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The Rooting Performances and Some Chemical Constituents of Rosemary (*Rosmarinus Officinalis*) Stem Cuttings as Influenced by IBA Concentration and Medium Type

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Abstract

A pot experiment was done at glass greenhouse conditions at Egyptian German Company for Agricultural Production (EGAP), Sharkia Governorate, Egypt during the two consecutive summer seasons of 2023 and 2024. This study aimed to enhancing the rooting performance (root length, root fresh and dry weights and root anatomy) as well as some chemical constituents in leaves and roots of rosemary stem cuttings. The main factor was rooting medium (coco peat, coco peat: perlite, coco peat: sand, peat moss, peat moss: perlite, peat moss: sand as 1: 1 V/V), while, the second factor was IBA concentration (0.0, 1250, 2500 and 3750 ppm), beside the combination influence between these two key factor. The obtained results referred to that planting rosemary in Coco peat medium combined with an IBA concentration of 3750 ppm is strongly recommended, as this interaction consistently produced the highest root fresh weight, dry weight, and root length across both seasons. To maximize leaf chemical content in rosemary, utilizing an IBA concentration of 3750 ppm in combination with Peat moss + Sand for total carbohydrates (28.665%) and Coco peat + Sand for potassium (1.923%) or Coco peat alone for nitrogen (2.735%) is recommended. Furthermore, applying 2500 ppm IBA in Coco peat + Sand provides the highest phosphorus accumulation.

Keywords: Rosemary, IBA, Rooting medium, anatomy, carbohydrates

1. Introduction

Rosmarinus officinalis L., commonly known as rosemary or *Hassalban*, belongs to the family Lamiaceae. It is an evergreen, aromatic shrub indigenous to the Mediterranean region, widely acclaimed for its substantial medicinal, economic, and industrial significance. Rosemary is rich in potent bio-active compounds - including carnosic acid, rosmarinic acid and essential oils rich in 1,8-cineole and camphor - which lend the plant remarkable antioxidant, antimicrobial, and anti-inflammatory properties (**Andrade *et al.*, 2018**; **Borges *et al.*, 2019**). These biochemical attributes make rosemary an essential raw material across the pharmaceutical, cosmetic, and food preservation industries. Furthermore, its essential oil is extensively used in aromatherapy and personal care products for its distinct fragrance and therapeutic benefits (**Al-Sereiti *et al.*, 1999**).

Although *Rosmarinus officinalis* can be propagated by both seeds and vegetative methods, seed propagation presents several major horticultural limitations. Studies show that rosemary seeds exhibit naturally poor and erratic germination rates—often below 30% to 50%—due to seed coat dormancy, low embryo viability, and high sensitivity to moisture and temperature fluctuations (**Geneve, 2004; El-Dengawy *et al.*, 2011**). Furthermore, because rosemary is an open-pollinated species, plants grown from seed exhibit significant genetic segregation and phenotypic variability, leading to inconsistent growth rates, unpredictable essential oil yields, and varying chemical profiles (monoterpene composition) among individual plants (**Tattini *et al*., 2006; Santos *et al*., 2016**). To overcome these constraints and satisfy commercial standards, propagation via stem cuttings is widely recognized as the superior method. Vegetative propagation using stem cuttings bypasses seed dormancy issues, ensures high rooting percentages when treated with proper auxins, and critically, maintains true-to-type clonal fidelity—preserving the desirable biochemical attributes and elite traits of the donor mother plant (**Hartmann *et al*., 2011**; **Monder and Kozakiewicz, 2019**).

The selection of an appropriate growing medium plays a pivotal role in the vegetative propagation and subsequent root establishment of stem cuttings. Growing media directly regulate physical and chemical properties essential for root development, including aeration, moisture retention capacity, bulk density, and nutrient availability (**Bilderback *et al*., 2005**). A balanced substrate provides adequate root anchorage while preventing hypoxia around the developing root system, thereby enhancing initial root initiation, stem elongation, and overall vegetative performance of cuttings (**Trevisan *et al*., 2008**).

To overcome delayed rooting and improve propagation efficiency, the application of exogenous rooting hormones—particularly auxin compounds—is standard practice in commercial horticulture. Among these, Indole-3-butyric acid (IBA) is widely recognized as the most effective synthetic auxin for promoting adventitious root formation in stem cuttings (**Hartmann *et al*., 2011**). IBA exhibits high stability against enzymatic degradation within plant tissues and triggers cell division in the cambium zone, accelerating both root initiation and early vascular connection (**Kundu *et al*., 2017**). Previous studies have demonstrated the synergistic effect of substrate formulation and IBA application on various aromatic and woody species. For instance, research by **Blythe *et al.* (2007)** and **El-Hawary *et al.* (2020)** confirmed that combining optimum growth media (such as peat moss and perlite mixtures) with targeted IBA concentrations significantly increases rooting percentage, root number and fresh/dry root mass in hard-to-root Lamiaceae cuttings.

Consequently, the primary objective of this study is to evaluate the influence of various growing media combinations and Indole-3-butyric acid (IBA) concentrations on the rooting efficiency, survival rate, and early shoot growth of rosemary stem cuttings. By optimizing these key propagation factors, this research aims to establish an effective protocol for the rapid, large-scale production of high-quality rosemary planting transplants.

2. Materials and Methods

Experimental Site and Plant Material

A pot experiment was conducted in the glass greenhouse of the Egyptian German Company for Agricultural Production (EGAP), Sharkia Governorate, Egypt (30°36' N; 31° 30' E), during two consecutive summer seasons of 2023 and 2024.

Tip stem cuttings of rosemary (*Rosmarinus officinalis* L.) were collected on February 24, 2023 and February 26, 2024, from 6–8-month-old healthy, field-grown mother plants located at the EGAP farm. Cuttings were standardized to a uniform length of approximately 10 cm, a stem diameter ranging from 0.50 to 0.75 cm, and retained 12–14 leaves per cutting.

Experimental Factors and Preparation of Treatments

The study evaluated the interactive effects of two main factors: Growing media and auxin (IBA) concentrations.

1. Rooting Media Formulations

Six substrate combinations were evaluated:

- M_1: 100 % Peat moss.

- M_2: Peat moss + Perlite (1:1, v/v).
- M_3: Peat moss + Sand (1:1, v/v).
- M_4: 100% Coco peat.
- M_5: Coco peat + Perlite (1:1, v/v).
- M_6: Coco peat + Sand (1:1, v/v).

2. Indole-3-Butyric Acid (IBA) Preparations

Indole-3-butyric acid (IBA) was applied as a talc-based powder formulation using pharmaceutical-grade talcum powder (magnesium silicate) as the carrier matrix. Four IBA concentrations were prepared:

- I_0: 0.0 ppm (Control).
- I_1: 1250 ppm.
- I_2: 2500 ppm.
- I_3: 3750 ppm.

Planting Procedure

Prior to planting, the basal ends (2–3 cm) of the standardized cuttings were briefly moistened with distilled water, dipped into the respective IBA powder formulations to ensure uniform coating, and immediately stuck into 16-cm diameter plastic pots pre-filled with the designated substrate treatments. Two cuttings were planted per pot.

Experimental Design and Layout

The experiment was arranged in a **Split-Plot Design** with three replications per treatment combination (**Gomez and Gomez, 1984**).

- **Main plots:** Six substrate types (M1–M6).
- **Sub-plots:** Four IBA concentrations (I0–I3).

Each experimental unit within a treatment comprised 48 cuttings (24 pots per replicate), yielding a total of 1,152 cuttings per season.

Data Collection

All vegetative growth parameters and adventitious rooting characteristics were evaluated 55 days post-planting:

A. Adventitious Rooting Characteristics

1. **Root length cm):** Measured from the stem base to the distal tip of the longest primary root.
2. **Root fresh weight per cutting (g):** Recorded after washing substrate residue off the root system and blotted dry with tissue paper.
3. **Root dry weight per cutting (g):** Determined following oven-drying at 70° C to a constant mass.
4. **Root anatomical Studies:**

The anatomical studies were carried out to follow the changes occurring in adventitious root of rosemary plant at the age of 120 days as affected by different types of soil of control plants (pet moss) and those of (coco peat) and (cocopeat + perlite) and plants treated with 7.5 ppm IBA. Specimens were killed and fixed for at least 48 hr. in FAA (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series, embedded in paraffin wax of 56ºC melting point, sectioned to a thickness of 20 microns, double stained with safranin and fast green, cleared in xylene and mounted in Canada balsam (**Nassar and El-Sahhar, 1998**). Sections were examined to detected histological manifestations of the chosen treatments and photomicrograph.

B. Chemical constituents

1- N, P and K percentages

In leaves of rosemary samples were for determining nitrogen, phosphorus and potassium percentages according to the methods of **AOAC (2005)**.

2. Total carbohydrate percentage

Total carbohydrate percentage was determined in rosemary leaves according to **Dubios *et al.* (1956)**.

Statistical Analysis

Data collected from both seasons were subjected to Analysis of Variance (ANOVA) using the Statistix 9.0 software package (**Analytical Software, 2008**) in accordance with the split-plot procedure outlined by **Gomez and Gomez (1984)**. Treatment means were statistically compared using the Least Significant Difference (L.S.D.) test at a probability level of P at 0.05.

3. Results and Discussion

Adventitious rooting characteristics

1. Root fresh and dry weights and root length

Effect of media type

Across both experimental seasons, media type exerted a statistically significant influence ($P \leq 0.05$) on all root physical and quantitative parameters (Tables 1, 2 and 3). Coco peat (alone) emerged as the overall superior substrate, recording the highest averages for root fresh weight (6.48 g in 1st season and 6.08 g in 2nd season), root length (22.08 cm in 1st season and 20.75 cm in 2nd season), and root dry weight in the first season (2.06 g), while coco peat + perlite gave the maximum root dry weight in the second season (1.85 g). Conversely, mixtures containing sand (specifically peat moss + sand and coco peat + sand) consistently exhibited the lowest values across all root biomass metrics.

The consistent superiority of organic fiber-based media, particularly coco peat, is directly linked to its balanced physical architecture, characterized by high capillary water retention coupled with optimal air-filled porosity. This micro-environment supports rapid metabolic respiration within the rhizosphere, encouraging vigorous cell expansion and linear elongation of adventitious roots without the risk of oxygen deficiency or hypoxia. Additionally, coco peat features low bulk density and minimal mechanical resistance, enabling root primordia to penetrate and elongate freely, resulting in longer root channels (Table 3) that accumulate substantial fresh biomass (Table 1) and structural dry matter (Table 2). In contrast, incorporation of sand increases overall bulk density and compaction, presenting physical barriers to root penetration while accelerating drainage, ultimately restricting both root elongation and biomass accumulation.

These findings are in strong alignment with **El-Ziat and Abdel-Wahab (2023)**, who demonstrated that coco peat media significantly enhanced root fresh/dry weights and length in stem cuttings of rosemary compared to sand-amended media. Similarly, **Silva *et al.* (2023)** highlighted that substrates providing low physical impedance paired with high moisture retention promote enhanced cell division within root apical meristems, directly boosting root structural biomass across *Lamiaceae* species.

Table 1. Effect of media type (M), IBA concentration (A) and their combinations (M×A) on root fresh weight (g) of rosemary (*Rosmarinus officinalis* L.) during the two seasons

Medium type	IBA concentration (ppm)				Mean (M)
	0.0	1250	2500	3750	
	First season				
Peat moss	3.75	5.10	5.53	6.30	**5.17**
Peat moss + Perlite	4.27	5.30	5.43	6.68	**5.42**
Peat moss + Sand	3.12	3.40	3.94	4.11	**3.64**
Coco peat	5.28	5.54	6.47	8.64	**6.48**
Coco peat + Perlite	4.19	4.52	5.60	6.10	**5.11**
Coco peat + Sand	3.65	4.26	5.11	6.58	**4.90**
Mean (A)	**4.04**	**4.69**	**5.35**	**6.40**	
L.S.D. at 5 %	**M= 0.19**		**A= 0.11**	**M×A= 0.31**	
	Second season				
Peat moss	3.65	4.18	4.87	5.74	**4.61**
Peat moss + Perlite	4.67	5.21	5.65	5.97	**5.37**
Peat moss + Sand	2.97	3.53	4.03	4.16	**3.67**
Coco peat	4.89	5.26	6.29	7.89	**6.08**
Coco peat + Perlite	4.57	5.04	5.88	6.34	**5.46**
Coco peat + Sand	4.02	4.51	4.85	5.73	**4.78**
Mean (A)	**4.13**	**4.62**	**5.26**	**5.97**	
L.S.D. at 5 %	**M= 0.04**		**A= 0.04**	**M×A= 0.10**	

Table 2. Effect of media type (M), IBA concentration (A) and their combinations (M×A) on root dry weight (g) of rosemary (*Rosmarinus officinalis* L.) during the two seasons

Medium type	IBA concentration (ppm)				Mean (M)
	0.0	1250	2500	3750	
	First season				
Peat moss	1.26	1.33	1.51	1.52	**1.41**
Peat moss + Perlite	1.36	1.42	1.45	1.52	**1.44**
Peat moss + Sand	1.26	1.29	1.30	1.38	**1.31**
Coco peat	1.70	1.88	2.24	2.35	**2.06**
Coco peat + Perlite	1.40	1.51	1.83	2.11	**1.71**
Coco peat + Sand	1.34	1.46	1.57	1.71	**1.52**
Mean (A)	**1.39**	**1.48**	**1.65**	**1.77**	
L.S.D. at 5 %	**M= 0.04**		**A= 0.04**	**M×A= 0.09**	
	Second season				
Peat moss	1.26	1.34	1.35	1.42	**1.34**
Peat moss + Perlite	1.27	1.34	1.39	1.45	**1.36**
Peat moss + Sand	1.21	1.31	1.38	1.47	**1.34**
Coco peat	1.53	1.61	1.82	2.06	**1.76**
Coco peat + Perlite	1.71	1.78	1.80	2.12	**1.85**
Coco peat + Sand	1.46	1.63	1.69	1.92	**1.68**
Mean (A)	**1.41**	**1.50**	**1.57**	**1.74**	
L.S.D. at 5 %	**M= 0.03**		**A= 0.02**	**M×A= 0.05**	

Table 3. Effect of media type (M), IBA concentration (A) and their combinations (M×A) on root length (cm) of rosemary (*Rosmarinus officinalis* L.) during the two seasons

Medium type	IBA concentration (ppm)				Mean (M)
	0.0	1250	2500	3750	
	First season				
Peat moss	17.00	20.67	22.33	24.33	**21.08**
Peat moss + Perlite	15.00	17.67	21.00	24.67	**19.58**
Peat moss + Sand	15.67	17.67	21.00	24.00	**19.58**
Coco peat	16.33	20.67	24.00	27.33	**22.08**
Coco peat + Perlite	16.00	19.00	22.33	27.00	**21.08**
Coco peat + Sand	14.33	14.67	17.00	21.67	**16.92**
Mean (A)	**15.72**	**18.39**	**21.28**	**24.83**	
L.S.D. at 5 %	**M= 0.39**	**A= 0.40**		**M×A= 0.94**	
	Second season				
Peat moss	16.67	18.67	20.67	22.33	**19.58**
Peat moss + Perlite	14.00	19.00	22.00	23.67	**19.67**
Peat moss + Sand	16.67	18.67	22.00	23.33	**20.17**
Coco peat	15.33	19.00	22.33	26.33	**20.75**
Coco peat + Perlite	16.33	17.33	21.33	24.67	**19.92**
Coco peat + Sand	14.67	16.00	18.33	21.67	**17.67**
Mean (A)	**15.61**	**18.11**	**21.11**	**23.67**	
L.S.D. at 5 %	**M= 0.66**	**A= 0.49**		**M×A= 1.22**	

Effect of IBA Concentration

Increasing Indole-3-butyric acid (IBA) concentration from 0.0 to 3750 ppm caused a progressive, statistically significant increase across all evaluated root parameters in both seasons. Treatment with 3750 ppm IBA yielded the highest seasonal averages for root fresh weight (6.40 g in 1st season and 5.97 g in 2nd season), root dry weight (1.77 g in 1st season and 1.74 g in 2nd season), and root length (24.83 cm in 1st season and 23.67 cm in 2nd season). Control treatments (0.0 ppm) consistently recorded the lowest quantitative values across all traits.

Exogenous application of IBA acts as a powerful morphogenetic trigger that accelerates adventitious root initiation and systemic root development. Auxins stimulate cambial activity, cell division, and cell elongation at the basal cutting zone. Higher IBA concentrations (3750 ppm) enhance hydrolytic activity, accelerating the mobilization of stored carbohydrates, proteins, and mineral reserves toward the root initiation sites. This metabolic sink activity promotes not only longer primary root axes (Table 3) but also increases root branching and lateral root density, which together substantially increase total fresh biomass (Table 1) and accumulated structural dry matter (Table 2).

These observations are supported by **Al-Menaie *et al.* (2022)** confirmed that elevated auxin levels directly stimulate cell wall loosening enzymes (e.g., expansions), leading to extensive root cell elongation and increased tissue fresh and dry weight in aromatic plant cuttings. Furthermore, **Hassan and Ibrahim (2024)**, who reported that applying 3000 ppm IBA to rosemary cuttings yielded maximal root length, branch numbers, and dry weight accumulation.

Interaction Effect between media type and IBA concentration

The interaction between media type and IBA concentration (M×A) significantly affected root fresh weight, root dry weight, and root length in both seasons ($P \leq 0.05$). The combination of coco peat + 3750 ppm IBA delivered the highest overall performance for root fresh weight (8.64 g in 1st season and 7.89 g in 2nd season) and root length (27.33 cm in 1st season and 26.33 cm in 2nd season), as well as root dry weight in the first season (2.35 g). For root dry weight in the second season, coco peat + perlite + 3750 ppm IBA recorded the highest value (2.12

g), closely followed by coco peat + 3000 ppm IBA (2.06 g). In contrast, non-treated controls (0.0 ppm IBA) in sand-containing or heavy media consistently produced the lowest values across all three traits.

The marked interactive enhancement reflects a strong physiological synergy between chemical growth stimulation (exogenous auxin) and physical environment optimization (substrate architecture). IBA at 3750 ppm provides the primary biochemical stimulus for rapid root primordial differentiation and cell elongation. Coco peat (alone or blended with perlite) provides the ideal physical substrate—low bulk density, ample aeration, and consistent moisture—enabling developing roots to express their full genetic and auxin-induced growth potential. As a result, roots elongate extensively without physical impedance, absorb water and nutrients efficiently, and build up high fresh and dry biomass.

This synergistic outcome corroborates the findings of **El-Ziat and Abdel-Wahab (2023)**, who observed peak rooting traits (length, fresh, and dry mass) when combining optimal organic substrates with high IBA dosages (3750 ppm). Likewise, **Hassan & Ibrahim (2024)** validated that integrating high-porosity media with target auxin treatments is essential for optimizing overall root architecture and dry mass accumulation in *Rosmarinus officinalis*.

2. Anatomy of adventitious root

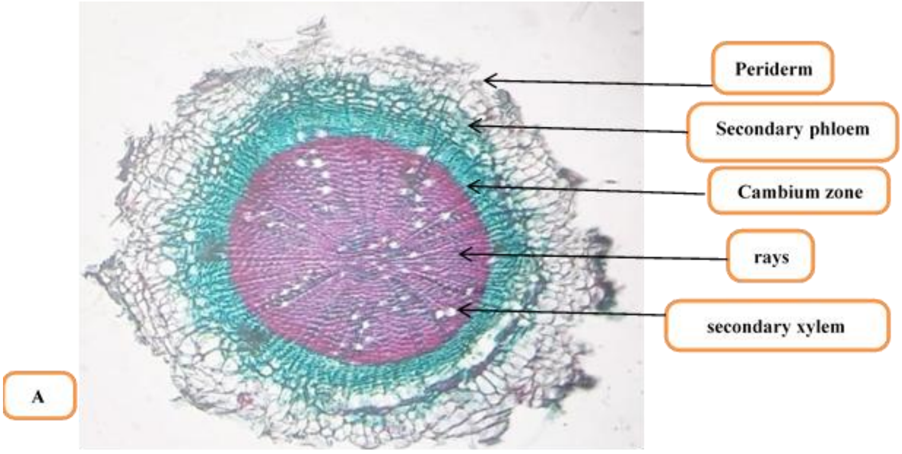
Microscopically measurements of certain histological characters of adventitious root of rosemary plant as affected by different types of soil of control plants (peat moss) and those of (coco peat) and (coco peat + perlite) and plants treated with 3750 ppm IBA in Table 4. Likewise, microphotographs depict these treatments are shown in Figure 1.

It is noted that sections taken from the roots of plants growing in coco peat increase root diameter of rosemary by 3 % more than the control. The increase in root diameter could be attributed mainly to the increase in all included tissues except periderm. The thickness of cambium, phloem tissue and xylem tissue were 50 , 5 and 8.6% more than the control, respectively. It is clear that the decrease in periderm and vessel diameter by 30, 16.7 % less than the control, respectively.

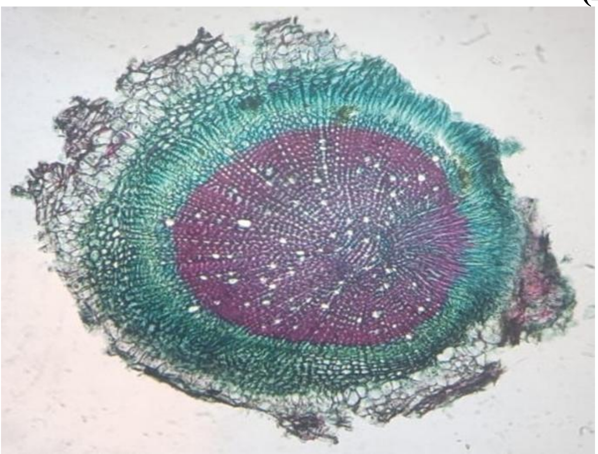
It is noted that sections taken from the roots of plants growing in coco peat + perlite increase root diameter of rosemary by 37.8% more than the control. The increase in root diameter could be attributed mainly to the increase in all included tissues. The thickness of cambium, phloem tissue and xylem tissue were 58.3, 34.8 and 52.1% more than the control, respectively. It is clear that the increase in vessel diameter by 16.6 % over the control, respectively.

Table 4. measurements in microns of certain histological features in transverse sections through adventitious root of rosemary plant at the age of 120 days, as affected by different types of media

Histological characters (µm)	Treatments								
	Peat moss (control)	Coco peat	± % to control	Coco peat + Perlite	± % to control	Coco peat + IBA 3750 ppm	± % to control	Coco peat + Perlite + IBA 3750 ppm	± % to control
Root diameter	863	889	3 +	1190	37.8+	930	7.7+	964	11.7+
Thickness of periderm	99	69.3	30.0 -	99	-	105.6	6.6+	118.8	20+
Thickness of cambium	39.6	59.4	50 +	62.7	58.3 +	52.8	33.3+	59.4	50+
Thickness of phloem tissue	66	69.3	5.0+	89	34.8 +	102.3	55 +	105.6	60+
Thickness of xylem tissue	227.7	247.5	8.6 +	346.5	52.1+	204.6	10-	198	13-
vessels diameter	19.8	16.5	16.7-	23.1	16.6 +	23.1	16.6 +	29.7	50 +



(A)



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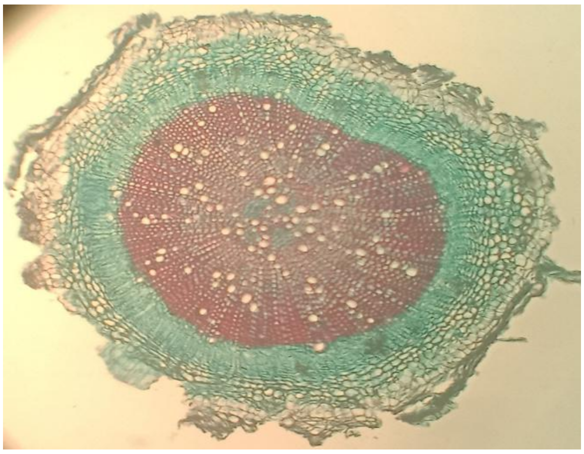

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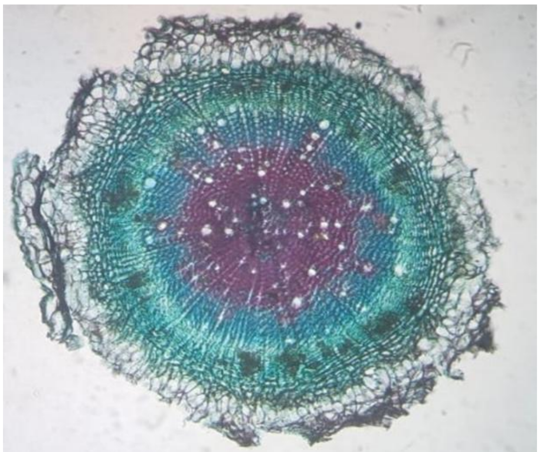

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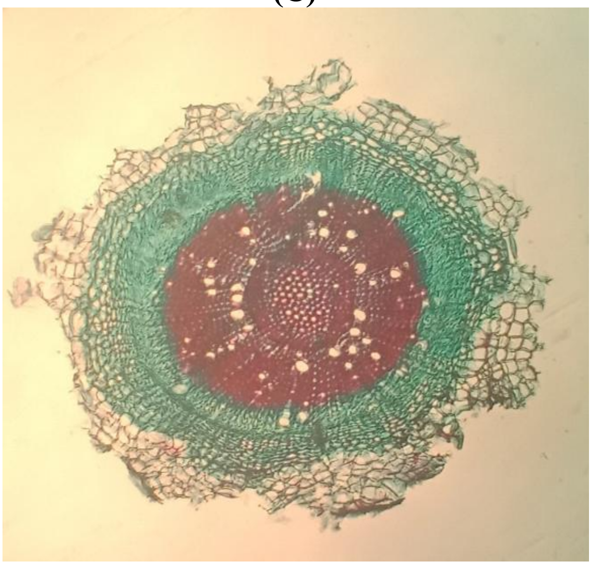

(E)

Fig. 1. Transverse sections through adventitious root of rosemary as affected by different types of soil of control plants (peat moss) and those of (coco peat) and (coco peat + perlite) and plants treated with 3750 ppm IBA (100^x) A. From plant grown in peat moss (control), B. From plant grown in coco peat, C. From plant grown in coco peat + perlite, D. From plant grown in coco peat and treated with 3750 ppm IBA and E. From plant grown in coco peat + perlite and treated with 3750 ppm IBA.

Sections taken from plants treated with 3750 ppm IBA and grown in coco peat increase in all included tissues except that of xylem tissue decrease by 10 %. Treated with 3750 ppm IBA increased root diameter by 7.7 % more than control. The increase in root diameter due to treated with 3750 ppm IBA, could be attributed to increase in periderm, cambium**,** phloem tissue were 6.6, 33.3 and 55% more than those control, respectively. It is seen that the increase in vessel diameter by 16.6 %.

Sections taken from plants treated with 3750 ppm IBA and grown in coco peat + perlite, it gave the same trend of result as in the previous treatment. Increase in all included tissues except that of xylem tissue decrease by 13 %. Treated with 3750 ppm IBA increased root diameter by 11.7% more than control. The increase in root diameter due to treated with 3750 ppm IBA, could be attributed to increase in periderm, cambium, phloem tissue was 20, 50 and 60% more than those control, respectively. It is seen that the increase in vessel diameter by 50 %.

By studying the anatomical changes of the adventitious root of rosemary plant, it was found that there was an increase in the root diameter, phloem tissue, and diameter of the xylem vessels as a result of growth in coco peat medium, weather perlite or IBA added to it. The obtained results could be supported by the findings of **Abass *et al.* (2024)** showed that sections taken from roots that have been treated with 3.0 g L^{-1} IBA and growing in medium composed of sand: pearlite showed the increases in histological parameters (root diameter, phloem tissue thickness, xylem tissue thickness and vessels diameter).

Chemical constituents

Effect of media type

The statistical analysis reveals significant differences among the tested growing media regarding leaf carbohydrate percentages (Table 5). The highest mean carbohydrate accumulation was observed in plants grown in Peat moss + Sand (25.435%), closely followed by coco peat + perlite (25.424%). Conversely, the lowest percentage was recorded in coco peat + sand (22.855%). The growing media significantly affected nitrogen accumulation in leaves. The highest overall mean nitrogen concentration was recorded in peat moss alone (2.569%), followed by peat moss + sand (2.508%). The lowest nitrogen accumulation was noted in coco peat + perlite (2.378%). Media type significantly influenced phosphorus percentages (Table 6). Peat moss + Perlite yielded the highest average phosphorus content (0.679%), followed by coco peat + sand (0.578%). The lowest mean phosphorus content was observed in Peat moss alone (0.396%). Distinct growing media significantly altered potassium accumulation (Table 6). The highest overall potassium percentages were found in coco peat + sand (1.710%) and Coco peat alone (1.700%). Peat moss + Sand resulted in the lowest potassium accumulation (1.514%).

The superiority of specific media mixtures—such as peat moss combined with sand or perlite, and coco peat combinations—stems from their balanced physical and chemical properties: A) Media incorporating perlite or sand provide optimal total porosity and aeration, preventing root hypoxia while maintaining adequate moisture. B) Peat moss and coco peat possess high cation exchange capacity (CEC) and organic matter content, which minimizes nutrient leaching and enhances the availability of potassium, nitrogen, and phosphorus to the root zone (**Bunt, 2012; Awang *et al*., 2009**).

Effect of IBA Concentration

Increasing the Indole-3-butyric acid (IBA) concentration led to a significant, step-wise increase in total carbohydrate content (Table 5). The control treatment (0.0 ppm IBA) produced the lowest overall mean (21.803%), whereas the highest concentration (3750 ppm IBA) yielded the maximum mean carbohydrate content (27.154%). Application of IBA linearly enhanced leaf nitrogen content (Table 5). Plants untreated with IBA (0.0 ppm) had the lowest nitrogen percentage (2.264%), which progressively increased with higher IBA doses, peaking at 3750 ppm IBA with a mean of 2.650%. Phosphorus levels increased significantly with escalating IBA concentrations (Table 6). The lowest mean was recorded in the control (0.386%), rising continuously to reach its maximum at 3750 ppm IBA (0.643%). Auxin application markedly increased leaf potassium levels in a dose-dependent manner. The baseline control (0.0 ppm IBA) resulted in 1.561% K, while applying 3750 ppm IBA maximized leaf potassium content to an average of 1.749% (noting the value 1.749%).

Table 5. Effect of media type (M), IBA concentration (A) and their combinations (M×A) on total carbohydrates and nitrogen (%) in leaves of rosemary (*Rosmarinus officinalis* L.) during the second season

Medium type	IBA concentration (ppm)				Mean (M)
	0.0	1250	2500	3750	
	Total carbohydrates (%)				
Peat moss	22.105	24.285	24.200	26.925	**24.378**
Peat moss + Perlite	21.690	24.330	25.235	27.395	**24.663**
Peat moss + Sand	21.865	23.075	28.135	28.665	**25.435**
Coco peat	22.685	22.785	24.595	26.445	**24.128**
Coco peat + Perlite	23.350	24.780	26.695	26.870	**25.424**
Coco peat + Sand	19.120	20.875	24.800	26.625	**22.855**
Mean (A)	**21.803**	**23.355**	**25.610**	**27.154**	
L.S.D. at 5 %	**M=0.082**	**A= 0.063**		**M×A= 0.156**	
	Total nitrogen (%)				
Peat moss	2.440	2.575	2.595	2.665	**2.569**
Peat moss + Perlite	2.145	2.410	2.605	2.685	**2.461**
Peat moss + Sand	2.405	2.505	2.545	2.575	**2.508**
Coco peat	2.195	2.435	2.605	2.735	**2.493**
Coco peat + Perlite	2.215	2.355	2.375	2.565	**2.378**
Coco peat + Sand	2.185	2.375	2.445	2.675	**2.420**
Mean (A)	**2.264**	**2.443**	**2.528**	**2.650**	
L.S.D. at 5 %	**M= 0.010**	**A= 0.008**		**M×A= 0.019**	

Table 6. Effect of media type (M), IBA concentration (A) and their combinations (M×A) on total phosphorus and potassium (%) in leaves of rosemary (*Rosmarinus officinalis* L.) during the two seasons

Medium type	IBA concentration (ppm)				Mean (M)
	0.0	1250	2500	3750	
	Total phosphorus (%)				
Peat moss	0.364	0.255	0.402	0.565	**0.396**
Peat moss + Perlite	0.613	0.668	0.677	0.760	**0.679**
Peat moss + Sand	0.305	0.353	0.371	0.734	**0.441**
Coco peat	0.345	0.386	0.424	0.463	**0.404**
Coco peat + Perlite	0.372	0.455	0.472	0.719	**0.504**
Coco peat + Sand	0.318	0.494	0.886	0.616	**0.578**
Mean (A)	**0.386**	**0.435**	**0.538**	**0.643**	
L.S.D. at 5 %	**M= 0.007**	**A= 0.005**		**M×A= 0.012**	
	Potassium (%)				
Peat moss	1.555	1.616	1.671	1.829	**1.668**
Peat moss + Perlite	1.503	1.527	1.611	1.663	**1.576**
Peat moss + Sand	1.508	1.520	1.518	1.511	**1.514**
Coco peat	1.618	1.628	1.700	1.754	**1.700**
Coco peat + Perlite	1.625	1.661	1.707	1.717	**1.677**
Coco peat + Sand	1.554	1.634	1.729	1.923	**1.710**
Mean (A)	**1.561**	**1.598**	**1.656**	**1.1749**	
L.S.D. at 5 %	**M= 0.032**	**A= 0.020**		**M×A= 0.053**	

The consistent increase in total carbohydrates, N, P, and K percentages following IBA applications can be attributed to the physiological role of auxins in enhancing root system development (adventitious root formation and lateral branching). A well-developed, extensive root network increases the active root surface area, thereby enhancing water uptake and the active/passive absorption of essential macro-nutrients (N, P and K) from the substrate. Furthermore, improved nutrient status directly stimulates photosynthetic efficiency, chlorophyll synthesis, and enzyme activity, leading to greater biosynthesis and accumulation of total soluble carbohydrates in leaf tissues (**Marschner, 2012**; **Hartmann *et al.*, 2014**).

Interaction Effect between media type and IBA concentration

The interaction between media type and IBA concentration was statistically significant (L.S.D. at 5% = 0.156). The combination of peat moss + sand with 3750 ppm IBA achieved the highest overall carbohydrate content (28.665%), whereas Coco peat + Sand without IBA (0.0 ppm) yielded the minimum value (19.120%). The interaction between factors was statistically significant (L.S.D. at 5% = 0.019). The combination of Coco peat alone at 3000 ppm IBA yielded the maximum leaf nitrogen content (2.735%), while Peat moss + Perlite at 0.0 ppm IBA recorded the lowest value (2.145%). The M × A interaction significantly altered leaf phosphorus content (L.S.D. at 5% = 0.012). The highest phosphorus level was registered under coco peat + Sand treated with 2000 ppm IBA (0.886%), followed by peat moss + perlite at 3750 ppm IBA (0.760%). The minimum value was observed in Peat moss alone at 1250 ppm IBA (0.255%). The interaction between media and IBA was statistically significant (L.S.D. at 5% = 0.053). The treatment combination of coco peat + sand with 3750 ppm IBA produced the highest leaf potassium concentration (1.923%), while peat moss + perlite at 0.0 ppm IBA registered the lowest (1.503%).

Likewise, **Bassiouny *et al.* (2020) and Gruda (2019)** observed that peat moss and coco peat blends significantly improve nutritional status and carbohydrate reserves in nursery-grown Lamiaceae species compared to single unblended substrates, owing to optimized water-air ratios in the root zone.Moreover, regarding IBA effect, the positive influence of exogenous IBA application on improving nutrient uptake (N, P, K) and leaf carbohydrate content has been corroborated by **El-Serafy *et al.* (2021)** on rosemary (*Rosmarinus officinalis* L.) and **Kewat *et al.* (2022)** on medicinal cuttings, who reported that IBA application stimulates photosynthetic sink activity and metabolic accumulation.

4. Conclusion

Based on the obtained results, combining growth media with higher IBA concentrations, particularly at 3750 ppm, significantly enhances both root growth traits and chemical constituents in rosemary. For superior root development - including fresh weight, dry weight and length - Coco peat combined with 3750 ppm IBA is the optimal recommendation. Additionally, for maximizing overall leaf nutritional content (carbohydrates, nitrogen, phosphorus and potassium), using 3750 ppm IBA in combination with coco peat or coco peat + sand produce the highest physiological performance.

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