



Modulating the Rhizosphere Medium and Indole – 3–butyric Acid Supply Influence Rooting, Nutrients and Biochemical Constituents and Histological Features of *Pedilanthus tithymaloids*

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Abstract

Pedilanthus tithymaloids is a popular temperate indoor succulent ornamental plant and is highly marketable. However, the appropriate propagation techniques should be adopted to solve the rooting issues of cuttings. Therefore, the present research was designed to specify the optimal indole – 3–butyric acid (IBA) concentration and rooting medium type for obtaining good rooting and healthy plants. Three IBA levels (0, 1.0, 2.0, and 3.0 g L⁻¹) and five growing mediums (sand, sand: clay, sand: peat moss, sand: perlite, and sand: vermiculite as 1:1, v/v) were used. The experiment was performed in a randomized complete block design in three replications. The obtained data illustrated that roots number per cutting and rooting percentage reached the maximum values by treating *P. tithymaloids* cuttings with 3.0 g L⁻¹ IBA x sand: perlite or sand: vermiculite medium. Cultivating the treated-IBA 3.0 g L⁻¹ cuttings of *P. tithymaloids* in sand: perlite showed the most enhancements in plant height, branches number and leaves number, leaf area and herb dry weight. However, the heaviest root dry weight was noticed with IBA 3.0 g L⁻¹ x sand: peat moss medium. Except sand medium, all other tested media in combination with IBA at 1.0, 2.0–3.0 g L⁻¹ showed remarkable stimulation effect on chlorophyll content. Sand: vermiculite mixture x IBA at 2.0 g L⁻¹ was the effective practice for improving carbohydrates percentage. By studying the anatomical changes of the adventitious root of *P. tithymaloids* plant, it was found that there was an increase in the root diameter, phloem and xylem tissues and xylem vessels diameter due to growing in sand: perlite x 3.0 g L⁻¹ IBA. In conclusion, dipping the cuttings bases of *P. tithymaloids* in 3.0 g L⁻¹ IBA and cultivation in sand: perlite considered the most effective combination practice for better rooting ability and growth and could be advisable for commercial production.

Keywords Growing medium · Growth regulation · Ornamentals · Rooting potential · Succulent plants · Root anatomy

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1 Introduction

Pedilanthus tithymaloids (devil's backbone) belongs to family *Euphorbiaceae*. The name "*Pedilanthus*" generated from the Greek words "pedil," which implies "shoe" and "anthos," that refers to "flower," because the red flower on this tall plant looks like a shoe from this upright succulent (Kemle 2016). The stems are thin and cylindrical, and the leaves are ovate with a thick, noticeable middle. Plants in full sun to light shade grown good and little to no water (Torre-Bueno 2016).

Cutting as a vegetative propagation mean is more favorable than the use of seeds in reproducing plants, since it supplies type-matched and early flowering of plants that produce high quality flowers (Santoso and Parwata 2014). Further, vegetative propagation is easy to implement at low cost (Waziri et al. 2015; Dawa et al. 2017). The first step in successful vegetative propagation of *Pedilanthus* is for the cuttings to be rooted effectively. Since *Pedilanthus* is a slow-rooting succulent plant, it requires effective treatments to accelerate rooting rate and enhance rooting percentage of stem cuts. Generally, the successful propagation via cutting is correlated with growth medium (Ashiono et al. 2017) and phytohormones (Bhardwaj et al. 2017).

Growing media are very important to plants because they hold up, give them water and nutrients, and let air get to the roots (Ali et al. 2024a; Lasheen et al. 2024). Caldwell et al. (1988) pointed out that the physical qualities of the rooting media and how they are used may affect how well cuttings can grow roots. Inappropriate growing media or rhizosphere is primarily a hindrance to the growth of roots, and therefore the above-ground parts of the plant (Abd El-Mageed et al. 2022; Abd-Elrahman et al. 2022; El-Metwally et al. 2022a; Makhoulouf et al. 2022). Accordingly, growing plant roots in poor rhizosphere medium subjects the crops to environmental stresses (Saady et al. 2020a; Salem et al. 2021; Shaaban et al. 2023). Certainly, such stresses lead to several critical troubles in physiological status related to photosynthesis process and dry matter accumulation (Saady et al. 2021a; El-Bially et al. 2022a; Ramadan et al. 2024a), water absorption (El-Bially et al. 2022b; Saady et al. 2023a; Ramadan et al. 2024b), and nutrient uptake and utilization (Saady et al. 2020b, 2023b; El-Metwally and Saady 2021). Therefore, it is so important to amend and adjust the soil medium to be stimulating for plant growth (Saady and El-Metwally 2019; Mubarak et al. 2021; Saady et al. 2021b). Frankly, there is no one best way to get cuttings to grow roots. Hartmann et al. (2011) reported that the right reproduction medium relies on the plant species, type of cutting, growing season, propagation method, expenditures, and abundance of medium components. Growing media is made up of sand and clayey soil, or one or more of a number of other materials, like

peat moss, perlite, vermiculite, leaf mold, manures, vermicompost or sewage sludge, etc., which modify the physico-chemical traits of the media mixtures influencing root growth habit and of plant nutrition (Habib 2012; Saady et al. 2020c; Doklega et al. 2024; Hadid et al. 2024). So, for each specie and variety, the best medium for rooting should be determined.

Concerning the plant growth regulation, it has been documented that the exogenous supply of various growth stimulators occurred remarkable enhancements in plant growth and productivity whether under normal or stressed environments (El-Metwally et al. 2022b; Rizk et al. 2023; Ali et al. 2024b). Since the endogenous supply of auxins is required for the early cell division, they have remarkable potential to commence the adventitious roots formation on stems (Hassan et al. 2018; Tawfik et al. 2022). Addition of indole-3-butyric acid (IBA) is a common utilized method in commercial production of plant rooting. This could be ascribed to the influential act of IBA for stimulating the accumulations and assimilation of nutrients, and consequently increased growth (Osman and El-Naggar 2014; Rajkumar et al. 2016).

Pedilanthus plant has not received sufficient studies on the relationship between plant hormones that stimulate rooting and the nature of the medium in which the roots grow. To obtain the most effective and practicable vegetative propagation of *P. tithymaloids* in order to provide successful nursery productivity, the integration between IBA and growing media was estimated. The current work hypothesized that there is a complementary effect between the growing medium type and IBA concentration for ameliorating rooting ability and growth of *P. tithymaloids*. Therefore, rooting ability, growth and chemical constituents of *P. tithymaloids* plants produced from IBA-treated cuttings and cultivated in various media mixtures were assessed.

2 Materials and Methods

The present work was performed at the Ornamental Nursery of El-Quassasin Horticultural Research Farm, Ismailia, Egypt, during the summer of 2021 and 2022. The study targeted to determine the influences of IBA and growing medium types on cuttings rooting ability, growth traits, biochemical constituents and root anatomy of *P. tithymaloids*. Growing medium types involved the use of sand, sand: clay, sand: peat moss, sand: perlite, and sand: vermiculite. The used substrates used in each medium mixture were a 1:1 volumetric ration. The properties of each prepared rooting media used are illustrated in Table 1. IBA was applied by soaking the cutting bases at the concentrations of (0.0, 1.0, 2.0 and 3.0 g L⁻¹). To prepare concentrations of IBA, the

Table 1 Properties of prepared medium types used in the experiment

Media type	Acidity (pH)	Electrical conductivity (dS m ⁻¹)	Organic matter (%)	Available nitrogen (mg kg ⁻¹)	Available phosphorus (mg kg ⁻¹)	Available potassium (mg kg ⁻¹)
Sand	7.3 ± 0.05	0.66 ± 0.06	0.08 ± 0.02	2210 ± 33	310 ± 13	420 ± 21
Sand + clay	7.5 ± 0.06	1.18 ± 0.05	1.42 ± 0.02	3644 ± 46	510 ± 16	722 ± 36
Sand + peat moss	6.4 ± 0.03	1.16 ± 0.02	1.99 ± 0.03	4466 ± 52	580 ± 15	780 ± 40
Sand + perlite	6.8 ± 0.02	0.33 ± 0.02	0.04 ± 0.01	1100 ± 25	150 ± 10	227 ± 17
Sand + vermiculite	7.6 ± 0.05	0.80 ± 0.04	0.59 ± 0.01	1105 ± 36	155 ± 11	210 ± 12

powder of IBA-potassium salt (C₁₂H₁₂NO₂K 98%) was exploited. Distilled water was used as a control treatment (without IBA, 0.0 g L⁻¹).

2.1 Plant Material and Culture Conditions

P. tithymaloids cuttings were obtained from uniform and healthy 3 month-old coppice shoots and 9 month-old seedlings rose in the nursery. On March 15 of 2021 and 2022 seasons, cuttings were obtained from the lateral part from mother plants and prepared for planting. After soaking the cuttings bases in IBA solution at the specified concentrations for 10 s, they were planted in pots (15 cm diameter and 15 cm length) filled with the specified tested media. The trial was performed in a randomized complete block design in three replications. Each treatment allocated in five pots per replicate and each pot had two cuttings.

2.2 Recorded Data

2.2.1 Rooting Ability and Growth Traits

The experiment was ended at 100 days from sowing and the percentage of rooting was recorded using Eq. 1. Further, root length, number of roots per cutting, root dry weight/cutting, and number of branches and leaves/cutting were recorded. Further, leaf area and herb dry weight (shoots + leaves)/cutting were measured.

$$\text{Rooted cuttings \%} = \frac{\text{number of the cuttings that formed roots}}{\text{total number of treated cuttings}} \times 100 \quad (1)$$

2.2.2 Nutrient and Biochemical Constituents

At the end of the experiments, total plant pigments (chlorophylls and carotenoids) were determined as mg/100 g fresh leaf weight (Cherry 1973). Total nitrogen, phosphorus, potassium, and carbohydrates were estimated in *P. tithymaloids* leaves as described by Chapman and Pratt (1978), Hucker and Catroux (1980), Brown and Lilleland (1946) and Dubois et al. (1956), respectively.

2.2.3 Anatomical Estimation

Depending on procedures illustrated by Nassar and El-Sahhar (1998), the anatomical assessment was performed to follow the changes occurred in adventitious root of *P. tithymaloids* plant at 60-day old. The anatomical estimation was performed only for efficient IBA level (3.0 g L⁻¹) under different rhizosphere media.

2.3 Statistical Analysis

Based on the procedures described by Casella (2008), the statistical analysis was carried out on the observed result data. Duncan's multiple range test was utilized to distinguish among treatment means at the 5% level of probability. For carrying out the data analysis and comparing means, software program 'Statistix version9', was used (Analytical software 2008)

3 Results

3.1 Rooting Ability

Result in Table 2 showed the significant response of rooting ability traits in *P. tithymaloids* to variations in soil media type and IBA concentration. In this connections, the highest roots number per cutting and rooting percentage of *P. tithymaloids* values in 2021 and 2022 seasons were obtained with sand: perlite and sand: vermiculite media compared to the other tested media. However, the longest roots were noticed with sand: peat moss medium in both seasons, significantly equaling sand: perlite and sand: vermiculite media in the 2nd season in this respect.

Using IBA at any concentration recorded significant enhancement regarding rooting ability of *P. tithymaloids* as comparing no IBA supply (distilled water). Moreover, the maximal roots number per cutting (8.3 and 8.8 roots), rooting percentage (88.7 and 89.1%) and root length (15.7 and 15.3 cm) were obtained when cutting bases were soaked in IBA at 3.0 g L⁻¹ for 10 s compared to control or the other concentrations during the two experimental seasons.

Table 2 Influence of growing media and indole-3-butyric acid (g L⁻¹) on roots number per cutting, rooting percentage and root length of *Pedilanthus tithymaloids* plant during 2021 and 2022 seasons

	Roots number per cutting					Rooting %					Root length (cm)					
	0.0	1.0	2.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	
	2021															
Sand	3.5f	4.5ef	6.5d	6.5d	5.2 C	77.5 g	80.0f	80.5f	80.5f	79.6 C	7.5j	10.5 h	12.5 fg	14.5c-e	11.2D	
Sand: clay	3.5f	5.0d-e	6.0de	6.5d	5.2 C	63.5i	73.0 h	77.5 g	82.5de	74.1D	8.0ij	9.5hi	13.0ef	14.0d-f	11.1D	
Sand: peat moss	4.5ef	5.5de	5.5de	6.5d	5.5 C	83.5d	83.5d	83.5d	88.5c	84.7B	13.5ef	15.5 cd	17.7ab	18.5a	16.3 A	
Sand: perlite	6.5d	8.5c	9.5bc	11.5a	9.0 A	81.5ef	88.5c	93.5b	96.5a	90.0 A	11.0gh	13.0ef	14.5c-e	15.5 cd	13.5 C	
Sand: vermiculite	5.5de	6.5d	8.5c	10.5ab	7.7B	81.0ef	89.5c	92.0b	95.5a	89.5 A	12.7e-g	14.5de	15.5 cd	16.2bc	14.7B	
Mean	4.7D	6.0 C	7.2B	8.3 A		77.4D	82.9 C	85.4B	88.7 A		10.5D	12.6 C	14.6B	15.7 A		
2022																
Sand	3.5hi	3.5hi	6.0e-g	7.0de	5.0 C	76.5gh	76.5gh	80.0ef	82.0d	78.7 C	8.5j	9.5ij	11.5gh	12.5 fg	10.5 C	
Sand: clay	3.0i	4.5 g-i	5.0f-h	7.0de	4.8 C	75.5 h	75.5 h	75.5 h	80.5d-f	76.7 C	9.0j	10.5hi	13.5d-f	14.5 cd	11.8B	
Sand: peat moss	4.0hi	6.0e-g	6.5d-f	7.5de	6.0B	81.5de	81.5de	81.5de	90.5b	83.7B	13.0ef	13.5d-f	15.5bc	17.7a	14.9 A	
Sand: perlite	7.5de	9.5bc	10.5ab	11.0ab	9.6 A	80.5d-f	85.5c	85.5c	97.5a	87.2 A	12.5 fg	14.0de	15.5bc	16.5ab	14.6 A	
Sand: vermiculite	6.5d-f	8.0 cd	10.5ab	11.5a	9.1 A	78.5 fg	88.5b	88.5b	95.0a	87.6 A	13.5d-f	14.0de	15.5bc	15.5bc	14.6 A	
Mean	4.9D	6.3 C	7.7B	8.8 A		78.5 C	81.5B	82.2B	89.1 A		11.50D	12.3 C	14.3B	15.3 A		

Values are the average of 3 replicates. Different letters within columns or rows indicate that there are significant differences among treatment means by Duncan's multiple range test at $p \leq 0.05$

Regarding the combination influence, roots number per cutting and rooting percentage reached the maximum values by treating *P. tithymaloids* cuttings with 3.0 g L⁻¹ IBA before culturing in sand: perlite or sand: vermiculite medium in the 1st and 2nd seasons, respectively (Table 2). Furthermore, cuttings which soaked in IBA at 2.0–3.0 g L⁻¹ and sown in medium comprised of sand: peat moss recorded the longest root.

3.2 Growth Traits

It is evident from the obtained data that plant height, leaves number and branches number (Table 3) and leaf area and herb dry weight (shoots + leaves) (Table 4) of *P. tithymaloids* recorded higher increases by using sand: perlite medium than to the other tested media in 2021 and 2022 seasons. While, the mixture of sand: peat moss was the efficient medium for recording the heaviest root dry biomass in both seasons.

Generally, increasing IBA concentrations gradually increased cuttings growth of *P. tithymaloids*. All measured growth traits of *P. tithymaloids* plant exhibited the best response achieving the highest increases by use IBA at 3.0 g L⁻¹. As averages of the two seasons, IBA at 3.0 g L⁻¹ treatment increased plant height, branches number, leaves number, leaf area, root dry weight and herb dry weight by about 1.36, 1.23, 1.5, 1.29, 3.17 and 1.41 times, respectively, compared to the control.

Cultivating the treated-IBA 3.0 g L⁻¹ cuttings of *P. tithymaloids* in sand: perlite showed the most enhancements in plant height, leaves number and branches number (Table 3) and leaf area and herb dry weight (Table 4) in both seasons. However, the heaviest root dry weight was produced with the interaction of IBA 3.0 g L⁻¹ x sand: peat moss medium in both seasons.

3.3 Nutrient Constituents

The obtained findings in Table 5 demonstrate that the maximal increase of total nitrogen % in *P. tithymaloids* leaves were obtained with sand: perlite mixture (in 2021 season) which statistically leveled with other media (in 2022 season, except the sand medium). Moreover, total phosphorus and potassium percentages recorded the highest values with sand: clay mixture compared to the other mixtures in both seasons.

From data presented in Table 5, it is clear that there the highest values in nitrogen, phosphorus and potassium were obtained with the treatment of 3.0 g L⁻¹ of IBA compared to the other concentration treatments. However, there were no noticeable differences between IBA at 3.0 g L⁻¹ and IBA at

Table 3 Influence of growing media and indole-3-butyric acid (g L^{-1}) on leaf area, roots dry weight and herb dry weight of *Pedilanthus tithymaloides* plant during 2021 and 2022 seasons

	Plant height (cm)				Branches number per plant				Leaves number per plant				Mean		
	0.0	1.0	2.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	0.0	1.0		2.0	3.0
2021															
Sand	15.5 g	17.50ef	20.5b	22.5a	19.0B	3.50hi	3.00i	4.50gh	4.00 g-i	3.75 C	30.50j	34.50i	37.00E		
Sand: clay	13.5 h	15.5 g	20.5b	22.5a	18.0 C	3.50hi	4.50gh	5.00 fg	5.00 fg	4.50 C	30.50j	34.50i	40.63D		
Sand: peat moss	11.5i	16.5 fg	17.5ef	19.5b-d	16.2D	3.50hi	4.50gh	5.00 fg	6.00ef	4.75 C	32.50ij		57.00d		
Sand: perlite	19.0 cd	19.0 cd	20.0bc	22.5a	20.1 A	8.50bc	8.00b-d	9.00b	11.00a	9.13 A	51.50e	56.50d	79.50a		
Sand: vermiculite	16.5 fg	15.5 g	18.5de	19.5b-d	17.5 C	7.00de	7.50 cd	6.00ef	8.00b-d	7.13B	41.00 h	52.50e	63.25 A		
Mean	15.2D	16.8 C	19.4B	21.3 A		5.20 C	5.50BC	5.90B	6.80 A				56.38B		
2022															
Sand	17.5ef	18.5de	20.5bc	20.5bc	19.2AB	2.50i	2.50i	3.50 h	4.50 g	3.25D	28.50k	33.50ij	36.38E		
Sand: clay	14.5 g	16.5f	21.0bc	22.0ab	18.5B	2.50i	2.50i	4.50 g	5.50f	3.75D			42.50D		
Sand: peat moss	12.5 h	14.5 g	17.5ef	18.5de	15.7 C	3.50 h	4.50 g	5.50f	5.50f	4.75 C	34.00ij	57.50d	48.13 C		
Sand: perlite	17.5ef	18.5de	21.5b	23.5a	20.2 A	9.50b	8.50c	9.50b	11.50a	9.75 A	53.50e	61.50c	85.00a		
Sand: vermiculite	17.5ef	18.5de	19.5 cd	20.5bc	19.0AB	7.50d	7.00de	6.50e	8.50c	7.38B	53.50e	64.00c	66.88 A		
Mean	15.9D	17.3 C	20.0B	21.0 A		5.10 C	5.00 C	5.90B	7.10 A				58.13B		

Values are the average of 3 replicates. Different letters within columns or rows indicate that there are significant differences among treatment means by Duncan's multiple range test at $p \leq 0.05$

2.0 g L^{-1} for nitrogen percentage in 2022 season and potassium percentage in 2021 season.

As for the interaction, sand: perlite mixture x IBA at 2.0 g L^{-1} and sand: vermiculite mixture x IBA at 3.0 g L^{-1} achieved the best influence enhancing nitrogen content in *P. tithymaloides* leaves in both seasons (Table 5). Pots filled with sand: clay mixture with IBA at 1.0 g L^{-1} in the first season or with IBA at 3.0 g L^{-1} in the second season exhibited the best effect on phosphorus content in *P. tithymaloides* leaves. While, sand: clay mixture x IBA at 2.0 g L^{-1} and sand: perlite mixture x IBA at 3.0 g L^{-1} (in 2021 season) as well as sand: peat moss mixture x IBA at 3.0 g L^{-1} (in 2022 season) were the effective combinations for improving leaf potassium content.

3.4 Biochemical Constituents

As shown in Table 6, sand: peat moss medium along with sand: perlite and sand: vermiculite media in both seasons and sand: caly in the 2nd exhibited the maximal chlorophyll content of *P. tithymaloides* plant. While, using sand: peat moss medium type significantly increased carotenoids content in leaves compared to the other media types in both seasons. Moreover, the highest values of total carbohydrates percentage (16.5%) were noticed when *P. tithymaloides* cuttings cultured in sand: vermiculite mixture in the two tested seasons.

IBA at 2.0 g L^{-1} was the efficient practice for improving chlorophyll and carotenoids contents in *P. tithymaloides* leaves in both seasons, equaling significantly IBA at 1.0 g L^{-1} in the first season and IBA at 3.0 g L^{-1} in the second one for chlorophyll content. On the other hand, carbohydrates percentage was higher with IBA at 3.0 g L^{-1} surpassing the other IBA levels in both seasons.

Except sand medium, all other tested media in combination with IBA at $1.0, 2.0\text{--}3.0 \text{ g L}^{-1}$ showed remarkable stimulation effect on chlorophyll in both seasons. Carotenoids were at the maximum level with application of sand: peat moss mixture x IBA at 0.0 g L^{-1} in 2021 season and sand: vermiculite mixture x IBA at 2.0 g L^{-1} in 2022 season. Sand: vermiculite mixture x IBA at 2.0 g L^{-1} practice possessed the best improvement in carbohydrates % in both seasons.

3.5 Anatomical Study

Sections taken from roots of sand: perlite medium illustrated that all histological parameters were greater than that of control treatment (sand: clay). In this respect the increase amounted to +21.7, +30.4, +12.5, +36.9, +37.5 and +10.0% in root diameter, periderm thickness, phloem tissue thickness, xylem tissue thickness, vessels number/xylem

Table 4 Influence of growing media and indole-3-butyric acid (g L⁻¹) on leaf nitrogen, phosphorus and potassium content of *Pedicularis tithymaloides* plant during 2021 and 2022 seasons

	Leaf area (cm ²)				Root dry weight (g)				Herb dry weight (shoots+ leaves) (g)							
	0.0	1.0	2.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	
	2021															
Sand	3.06k	3.17jk	3.29ij	3.35 h-j	3.22E	0.09 m	0.13kl	0.17 h-j	0.19 g-i	0.14 C	1.18 L	1.20 L	1.24kl	1.29j-l	1.22E	
Sand: clay	3.33 h-j	3.40 g-i	3.48gh	3.56 g	3.44D	0.12 lm	0.14j-l	0.14j-l	0.20 fg	0.15 C	1.22kl	1.31jk	1.39ij	1.50i	1.35D	
Sand: peat moss	3.31 h-j	3.43 g-i	4.25f	4.45f	3.86 C	0.16i-k	0.24e	0.28d	0.76a	0.36 A	1.20 L	1.47i	2.02 h	2.11gh	1.70 C	
Sand: perlite	5.18e	6.38d	7.17b	7.50a	6.55 A	0.18 g-i	0.20F-h	0.30d	0.59b	0.31B	2.83d	3.25c	4.10b	4.22a	3.60 A	
Sand: vermiculite	5.13e	6.22d	6.83c	7.25a	6.36B	0.22ef	0.24e	0.28d	0.48c	0.30B	2.05 h	2.16 g	2.45f	2.65e	2.33B	
Mean	4.00D	4.52 C	5.00B	5.22 A		0.15D	0.19 C	0.23B	0.44 A		1.69D	1.88 C	2.24B	2.35 A		
2022																
Sand	3.10o	3.12mn	3.39mn	3.42 lm	3.26D	0.10 L	0.12k	0.15j	0.18gh	0.14E	1.21 m	1.23 m	1.27 lm	1.28 lm	1.24E	
Sand: clay	3.30n	3.38jk	3.59jk	3.65ij	3.48 C	0.13k	0.17hi	0.19 g	0.22f	0.17D	1.18 m	1.33kl	1.40jk	1.44j	1.35D	
Sand: peat moss	3.51kl	3.73 h	4.62 h	4.60 h	4.11B	0.19 g	0.32d	0.35c	0.69a	0.38 A	1.20 m	1.55i	1.83 h	1.93 g	1.63 C	
Sand: perlite	5.22f	5.78c	7.02c	7.34a	6.34 A	0.15ij	0.22f	0.33d	0.69a	0.34B	2.37e	3.08c	3.33b	4.02a	3.20 A	
Sand: vermiculite	5.11 g	6.43c	6.94c	7.14b	6.40 A	0.18gh	0.22f	0.25e	0.61b	0.31 C	2.01 g	2.20f	2.46e	2.69d	2.34B	
Mean	4.05D	4.48 C	5.11B	5.23 A		0.14D	0.21 C	0.25B	0.48 A		1.59D	1.87 C	2.06B	2.27 A		

Values are the average of 3 replicates. Different letters within columns or rows indicate that there are significant differences among treatment means by Duncan's multiple range test at $p \leq 0.05$

row and vessels diameter, respectively (Table 7). Also, sections taken from roots that have been treated with 3.0 g L⁻¹ IBA and growing in medium composed of sand: perlite (Fig. 1) showed the increases in all histological parameters except periderm thickness and vessels number/xylem row. Herein, the increments due to sand: perlite mixture x IBA at 3.0 g L⁻¹ were +31.3, +19.5, +16.6 and +9.1% for root diameter, phloem tissue thickness, xylem tissue thickness, and vessels diameter, respectively greater than the control. While, periderm thickness and vessels number/xylem row reduced by -25.5 and -21.8%, respectively.

4 Discussion

Determining the most appropriate medium for effective rooting mainly depends on the properties of substrate used in media preparation (Sardoei 2014). The prosperous propagation media provide proper level of nutrients, moisture and aeration to cuttings for better rooting potential (Manila et al. 2017; Dawa et al. 2018). Contrariwise, existence of plant roots in medium having inadequate essential resources causes abiotic stress on the crop plants (El-Metwally et al. 2022c; Salem et al. 2022; Helal et al. 2024). The current research proved the better potential of sand: perlite, sand: vermiculite and sand: peat moss media to enhance roots number, rooting percentage and root length of *P. tithymaloides* cuttings. In this respect, Hartmann et al. (2014) stated that the good media should involve coarse mineral constituents such as coarse sand or perlite for achieving proper aeration in root zone. Good oxygen availability in growing medium is the major influencer metabolic events and stimulating formation of roots (Yeboah and Amoah 2009). Rooting of cuttings was remarkably stimulated by using media comprising of sand, perlite, vermiculite and peat (Jaleta and Sulaiman 2019). Sand is commonly used as a substrate in preparing rooting media for plants that reproduce by vegetative propagation. However, the properties of sand were improved by mixing with several substrates, thus stimulating the rooting of cuttings (Wei et al. 2017). Soil matrix traits expressed in physico-mechanical structure were improved due to perlite addition (Fang et al. 2017). Thus, perlite positively modified air permeability and water holding of the soil medium (He and Xiong 2019). Consequently, it has been reported that perlite was the most influential rooting media for peach cuttings (Tsipouridis et al. 2004), and showed the highest rooting percentage in *Cotinus obovatus* cuttings (Denny and Arnold 2001). Vermiculite was the effective substrate in enhancing rooting rate in cuttings of several crops (Rajkumar et al. 2016, Manila et al. 2017; Peña-Baracaldo et al. 2018). The superiority of vermiculite for stimulating rooting of cuttings could be ascribed to its ability to retain water and

Table 5 Influence of growing media and indole-3-butyric acid (g L⁻¹) on chlorophyll, carotenoids and carbohydrates of *Pedilanthus tithymaloides* plant during 2021 and 2022 seasons

	Nitrogen %				Phosphorus %				Potassium %				Mean		
	0.0	1.0	2.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	0.0	1.0		2.0	3.0
2021															
Sand	2.11 L	2.16k	2.21gh	2.20hi	2.17 C	0.43 fg	0.43gh	0.41k	0.44c	0.43BC	2.64 h	2.46 m	2.66 fg	2.63hi	2.60D
Sand: clay	2.23ef	2.24de	2.20 g-i	2.24de	2.22B	0.42ij	0.46a	0.45b	0.45b	0.45 A	2.63hi	2.68de	2.72a	2.68de	2.67 A
Sand: peat moss	2.18j	2.23ef	2.25 cd	2.26bc	2.23B	0.42jk	0.42ij	0.43d-g	0.43d-f	0.42 C	2.55k	2.62i	2.68de	2.70bc	2.63B
Sand: perlite	2.22 fg	2.26bc	2.28a	2.26bc	2.25 A	0.42ij	0.42hi	0.44c	0.43 cd	0.43 B	2.55jk	2.64gh	2.66 fg	2.71ab	2.64B
Sand: vermiculite	2.20 g-i	2.19ij	2.25 cd	2.27ab	2.23B	0.42jk	0.43e-g	0.43 cd	0.43c-e	0.43 B	2.53 L	2.57j	2.67ef	2.69dc	2.61 C
Mean	2.19D	2.21 C	2.23B	2.24 A	2.23 A	0.42 C	0.43B	0.43B	0.44 A	0.43 B	2.58 C	2.59B	2.67 A	2.68 A	2.61 C
2022															
Sand	2.13i	2.18 h	2.19gh	2.19gh	2.17B	0.42kl	0.43hi	0.43jk	0.43de	0.43D	2.55ij	2.48k	2.59gh	2.65d	2.56E
Sand: clay	2.22d-f	2.21e-g	2.23c-e	2.25a-c	2.22 A	0.41n	0.45b	0.44c	0.46a	0.44 A	2.56ij	2.66 cd	2.73a	2.70b	2.66 A
Sand: peat moss	2.19gh	2.22d-f	2.24b-d	2.25a-c	2.22 A	0.42kl	0.43ij	0.43gh	0.43gh	0.43D	2.54j	2.60 fg	2.64de	2.73a	2.63 C
Sand: perlite	2.20f-h	2.25a-c	2.27a	2.25a-c	2.24 A	0.42 lm	0.43jk	0.44c	0.44d	0.43 B	2.58 g-i	2.62ef	2.68bc	2.70b	2.64B
Sand: vermiculite	2.20f-h	2.22d-f	2.24b-d	2.26ab	2.23 A	0.42 m	0.43 fg	0.43ef	0.43de	0.43 C	2.56 h-j	2.55ij	2.66 cd	2.68bc	2.61D
Mean	2.18 C	2.21B	2.23 A	2.24 A	2.23 A	0.42D	0.437 C	0.43B	0.44 A	0.43 C	2.56D	2.58 C	2.66B	2.69 A	2.61D

Values are the average of 3 replicates. Different letters within columns or rows indicate that there are significant differences among treatment means by Duncan's multiple range test at $p \leq 0.05$

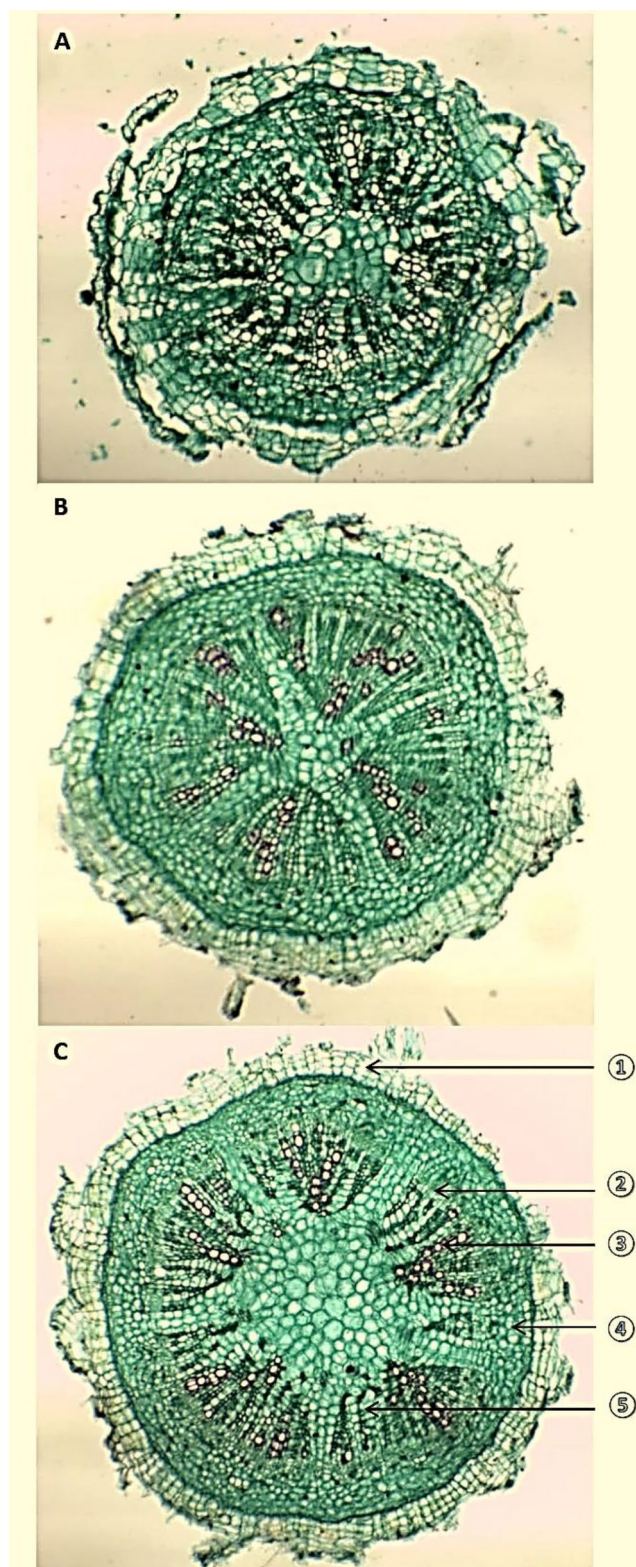


Fig. 1 Transverse sections through adventitious root of *Pedilanthus tithymaloides* cutting as affected by different growing media (40 x). **A:** sand: clay (control), **B:** sand: perlite, **C:** sand: perlite x indole-3-butyric acid at 3.0 g L⁻¹. ①: periderm, ②: cambium zone, ③: secondary xylem, ④: secondary xylem, ⑤: rays

Table 6 Influence of growing media and indole-3-butyric acid (g L^{-1}) on chlorophyll, carotenoids and carbohydrates of *Pedilanthus tithymaloides* plant during 2021 and 2022 seasons

	Chlorophyll ($\text{mg}/100 \text{ g fresh weight}$)						Carotenoids ($\text{mg}/100 \text{ g fresh weight}$)						Carbohydrates %									
	0.0		1.0		2.0		3.0		Mean		0.0		1.0		2.0		3.0		Mean			
	0.0	1.0	1.0	2.0	2.0	3.0	3.0	Mean	0.0	1.0	1.0	2.0	2.0	3.0	3.0	Mean	0.0	1.0	2.0	3.0	Mean	
2021																						
Sand	1.27e	1.43a-d	1.36de	1.36c-e	1.36 C	0.41ef	0.25k	0.33ij	0.33ij	0.33E	15.4 m	15.6 L	15.67 L	15.7jk	15.6E							
Sand: clay	1.39b-e	1.42a-d	1.53a	1.41a-d	1.44B	0.39gh	0.38 h	0.40 fg	0.34i	0.38D	15.6kl	15.7j	15.8i	16.1 g	15.8D							
Sand: peat moss	1.51ab	1.52ab	1.47a-d	1.53a	1.51 A	0.53a	0.43d	0.47c	0.40 fg	0.46 A	15.7j	15.9 h	16.7b	16.5d	16.2 C							
Sand: perlite	1.44a-d	1.52ab	1.49a-c	1.51ab	1.49AB	0.41ef	0.43d	0.46c	0.32j	0.41 C	15.8i	16.3e	16.5d	16.6d	16.3B							
Sand: vermiculite	1.37c-e	1.48a-d	1.54a	1.46a-d	1.46AB	0.38 h	0.42de	0.50b	0.40 g	0.42B	16.1 g	16.2f	16.6c	16.9a	16.5 A							
Mean	1.40B	1.47 A	1.48 A	1.46AB		0.43 A	0.38B	0.43 A	0.63 C		15.7D	15.9 C	16.3B	16.4 A								
2022																						
Sand	1.28f	1.35d-f	1.37c-f	1.42b-f	1.35B	0.32k	0.27 L	0.35j	0.36i	0.32D	15.5n	15.6 lm	15.7 L	16.0j	15.7E							
Sand: clay	1.40b-f	1.31ef	1.65a	1.42b-f	1.44AB	0.40 fg	0.37i	0.38 h	0.37i	0.38 C	15.5n	16.1i	16.0j	16.2 h	15.9D							
Sand: peat moss	1.50a-d	1.58ab	1.51a-d	1.54a-c	1.53 A	0.45 cd	0.43e	0.46b	0.39gh	0.43 A	15.6 m	15.8k	16.7c	16.6d	16.2 C							
Sand: perlite	1.45b-f	1.51a-d	1.51a-d	1.53a-d	1.50 A	0.40 fg	0.44d	0.45bc	0.33k	0.41B	16.0j	16.2 g	16.4f	16.6c	16.3B							
Sand: vermiculite	1.39c-f	1.47a-e	1.53a-d	1.51a-d	1.48AB	0.37i	0.41f	0.47a	0.37i	0.41B	15.8k	16.5e	16.7b	16.8a	16.5 A							
Mean	1.41B	1.44AB	1.51 A	1.48 A		0.39B	0.38 C	0.42 A	0.36D		15.7D	16.1 C	16.3B	16.4 A								

Values are the average of 3 replicates. Different letters within columns or rows indicate that there are significant differences among treatment means by Duncan's multiple range test at $p \leq 0.05$

progressively provide the nutrients while the potentiality to improve aeration and drainage (Manila et al. 2017). Regarding the organic substrates, fertilizers originated from natural and organic origin are a stable source of organic acids and minerals while regulating the biological and chemical properties of rhizosphere (Elgala et al. 2022; El-Ziat et al. 2024). Therefore, plant growth was enhanced with the soil medium amended by organic matter (Moreira et al. 2014; Ramadan et al. 2023). In this concern, because peat moss is of organic origin it can modify the properties of sandy soil, as it reduces the soil bulk density while improving soil porosity, availability of nutrients, and moisture holding capacity (Mostafa et al. 2019). Peat moss is an appropriate medium for propagating various horticultural plants since it has proper physico-chemical properties, which include porosity, density, water retention, exchange capacity, acidity and carbon: nitrogen ratio (Abad et al. 2001). Similar findings were documented by Minj et al. (2023) for *Bougainvillea* cuttings.

Despite the individual use of different substrates revealed success in cutting rooting and growth, mixing various substrates possessed better findings. In this context, root traits of *P. tithymaloides* cuttings substantially improved in media which prepared by combining different substrates such as sand plus perlite, vermiculite or peat moss. In some plant species, cultivating cuttings in medium contains mixtures of peat moss + perlite or peat moss + sand has significant potential for rooting and growth (Exadaktylou et al. 2009; Jaleta and Sulaiman 2019). This result coincides with the resulting effect of sand: perlite, sand: vermiculite and sand: peat moss media in stimulating root and growth traits of *P. tithymaloides*. The improvement in rooting ability of cuttings in *P. tithymaloides* owing to sand: perlite, sand: vermiculite and sand: peat moss media could be correlated with proper aeration and water retaining (Ercişli et al. 2002). As the rooting ability of cuttings grown in a suitable medium is improved, the growth of developed plants is expected to be superior. Accordingly, plant height, branches number, leaves number, leaf area and herb dry weight (Tables 3 and 4) as well as leaf nutrients content (Table 5) showed significant increases with various substrate mixtures, especially sand: perlite, greater than sand medium. Growth, yield and quality are closely associated with the growing media (Ta et al. 2011). It should be noted that medium included perlite was more suitable for rooting of *P. tithymaloides* cuttings while better plant growth. In this respect, Zhao et al. (2016) stated that perlite is typified by lower bulk density as well as higher pores and water holding capacity than vermiculite. Thus, adding perlite to the growing medium is significant to ensure better root formation and growth and nutrients absorption (Ma et al. 2017). Further, discrepancy in effect between different media of rooting might be linked

Table 7 Counts and measurements in microns of certain histological features in transverse sections through adventitious root of *Pedilanthus tithymaloids* plant at the age of 60 days, as affected by different growing media and indole–3–butyric acid (IBA) at 3.0 g L⁻¹ (means of three sections from three specimens)

Histological feature	Sand: clay	Sand: perlite	± % of Sand: clay	Sand: perlite x 3.0 g L ⁻¹ IBA	± % of Sand::perlite
Root diameter (μm)	1138.5	1386.0	+21.7	1821	+31.3
Periderm thickness (μm)	118.8	155	+30.4	115.5	-25.5
Phloem tissue thickness (μm)	105.6	118.8	+12.5	142	+19.5
Xylem tissue thickness (μm)	188	257.4	+36.9	300.3	+16.6
Vessels number/xylem row	4.0	5.5	+37.5	4.3	-21.8
Vessels diameter (μm)	33.0	36.3	+10.0	39.6	+9.1

to the substrate's direct impact on the cutting's basal region (Khayyat et al. 2007).

In accordance to the results of the present work, IBA as a growth promoter has a hormonal effects and positively affected the rooting ability, growth and nutrients content of *P. tithymaloids*. Plant hormones act as stimulators for primary meristematic activities and augment the growth and development of the formed roots (Hartmann et al. 2011). Since growth regulators activate cell division and cell elongation, IBA treatment caused a distinctive growth of roots (Kashyap 2015). As shown in Table 2, increasing IBA concentrations resulted in increased root number, percentage and length of *P. tithymaloids* cuttings. The increased root length with increasing IBA levels could be attributed to the earlier onset of roots as nutrient uptake and utilization are improved (Jadhav 2007). The effect of IBA may be caused by the fact that applying auxin brings assimilates to the base of the cutting and speeds up meristem differentiation, which is probably the most well-known way to help cuttings grow roots (Tchoundjeu et al. 2002). Furthermore, IBA as stimulator for hydrolysis and movement of nitrogenous substances and carbohydrates at cutting bases could accelerate cell division and elongation, increasing root development (Singh et al. 2003; Hartmann et al. 2007). In several plant species, IBA promoted root formation (Shirzad et al. 2012), while enhancing roots number, length and root biomass (Galavi et al. 2013). The response of rooting ability to auxins application have been reported by Rossa et al. (2019) on *Euphorbia phosphorea* Mart and *Euphorbia enterophora* Drake cuttings, Abdel-Rahman et al. (2020) on *Conocarpus erectus* and Singh et al. (2023) on dahlia cuttings. External supply of IBA resulted in significant nutrient accumulations and assimilation, thus enhancing growth parameters (Rajkumar et al. 2016). Remarkable success in rooting and preventing death of cuttings were achieved by application of auxin (Štefančič et al. 2007; Kasim and Rayya 2009). The beneficial act of auxin on rooting could be also ascribed to auxin-based food provision, and increased peroxidase activity and protein content (Husen and Pal 2007). Since IBA enhanced rooting ability and produced more healthy roots of *P. tithymaloids* cuttings, a subsequent plant growth improved. Herein, auxin as a crucial plant hormone

encourages the production of lateral roots, which is reflected in the growth of shoots and leaves (Fukaki et al. 2007). On the other hand plant stimulators adjust the physiological status and improve growth and quality of the economical products (El-Bially et al. 2018; El-Metwally et al. 2021). Herein, the rise in the growth parameters with auxin supply may be attributable to a higher rooting percentage and root count, which are reflected in higher measurements of the leaves and shoots (Jamal et al. 2016). In addition, Deb et al. (2009) showed that chlorophyll of lemon leaves was improved via treating cuttings with IBA. Similar results were reported by Osman and El-Naggar (2014) on *Jatropha curcas* L. and *Euphorbia tirucalli* cuttings.

There were synergistic interaction effects between growing medium and IBA level on *P. tithymaloids* cuttings, plant growth and leaf nutrient content. Generally, cuttings of *P. tithymaloids* cultivated in sand: perlite or sand: vermiculite media and treated by IBA at 3.0 g L⁻¹ exhibited the potency for rooting ability, plant growth and leaf nitrogen content. Also, soaking cuttings in IBA at 3.0 g L⁻¹ with cultivation in sand: clay mixture and sand: peat moss achieved the highest phosphorus and potassium contents, respectively. The favorable integral effect between auxins application and growing media on rooting and growth of cuttings in different species was recorded (Aghdaei et al. 2018; Rani et al. 2018; Kumar et al. 2019; Maanik and Sharma 2022; Voruganti et al. 2022). In this situation, Hae and Funnah (2011) on *Dovyalis caffra* stem cuttings and Hamad et al. (2017) on *Pachira aquatica* cuttings reported similar results. For instance, the combination between vermiculite and naphthalene acetic acid or IBA showed enhancements in rooting and growth of cuttings in different species (Rajkumar et al. 2017; Peña-Baracaldo et al. 2018). In the same line, Mohammed et al. (2020) noted that the combinations of different media plus IBA at 3.0 g L⁻¹ scored the maximal NPK and carbohydrates of *Ficus benjamina*.

5 Conclusions

The present work furnishes initiatory findings related to the propagation of stem cuttings of *Pedilanthus tithymaloids*. There was a distinctive integral effect between the type of growing medium and indole – 3–butyric acid in stimulating rooting ability while promotion of commercial qualities of *Pedilanthus tithymaloids* as an ornamental plant. In general, the obtained findings clearly demonstrated that sand: perlite, sand: vermiculite or sand: peat moss are considered appropriate media for improving cuts rooting ability, and growth and nutrients contents of *Pedilanthus tithymaloids* plants. However, sand: perlite was the most efficient medium in producing the maximum values of the market-related traits such as branches and leaves per plant as well as the anatomical parameters. Also, the progressive increase in indole – 3–butyric acid levels was synchronizing with high ability of cuttings rooting and plant growth. Accordingly, the current study could recommended the growers propagate *Pedilanthus tithymaloids* plant via dipping cuttings in indole – 3–butyric acid solution (3.0 g L^{-1}) and planting in sand: perlite mixture as an excellent growing medium.

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Declarations

Conflict of Interest There is no conflict of interests has been declared by the authors of the present research.

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