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Vegetative Propagation of *Zamioculcas zamiifolia* (G. Lodd.) Engl. Managed by IBA Application, Substrate Quality, and Leaflet Cutting

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ABSTRACT

Zamiifolia or ZZ is a luxury indoor ornamental plant that has received considerable attention in the past two decades. Due to its high economic value, its propagation is highly valuable. This research had two experiments to investigate rooting and rhizome production by leaf cuttings. The first experiment was a factorial with two factors, i.e., IBA concentrations (0, 250, and 500 mg L^{-1}) and three positions of leaflet cuttings on the main axis of the leaf (apical, middle, and basal). The results showed that the number of rhizomes and roots increased in response to 250 and 500 mg L^{-1} IBA, respectively, compared to the other treatments. In the second experiment, the effects of three propagation media were investigated, i.e., perlite: sand: cocopeat (50: 25: 50), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30), as well as three positions of leaflet cuttings on the main axis of the leaf (apical, middle and basal). The results indicated that the cocopeat: perlite medium (50: 50) significantly increased the rhizome length, rhizome width, root formation, root length, and shoot count. In conclusion, the optimal recommendation for rhizome formation/rooting substrate was cocopeat plus perlite. IBA application can highly benefit the vegetative propagation of this valuable ornamental plant.

Introduction

ZZ (*Zamioculcas zamiifolia* (G. Lodd.) Engl.) is an ornamental stem-less monocotyledonous plant that belongs to the Araceae family (Feng et al., 2006). This evergreen plant is native to eastern Africa (Lopez et al., 2009) and is one of the relatively new potted plants in the world due to its attractiveness (Seneviratne et al., 2020; Mayers, 2023). It is also known as "Money tree", "Eternity Plant", "Zanzibar Gem" and "Golden tree" (Nirmala et al., 2019). ZZ acclimatizes to environmental conditions, protects itself against pathogenic factors, and has the capacity to copes with climate change (Seneviratne et al., 2020). Furthermore, *Z. zamiifolia* became a popular indoor plant in a short time due to its unique appearance and ability to resist low light, drought, diseases, and pests (Chen and Henny, 2003). Moreover, *Zamioculcus zamiifolia* is a resourceful plant for ozone phytoremediation (Pheomphun et al., 219). Previous research showed that ZZ is a suitable plant for removing non-polar VOCs, such as benzene, toluene, ethylbenzene, and xylene from contaminated indoor air (Ullah et al., 2021).

ZZ is usually trees are propagated by rhizome division and leaf cuttings (Mayers, 2023; Nirmala et al., 2019). Production through leaf cuttings involves rhizome formation, followed by root formation, and finally leaf formation (Chen and

Henny, 2003).
Few studies have considered specific aspects of this plant so far, especially on its vegetative propagation. Seneviratne et al. (2013) investigated propagation methods with leaf cuttings in different cultivation substrates, reporting that basal leaflets without petioles were the most effective cuttings for asexual propagation. Additionally, Lopez et al. (2009) explored the possibility of *Z. zamiifolia* asexual propagation of by single, apical, and basal, or axial leaflets. It was found that the apical leaflet had weak propagative ability and insignificant reproduction under a 16-hour photoperiod at 29-32 °C.
Furthermore, previous research has addressed the effects of leaflet position and size on rooting and rhizome formation. A comparison was made between the rooting and rhizome formation of leaflet cuttings from the apical, middle, and basal parts of the petiole in plants. The results showed that the leaves of its different petiole can form roots and form rhizomes equally well (Thongkham and Phavaphutanon, 2018). Badizadegan et al. (2023) examined the application of chitosan levels (0, 250, and 500 mg L^{-1}) on the positioning of leaflet cuttings along the main axis of the mother leaf (apical, middle, and basal). They indicated that the type of leaflet cutting significantly affected the width and number of rhizomes. Correspondingly, chitosan application at concentrations of 250 and 500 mg L^{-1} increased rhizome count, width, and of roots count.
Nirmala et al. (2019) examined six propagules and six substrates for in vivo propagation of *Zamioculcas zamiifolia*. They found that two leaflets with rachis were optimal propagation materials among the propagules, with early rhizome initiation, larger rhizomes and more roots. However, more rhizomes formed when the upper half of the leaves were used as propagules. Among the substrates, sphagnum moss supported early rhizome initiation. Larger rhizomes formed produced in vermicompost and coir pith substrates. More roots with maximum root length formed in sphagnum moss.
The propagation rate of this species is low in conventional propagation methods. Therefore, the current study aimed to develop a new method to accelerate ZZ vegetative propagation of ZZ by IBA application in combination with, appropriate selections of leaflet cuttings and rhizome/rooting media.

Material and Methods

This study was conducted in a laboratory at the Horticultural Science and Engineering Department, Faculty of Agriculture and Environmental Sciences, Arak University, 2021-2022.

Plant materials

Stock *Z. zamifolia* pots were purchased from a producer of indoor plants in Mahalat, Iran. The selected ZZ plants had uniform development and were at similar growth stages based on their size, age, and leaf count. Each mother-leaf had at least 15 leaflets which divided into three groups: apical leaflets (juvenile leaflets), middle leaflets, and basal leaflets (mature leaflets). These leaflets were separated from the mother plants and were plugged vertically into individual pots after the application of treatments (Badizadegan et al., 2023).

Treatments

This research consisted of two experiments. This first experiment aimed to evaluate the effect of IBA (Merck Company) at three concentrations (0, 250, and 500 mg L^{-1}) and the type of leaflet cuttings positioned on the main leaf axis of the *Z. zamiifolia* (apical, middle, and basal leaflets). The experiment was conducted as a factorial arrangement based on a completely randomized design with three replicates. IBA solutions were prepared by accurately weighing 250 and 500 mg L^{-1} of IBA in ethanol. Distilled water was added to achieve the desired volume of the solutions. Finally, leaflet cuttings were immersed in these solutions for 5 s. To prevent fungal decay, the leaflet cuttings were placed inside the commercial fungicide solution of Captan (ARIASHIMI, Iran) for five seconds. Lastly, the treated cuttings were planted in uniform 4-inch pots containing a substrate of 50:50% cocopeat and perlite (v/v). Distilled water was used as a control. In the second experiment, three propagation media were used i.e., 50:25:25; perlite:sand:cocopeat, 50:50; cocopeat:perlite, and 70:30; cocopeat:perlite. Also, three types of leaflet cuttings were used from the main axis of the plant (apical, middle, and basal leaflets).

Measurements

Four months after propagation, leaf cuttings were removed from the substrate to investigate their morphological characteristics. The rhizomes were cleaned before conducting the measurements without damaging the roots. Several morphological traits including the number of rhizomes, the length and width of rhizomes, the number of roots, the length of the longest root, and the quality of the mother-leaf cuttings were examined. The length and width of the rhizomes, and, the length of the roots were measured with a caliper. The quality of the mother-leaf cuttings was scored visually using a color scale of 1-5 (1: full yellow color or dry, 2: 25% green, 3: 50% green, 4: 75% green, and 5: full green) according to a previous study (Badizadegan et al., 2023).

Propagation conditions
The pots were maintained in a propagation room. The light intensity in the laboratory was 10 µ mol m^{-2} s^{-1} sourced from cool-white fluorescent lamps. The ambient temperature of the laboratory was 25 ± 2 °C and the relative humidity was 60-70%.

Experimental design and data analysis
The experiments were carried out as a factorial arrangement in a completely randomized design (CRD) with three replications. SAS software was used for analyzing the data via ANOVA statistical analysis. Duncan's multiple range test (DMRT) was performed to determine the significance of the statistical differences at 5% probability level.

Results
First experiment
The analysis of variance revealed that the effect of IBA concentrations was significant on all of the evaluated traits, except for root and shoot counts ($P \leq 0.01$). Leaflet type affected the rhizome count and width and the longest root length, significantly ($P \leq 0.01$). Also, the interaction between IBA levels and types of leaflets had significant effects on traits, except for shoot count and mother-leaf quality ($P \leq 0.01$).

Rhizome count
The results indicated that the 250 mg L^{-1} IBA in combination with middle leaflet cuttings caused the highest rhizome number (2 rhizomes). However, all of treatments produced one rhizome per each leaflet cutting (Table 1).

Rhizome width
With increasing concentrations of IBA, the width of produced the rhizomes increased. Also, apical leaflet cuttings had the highest rhizome count. Combinations of IBA and leaflet position showed that the treatment of 250 mg L^{-1} IBA on basal leaflet caused the highest rhizome width (13.3 mm). This was not different from the effect of 500 mg L^{-1} IBA. The lowest rhizome width was observed in 250 mg L^{-1} IBA in combination with middle leaflet cuttings (2 mm). As mentioned before, this treatment produced the highest rhizome count. Therefore, the formation of a higher number of rhizomes corresponded with smaller rhizomes (Table 1).

Rhizome length
Similar to previous results, increasing the IBA concentrations significantly affected rhizome length. The highest rhizome length was obtained in 250 mg L^{-1} IBA with middle leaflet cuttings (22 mm) and the lowest value was observed in this leaflet without IBA application (9.7 mm) (Table 1).

Root count and length
According to the comparison of mean values, the maximum root count per rhizome was produced by the application of 500 mg L^{-1} IBA when using middle leaflet cuttings (9). The lowest amount of this trait was in basal leaflets without using IBA (2 roots) (Table 1). The highest root length was produced in basal leaflet cuttings treated with 500 mg L^{-1} IBA (70.1 Mm). This combination was not different from most of the other treatments. The minimum root length (2 mm) was observed in middle leaflets when using 250 mg L^{-1} IBA (Table 1).

Mother-leaf quality
The analysis of variance revealed that the IBA considerably affected the mother-leaf quality. By increasing the IBA levels, the quality of mother-leaf increased. The application of 500 mg L^{-1} IBA induced maximum mother-leaf maintenance (Fig. 1).

Second experiment
The analysis of variance revealed that the propagation substrates significantly affected all of the evaluated traits, except for rhizome count ($P \leq 0.01$). However, the type of leaflet cuttings and their combination with substrates had no significant effect on the evaluated morphological traits during the propagation period.

Rhizome width
An equal mixture of perlite and cocopeat enhanced the rhizome width (8.54 mm) compared to the other media. Conversely, using sand as the propagation medium led to minimal rhizome width (4.8 mm) (Fig. 2).

Rhizome length
Corresponding to the rhizome width, the maximum rhizome length (10.3 mm) occurred in response to an equal proportion of perlite and cocopeat (50:50). Likewise, the shortest rhizome length was formed in a substrate of sand (5.9 mm) (Fig. 3).

Table 1. Mean comparisons of the effect of IBA levels and leaflet cutting types on vegetative propagation of Zamiifolia after four months.

IBA (mgL^{-1})	Leaflet cutting types	Rhizome number	Rhizome wide (mm)	Rhizome length (mm)	Root number	The longest root length (mm)
0	Apical	1^{b}	12.68^{a}	15.89^{b}	8.33^{ab}	48.49^{a}
	Middle	1^{b}	3.89^{b}	9.73^{c}	2.5^{abc}	12^{b}
	Basal	1^{b}	3^{b}	13^{bc}	1^{c}	3^{b}
250	Apical	1^{b}	13.07^{a}	16.51^{b}	8.5^{ab}	52.26^{a}
	Middle	2^{a}	2^{b}	22^{a}	2^{c}	2^{b}
	Basal	1^{b}	13.29^{a}	15.38^{b}	8.5^{ab}	53.63^{a}
500	Apical	1^{b}	10.71^{a}	13.77^{bc}	4.66^{abc}	48.49^{a}
	Middle	1^{b}	12.51^{a}	15.79^{b}	9^{a}	50.79^{a}
	Basal	1^{b}	13.35^{a}	15b	5.66^{abc}	70.96^{a}

Similar letters in each column indicate no significant difference at 5% probability level.

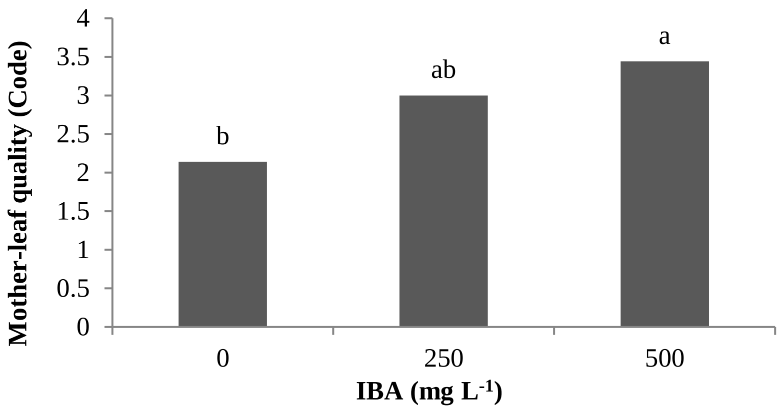

Fig. 1. Effect of different levels of IBA (0, 250, and 500 mg L^{-1}) on quality of mother-leaf in Zamiifolia propagation by leaflet cutting, four months from planting.

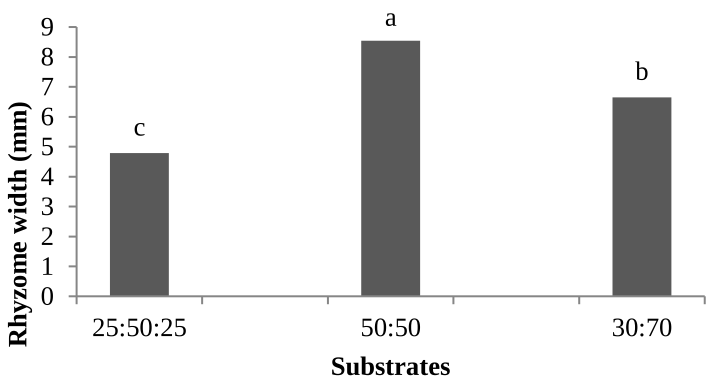

Fig. 2. Effect of different propagation substrates (perlite: sand: cocopeat (50: 25: 25), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30)) on rhizome width (mm) in Zamiifolia propagation by leaflet cutting, four months from planting.

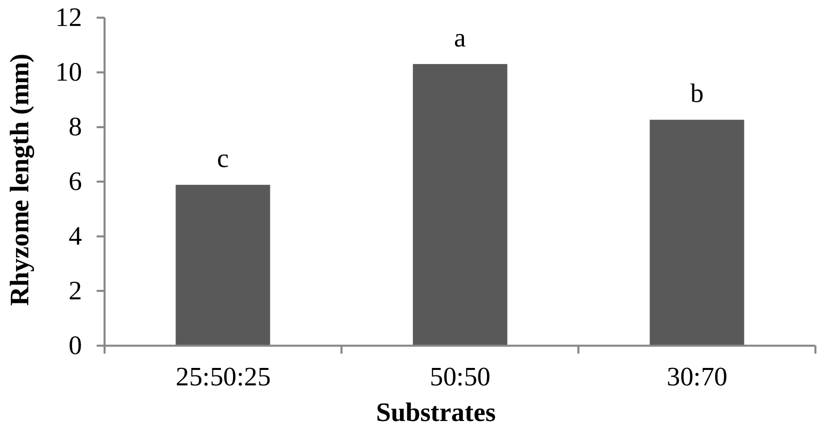

Fig. 3. Effect of different propagation substrates (perlite: sand: cocopeat (50: 25: 25), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30)) on rhizome length (mm) in Zamiifolia propagation by leaflet cutting, four months from planting.

Root count

The analysis of variance showed that substrate types considerably affected the number of roots taking form. The highest root count (4.4 per rhizome) was obtained in an equal mixture of perlite and cocopeat (50:50). Nonetheless, the lowest root count (2.7 per rhizome) formed in the substrate with 25% sand (2.7). These results are similar to the rhizome width and length (Fig. 4).

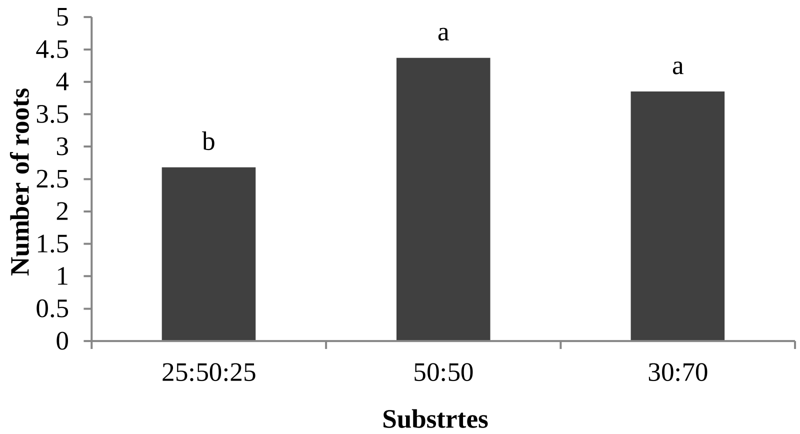

Fig. 4. Effect of different propagation substrates (perlite: sand: cocopeat (50: 25: 25), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30)) on the number of roots in Zamiifolia propagation by leaflet cutting, four months from planting.

Root length

Similar to previous results in the second experiment, the medium with 50% perlite in combination with 50% cocopeat produced the maximum root length (38.7 mm). Also, the medium with 25% sand, 50% perlite, and 25% cocopeat produced the shortest roots (14.7 mm) (Fig. 5).

Mother-leaf quality

As shown in Figure 6, the results indicated that the best quality of mother-leaves was achieved in the medium with 30% perlite and 70% cocopeat as well as in the medium containing 25% sand, 50% perlite, and 25% cocopeat. Conversely, the lowest quality was observed in the medium with 50% perlite and 50% cocopeat (Fig. 6). These results are in contrast with previous results on the other measured traits.

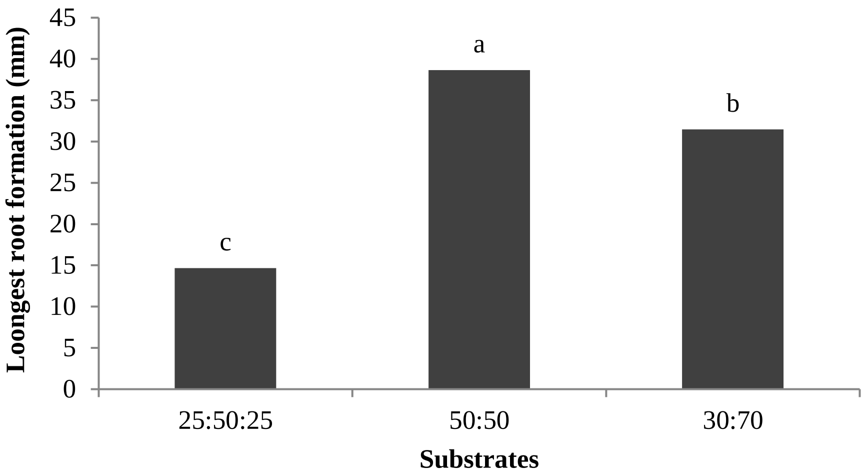

Fig. 5. Effect of different propagation substrates (perlite: sand: cocopeat (50: 25: 25), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30)) on the longest root formation (mm) Zamiifolia propagation by leaflet cutting four months from planting.

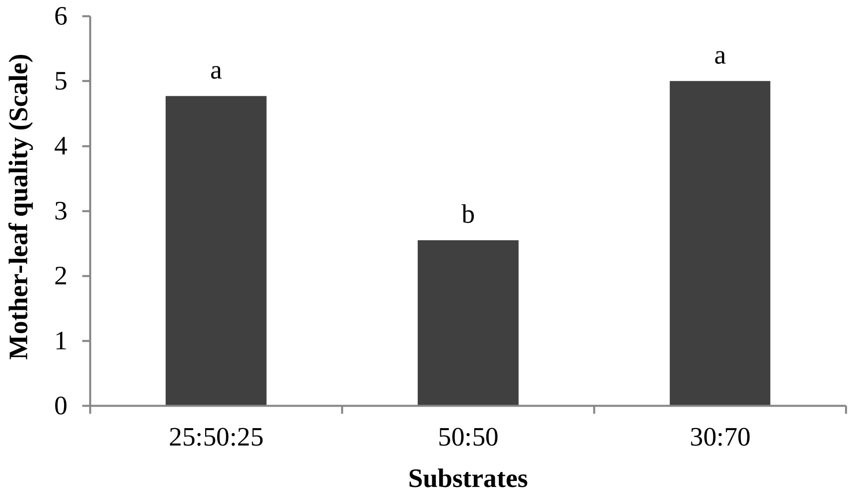

Fig. 6. Effect of different propagation substrates (perlite: sand: cocopeat (50: 25: 25), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30)) on mother-leaf quality in Zamiifolia propagation by leaflet cutting, four months from planting.

Shoot formation

The results showed that substrate types significantly affected the extent of shoot formation. According to the comparison of mean values, the highest number of shoots (1.8 per rhizome) occurred in the medium containing sand (50%) and perlite (50%). The medium with sand formed the fewest shoots number (less than one shoot per rhizome) (Fig. 7).

Discussion

Z. zamifolia usually propagates new plantlets through leaf propagules in a specific way. According to our observations, white tissues appeared at the end of the leaf cuttings a few days after obtaining them. Subsequently, these white tissues became round and turned into rhizomes, and finally, adventitious roots formed on these rhizomes (Fig. 8).

Reports have shown that growth hormones may play a role in rhizome formation. In *Z. zamiifolia* plants, the leaves are responsible for rhizome growth and development (Cutter, 1962). Using 250 mg L-1 IBA on middle leaflets produced the maximum rhizome formation in this study. Confirming these findings, Mirzakhani and Naderi (2010) reported that the application of 300 mg L^{-1} IBA increased the number of bulbs in Lilium ledebourii. Also, all types of leaflets produced one rhizome. However, middle leaflets produced the highest rhizome per leaflet cutting. Also, authors recently indicated that the types of leaflet cuttings (especially middle leaflets) significantly affected the number of rhizomes (Badizadegan et al., 2023). Conversely, Thongkham and Phavaphutanon (2018) reported that leaves taken from different positions (apical, middle, and basal) can root and form rhizomes equally.

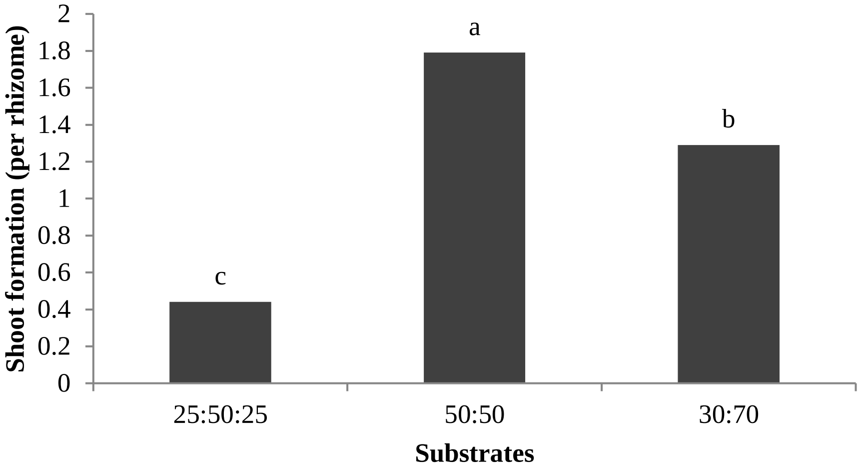

Fig. 7. Effect of different propagation substrates (perlite: sand: cocopeat (50: 25: 25), cocopeat: perlite (50: 50), and cocopeat: perlite (70: 30)) on shoot formation (per rhizome) in Zamiifolia propagation by leaflet cutting, four months from planting.

Fig. 8. Developmental stages of root and rhizome formation in Zamiifolia propagation by leaflet cutting (from left to right; swelling of cutting place, formation of rhizome and appearance of first root, enlargement of rhizome and roots, extension of rhizome and formation of more roots).

The formation of rhizomes in all leaf propagules showed that there are sufficient substances such as auxins, rooting cofactors, and carbohydrates in the apical, middle, and basal leaves for the formation of roots and rhizomes (Thongkham and Phavaphutanon, 2018). These results demonstrated the positive effects of IBA on the width and length of rhizomes, although the apical leaflets produced the highest width with or without IBA. Lopez et al. (2009) confirmed that using of apical leaflet propagules produced more rhizomes than basal leaflets thus confirming our results. Also, authors recently indicated that apical leaflet cuttings caused the largest rhizome width (5.73 mm), significantly larger than the middle and basal leaflets (Badizadegan et al., 2023). Our findings showed that using of IBA on middle and basal leaflets enhanced the root count and length. Many reports have shown the positive effects of auxin on the rooting of ornamental plants. The positive effect of IBA on the rooting of propagules can be attributed to the action of auxins, which stimulate the primary division of root initiator cells. Auxins are key signaling molecules that play a fundamental role in regulating various developmental processes in plants. These processes include rhizome and/or root formation and their subsequent growth (Zhang et al., 2022). It has been confirmed that the cell division of adventitious roots depends on either internal or external auxin (Solgi and Sahraei, 2022). According to Hartmann et al. (2002), IBA is the best auxin for general use because it is nontoxic to plants and is effective in promoting the rooting of a large number of plant species. IBA, is a major artificial auxin and is partially transformed to IAA when applied on propagules to stimulate rooting. External applications of IBA may also increase rooting by increasing internal IBA or synergistically altering

the action of IAA or may enhance the endogenous synthesis of IAA. Furthermore, IBA increases tissue sensitivity to IAA, thus increasing rooting (Henrique et al., 2006).
In this experiment, the mother leaves treated with 500 and 250 mg L^{-1} IBA showed the highest quality. Based on the evidence that IBA improves the traits related to rhizomes and roots, it can be concluded that mother leaves are essential for root and rhizome formation. This positive role of mother leaves may be explained by the sink-source relationship. Mother leaves supply phytohormon compounds and nutrients for the development of roots and rhizomes (Cutter, 1962). Similarly, according to Badizadegan et al. (2023), maintaining healthy mother leaves significantly improves rhizome formation, subsequent root formation, and in zamiifolia growth.
The choice of culture substrate significantly influences the rooting capacity of many ornamental plants (Shokri et al., 2014). Substrates for rooting play a pivotal role not only in root initiation but also in the subsequent development of roots. The presence of organic matter and high cation exchange capacity in substrates can enhance nutrient and water retention, creating favorable conditions for root growth in cuttings (Nair et al., 2008). However, it has been reported that using cocopeat as an organic material individually may not be ideal due to its compactness and reduced aeration (Noguera et al., 2000). Combining cocopeat with other substrates such as sand or perlite can improve its physical and chemical properties, ensuring sufficient oxygen supply to the cuttings and adequate moisture retention. Therefore, it can enhance substrate efficiency (Shokri et al., 2014). The results of this experiment demonstrated that when a substrate consisting of 50% cocopeat and 50% perlite was utilized, there was a significant increase in the length and width of rhizomes, the number of roots, maximum root length, and the shoots taking form. Similarly, the combination of perlite with cocopeat in comparison with perlite alone promoted leaf surface area, leaf count, and overall plant performance in gerbera plants (De Paradiso and Pascale, 2008). Moreover, our findings are consistent with those reported by Abshahi et al. (2019) which suggested the optimal substrate mixture of perlite and cocopat (1:1) for rooting *Juniperus Sabina* cuttings.
Seneviratne et al. (2013) indicated that a substrate composed of sand and compost was the most effective medium for root induction in zamiifolia. Contrary to the results of this research, it was demonstrated that in the case of Schefflera ornamental plants, the use of perlite as a sole substrate led to an increase in root initiation (Bidarnamani et al., 2016). Additionally, an investigation into the impact of cocopeat as a substrate on the growth and development of *Celosia cristata* roots revealed that cocopeat alone provided better aeration compared to a mixture of cocopeat and perlite (70: 30) (Awang et al., 2009).
In this research, the substrate containing sand, perlite, and cocopeat exhibited the lowest values of rhizome length and width, root count, and maximum root length. Sand substrates, due to their low cation exchange capacity, are not a suitable propagation medium. The combination of sand and perlite, separately or in combination, resulted in higher salinity levels, making the substrate unsuitable and necessitating frequent leaching. While perlite supports substrate aeration, it lacks nutritional content, has poor moisture retention capacity, and is inconsistent because of variable salt contents. When sand and perlite are combined, they reduce root initiation (Shokri et al., 2014).

Conclusion
This is the first report on the application of IBA on three types of Zamiifolia leaflet cuttings. IBA improved the vegetative propagation of Zamiifolia by leaflet cutting. Also, mother leaves treated with 500 mg L^{-1} IBA showed the highest quality. Rhizome formation and subsequent root formations were vital characteristics enhanced by IBA. Although the influence of leaflet position on most of the characteristics was not significant, the middle leaflets produced the highest rhizome count. Zamiifolia produced rhizomes with acceptable traits in the propagation medium containing equal portions of perlite and cocopeat. Our findings suggested that IBA and cocopeat-perlite substrates can accelerate Zamiifolia propagation.

Conflict of Interest
The authors indicate no conflict of interest in this work.

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