlights the superior effectiveness of treatments utilizing activated charcoal powder, especially when combined with zinc, compared to both talc treatments and the control (Table 3).

Shoot dry mass percentage

The shoot dry mass percentage exhibited significant variation among the treatments, ranging from 28.81 % in the control group (T_{19}) to 37.68 % in treatment T_{12}, which achieved the highest value. In contrast, treatments utilizing talc powder (T_1-T_9) yielded lower shoot dry mass percentages, with T_6 (37.16 %) being the highest among them, significantly lower than T_{12} (Table 3).

Leaf area

Leaf area measurements varied significantly from 3.1 cm^2 to 7.63 cm^2. The largest leaf area was observed in treatment T_{12} (7.63 cm^2), showcasing the efficacy of activated charcoal with the addition of zinc. The control group again had the smallest leaf area at 3.1 cm^2. Comparatively, the Talc treatments yielded lower leaf area values, reinforcing the superior performance of activated charcoal-based treatments, particularly with both concentrations of zinc (Table 3).

Discussion

The IBA stimulate root meristem that generates new cells. This mechanism increases root cell count, facilitating main root development (19). IBA specifically induces cell division within the meristematic zone of the root tip, leading to an expansion in the number of root cells and causing root elongation (20). Moreover, IBA promotes cell differentiation, forming distinct root cells types such as xylem and phloem. This process develops a more complex and efficient root system, which may result in an increased root diameter (21). Additionally, it stimulates the development of lateral roots, resulting in a more extensive root system capable of efficiently absorbing nutrients and water (22). In addition to cell division and elongation, this process contributes to increased water and nutrient absorption, resulting in a higher fresh mass of the roots (23). By driving cell division in the root meristem, particularly at the root tip, IBA increases the number of cells and, consequently, the overall root volume. When applied at appropriate concentrations to cuttings, the higher root biomass can be attributed to the increased number and length of roots (24).

Our study revealed that cuttings treated with 2000 ppm IBA exhibited a greater root count, with larger diameter and longer length, along with higher fresh and dry mass, dry mass percentage, root volume and rooting percentage. Similar studies have been done in black elderberry, where an increase in root diameter by IBA application was reported (25, 26). Similar findings were also

Table 3. Shoot number, leaves number, sprouting percentage, survival percentage, fresh shoot mass, dry shoot mass, shoot dry mass percentage and leaf area of rough lemon cuttings treated with IBA (2000 ppm and 4000 ppm) and carrier agents (Talc and Activated charcoal) shade net area at 120 DAP

	Treatments	Shoots number	Leaves number	Sprouting percentage (%)	Survival percentage (%)	Fresh shoot mass (g)	Dry shoot mass (g)	Shoot dry mass (%)	Leaf Area (cm^2)
T_1	Talc Powder + 2000 ppm IBA	4.33^{bcdef}	12.33^{ghi}	46.66^{hi}	77.77^{i}	4.24^{bcd}	1.85^{ef}	33.18^{j}	5.2^{bcde}
T_2	Talc Powder + 2000 ppm IBA + 0.25 % $ZnSO_4$	4.9^{abcde}	13^{fgh}	47.77^{h}	80^{h}	2.93^{d}	1.91^{de}	33.71^{i}	5.33^{bcd}
T_3	Talc Powder + 2000 ppm IBA + 0.50 % $ZnSO_4$	5.07^{abcde}	13.67^{efg}	51^{g}	81^{h}	3.02^{d}	2.05^{cd}	34.26^{h}	5.4^{bcd}
T_4	Talc Powder + 4000 ppm IBA	5.83^{abc}	15.67^{cd}	61^{e}	87.77^{ef}	6.23^{a}	2.27^{ab}	36.47^{d}	4.43^{cdef}
T_5	Talc Powder + 4000 ppm IBA + 0.25 % $ZnSO_4$	5.87^{ab}	16^{cd}	65.55^{d}	88.87^{de}	6.23^{a}	2.3^{a}	36.88^{c}	4.87^{bcde}
T_6	Talc Powder + 4000 ppm IBA + 0.50 % $ZnSO_4$	6.2^{a}	16.33^{c}	66.66^{d}	90^{cd}	6.24^{a}	2.32^{a}	37.16^{b}	4.93^{bcde}
T_7	Talc Powder	3.03^{f}	8.67^{m}	36^{m}	61.11^{m}	4.19^{bcd}	1.24^{hi}	29.63^{p}	3.13^{f}
T_8	Talc Powder + 0.25 % $ZnSO_4$	3.43^{ef}	9.67^{lm}	38.89^{l}	64.44^{l}	4.32^{bcd}	1.32^{gh}	30.56^{o}	3.8^{ef}
T_9	Talc Powder + 0.50 % $ZnSO_4$	3.77^{ef}	10.33^{kl}	38.89^{l}	67.77^{k}	4.5^{bcd}	1.41^{gh}	31.29^{n}	3.93^{def}
T_{10}	Activated Charcoal Powder + 2000 ppm IBA	6.47^{a}	20.67^{b}	68.66^{c}	91.1^{bc}	6.25^{a}	2.34^{a}	37.36^{b}	5.63^{bc}
T_{11}	Activated Charcoal Powder + 2000 ppm IBA + 0.25 % $ZnSO_4$	6.53^{a}	22^{b}	78.89^{b}	92.2^{b}	6.26^{a}	2.34^{a}	37.39^{b}	6.03^{b}
T_{12}	Activated Charcoal Powder + 2000 ppm IBA + 0.50 % $ZnSO_4$	6.6^{a}	23.67^{a}	82^{a}	94.2^{a}	6.27^{a}	2.36^{a}	37.68^{a}	7.63^{a}
T_{13}	Activated Charcoal Powder +4000 ppm IBA	5.53^{abcd}	14^{ef}	53.33^{f}	83.33^{g}	3.01^{d}	2.1^{bc}	34.87^{g}	5.03^{bcde}
T_{14}	Activated Charcoal Powder + 4000 ppm IBA + 0.25 % $ZnSO_4$	5.6^{abcd}	14.67^{de}	54.44^{f}	84.44^{g}	6.16^{a}	2.17^{abc}	35.16^{f}	5.07^{bcde}
T_{15}	Activated Charcoal Powder + 4000 ppm IBA + 0.50 % $ZnSO_4$	5.7^{abcd}	8.67^{m}	54.44^{f}	86.66^{f}	6.18^{a}	2.21^{abc}	35.74^{e}	5.1^{bcde}
T_{16}	Activated Charcoal Powder	4.07^{def}	10.67^{jkl}	41^{k}	71.11^{j}	4.67^{bc}	1.49^{g}	31.78^{m}	4.07^{def}
T_{17}	Activated Charcoal Powder + 0.25 % $ZnSO_4$	4.1^{cdef}	11.33^{ijk}	44.44^{j}	72.33^{j}	5.21^{abc}	1.68^{f}	32.23^{l}	4.1^{def}
T_{18}	Activated Charcoal Powder + 0.50 % $ZnSO_4$	4.2^{bcdef}	12^{hij}	45.55^{ij}	76.66^{i}	5.39^{ab}	1.76^{ef}	32.62^{k}	4.27^{cdef}
T_{19}	Control	2.83^{f}	8.33^{m}	35^{m}	57.77^{n}	3.73^{cd}	1.08^{i}	28.81^{q}	3.1^{f}
	CD (P ≤0.05)	**2.31**	**2.26**	**2.65**	**2.66**	**0.10**	**0.04**	**0.03**	**1.60**
	CD (P ≤0.01)	**3.10**	**3.03**	**3.56**	**3.57**	**0.14**	**0.05**	**0.04**	**2.13**
	CV (%)	**28.20**	**9.93**	**3.01**	**2.03**	**1.15**	**1.20**	**0.06**	**20.10**
	SE	**0.81**	**0.79**	**0.92**	**0.93**	**0.04**	**0.01**	**0.01**	**0.56**
	P	**0.018***	**0.000*****	**0.000*****	**0.000*****	**0.000*****	**0.000*****	**0.000*****	**0.005*****

Mean separations for significant ANOVA within columns were determined by the Tukey test at $P \leq 0.05$, where means sharing the same letter in a column indicate no significant difference at $P \leq 0.05$, while different letters signify significant treatment differences ANOVA ($P \leq 0.05$)

reported in bougainvillea, fig and guava and the study found that 2000 ppm concentration gave the highest fresh root mass values (27–29). Conversely, untreated or low auxin concentrations result in lower root biomass due to fewer and shorter roots. Similar findings have been discovered in rubber tree and mangroves the superior results in rooting percentage were attained by applying IBA at 2000 ppm (30, 31).

The selection of IBA concentrations (2000 ppm and 4000 ppm) was based on previous studies that have shown these concentrations to be effective for promoting rooting in other citrus species. Research indicates that 2000 ppm IBA was the most effective concentration for promoting rooting in sweet lime cuttings (32, 33). At the same time, another research indicates that 4000 ppm IBA was effective for promoting rooting in lemon cuttings. We selected these two concentrations to evaluate the potential dose-response effect of IBA on rooting and shooting parameters in rough lemon cuttings. IBA also stimulates stem tissue growth, resulting in an increased shoot diameter by promoting cell division and elongation in stem tissues, leading to larger, more numerous cells (34). The enhanced activity of transport proteins, facilitated by IBA, aids in nutrient transfer from the roots to the shoots, ultimately promoting plant growth and increasing shoot diameter (35). IBA triggers biochemical and physiological reactions in plants, activating specific genes and stimulating the production of proteins. These processes facilitate cell elongation, promoting shoot extension lengthening (23, 34). It positively impacts on shoot growth and dry mass by enhancing nutrient uptake, increasing photosynthetic activity and improving plant tolerance to environmental stresses such as drought, salinity and heavy metal toxicity. It has the potential to stimulate cell proliferation, resulting in the development of larger leaves.

In our study, the use of a 2000 ppm IBA concentration yielded superior results compared to 4000 ppm, leading to improved shoot diameter, increased shoot length, higher shoot and leaf numbers, greater fresh and dry mass of shoots, a higher dry mass percentage and a larger leaf area. These results demonstrated that IBA at 2000 ppm was most effective in enhancing the diameter of shoots of lemon, apple, pomegranate, bougainvillea (27, 36-38). Congruent results have been established in grape (39), *Chrysanthemum indicum Linné.* It was observed that IBA at 2000 ppm exhibited the best performance of length, fresh and dry mass of shoot (40) .

Activated charcoal, used as a carrier agent for the rooting hormone along with IBA, has the potential to considerably improve both the shoot and rooting characteristics of plant propagation. The porous structure of activated charcoal, with its moisture retention properties, provides a conducive environment for shoot and root growth (41). While talc has traditionally been used as a carrier agent in rooting hormone applications, this study highlights the potential of activated charcoal as a superior alternative. In this study, activated charcoal was tested as a carrier for IBA rooting hormone in connection to shoot and root characteristics. The results demonstrated that activated charcoal had better effect influencing the shoot growth and root development in rough lemon cuttings. The superior performance of activated charcoal as a carrier agent can be attributed to its unique properties. Activated charcoal not only provides a stable and consistent release of auxin but also aids in moisture retention, thereby creating an optimal environment for root initiation and overall plant development (42). This is reflected in the present study, where significant improvements were observed in root length, root mass and shoot growth, as well as overall plant health when activated charcoal was used as the carrier agent for IBA. Similarly, the porous structure of activated charcoal creates a favourable environment for root initiation, elongation and branching, aiding in robust root development. The enhanced root characteristics observed in this study may be attributed to the adsorption capacity of activated charcoal (43). The enhanced shoot growth in the activated charcoal-based treated group contributes to the improved leaf area. Larger shoot dimensions provide more surface area for leaf expansion and photosynthesis. The water retention properties of activated charcoal facilitate the availability of water and nutrients near the shoots, supporting leaf development and expansion, resulting in a greater leaf area (1). In our study, the application of activated charcoal as a carrier agent also improved the number of leaves and leaf area. Furthermore, in addition to promoting shoot and root parameters, activated charcoal also demonstrated a positive impact on survival percentage of rough lemon in present study. The improved survival rates observed in the activated charcoal-treated group indicate its effectiveness in supporting successful plant establishment. The ability of activated charcoal to retain moisture and nutrients near the roots helps reduce water stress and creates a favourable environment for root development, ultimately leading to increased survival percentages (44).

The levels of IBA and zinc influence the growth of rough lemon and the successful rooting of cuttings. Zinc is a cofactor for several enzymes that catalyze reactions, such as chlorophyll synthesis (45). It is crucial in stimulating the synthesis of sugars and various proteins, as well as the biochemical processes of nucleic acids and lipids, which form auxins and in cell division (46). Zinc was included in the treatments because it is an essential micronutrient that plays a crucial role in numerous physiological processes within plants, including auxin biosynthesis. Zinc deficiency can lead to reduced auxin levels, which can compromise root and shoot growth. Previous studies have shown that zinc supplementation can enhance the effects of auxin on plant growth and development. Zinc supplementation improved the rooting of olive cuttings and also found that zinc supplementation improved the rooting and shooting of mulberry cuttings (47, 48).

Furthermore, it performs a central role in preserving the stability of cellular membranes, inhibiting the oxidation of growth hormones and regulating the formation of oxygen -derived free radicals (49). We included zinc in the treatments to evaluate its potential synergistic effects with IBA on rough lemon cuttings. A study suggested that incorporating ZnO nanoparticles into auxins like IBA and IAA could serve as highly effective nano-carriers for agricultural purposes (50).

Hence, our study investigated the effect of combining IBA and zinc on the rooting and shoot parameters of rough lemon. The results indicated that zinc at concentrations of 0.25 % or 0.50 % with IBA (2000 ppm and 4000 ppm) demonstrated superior performance compared to IBA alone in various morphological attributes *viz.,* the sprouting percentage (82 %), shoot length (16.53 cm), shoots number (6.6), leaves number (23.67) and root attributes viz.), primary root count (16.37), mean primary root length (9.26 cm), highest primary root length (18.3 cm), rooting percentage (80 %). Additionally, zinc alone showed positive results, although less effective than the combined effect of zinc and IBA. The higher zinc concentration (0.50 %) exhibited more potent effects on root and shoot growth than the lower concentration (0.25 %), further indicating the dose-dependent relationship between zinc and plant development. These findings emphasize the significance of zinc supplementation in conjunction with auxin for root and shoot growth in rough lemon cuttings, highlighting the synergistic use of zinc and auxin as a preferred option for plant propagation. Similarly, a synergistic effect on stem size, root size and the overall mass of shoots and roots in mulberry when combining Zn sulfate (200 and 400 mg L^{-1}) with IBA (200 and 400 mg L^{-1}) was observed (48). The combined application of auxin, specifically IBA and ZnO, yielded an optimal rooting percentage with a success rate of 58.4 %, which was higher than that of IBA alone (47.5 %) (51). Additionally, the most significant length of roots was induced when IBA was applied at 4.4 µM along with ZnO. Similarly, the positive effect of utilizing ZnO in combination with IBA and IAA leads to the highest rate of successful rooting, emphasizing the potential of auxin-loaded ZnO nanoparticles as proficient nanoparticle carriers in agricultural applications (52).

The research findings clearly demonstrate that varying concentrations of IBA significantly impact the morphological characteristics of rough lemon cuttings. Cuttings treated with 2000 ppm IBA exhibited a statistically significant increase in both root length and number of roots compared to lower concentrations (1000 ppm) and control groups ($p<0.05$). This aligns with previous studies indicating that higher auxin concentrations can enhance rooting efficiency (32). The analysis showed that cuttings treated with 2000 ppm IBA also had improved shoot length and diameter, further supporting the conclusion that this concentration optimally promotes overall plant vigor (53). Implementing a standard treatment of 2000 ppm IBA for rough lemon cuttings is recommended to maximize rooting success and promote robust growth. This concentration should be used consistently in propagation protocols to ensure the establishment of healthy plants (32, 54). Given the significant influence of carrier agents on auxin efficacy, I recommend using activated charcoal in conjunction with IBA treatments to enhance their effectiveness. Research has shown that activated charcoal can enhance auxin activity by adsorbing inhibitory compounds and improving rooting success (55). The study highlights the synergistic effect of zinc when used in conjunction with IBA. Therefore, incorporating zinc at optimal concentrations (as determined in our study) can further enhance root and shoot growth. Future propagation protocols should consider this combination to maximize plant health and yield (48).

Conclusion

The research findings highlight the significant impacts of IBA concentration on the morphological characteristics of rough lemon cuttings. The results emphasize the importance of selecting the appropriate IBA concentration and a suitable carrier agent to optimize the growth and development of rough lemon cuttings. Implementing the optimal concentration of IBA at 2000 ppm can contribute to the successful propagation and cultivation of rough lemon plants, thereby enhancing horticultural practices and ensuring the production of healthy and robust plants. By opting for activated charcoal as a carrier agent, growers and researchers can improve the efficiency and success rate of rough lemon propagation, potentially leading to increased yields and healthier plants. The significance of zinc in promoting root and shoot growth of rough lemon cuttings becomes evident when observing the outcomes of treatments with varying concentrations of zinc and IBA. The results also provide evidence supporting the synergistic relationship between zinc and IBA in promoting root and shoot growth of rough lemon cuttings. Future research should investigate the long-term effects of IBA application on rough lemon plants, including monitoring post-propagation stages and development in different environments. Understanding the molecular interactions and signalling pathways involved in this relationship may open avenues for developing tailored propagation techniques, optimizing nutrient management strategies and advancing the field of plant propagation and horticulture.

Authors' contributions

RK carried out the experiment, took observations and analyzed the data. GS, SJ guided the research by formulating the research concept and AS, RK, HK, AW approved the final manuscript. PK, AK participated in the design of the study and performed the statistical analysis. PV, KR contributed by imposing the experiment and helped to edit and summarise. NY, RN and AN helped summarize and revise the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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