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Scientific Article

Effect of chitosan on propagation of zamiifolia as tropical ornamental indoor plant by leaf cutting

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Abstract

Zamiifolia (*Zamioculcas zamiifolia*) is an ornamental and perennial medicinal plant, which belongs to Araceae family. This plant holds a prominent place in the economic significance of this particular plant family. This study aimed to examine the process of root development and rhizome production through leaf cuttings of zamiifoliain the this experiment. This experiment was conducted as factorial design within a completely randomized framework design with three replications. Experimental treatments composed of chitosan application at three levels (0, 250 and 500 mg L^{-1}), and the positioning of leaflet cuttings along the main axis of the mother leaf (apical, middle, and basal). After the application of the treatments, the leaflet cuttings were subjected to a four-month rooting period. This rooting process took place in a growth medium consisting of a balanced mixture of perlite and cocopeat mixed in a volume ratio of 1:1. The results exhibited significant effects of different chitosan concentrations on several parameters, including rhizome number, rhizome width, the number of roots, and the quality of the mother leaf. In addition, the type of leaflet cutting demonstrated a significant influence on the width and number of rhizomes. These results demonstrated that the application of chitosan at concentrations of 250 and 500 mg L^{-1} had a positive effect and resulted in increased rhizome number, rhizome width, and number of roots. Overall, it can be concluded that chitosan can promote the growth and development of zamiifolia by stimulating rhizome production and improving root proliferation.

Keywords: cutting position, mother leaf, rhizome, rooting, substrate.

Resumo

Efeito da quitosana na propagação por estaquia foliar da planta de interior tropical zamioculca

Zamioculca (*Zamioculcas zamiifolia*) é uma planta perene de importância ornamental e medicinal, ocupando lugar de destaque na família Araceae. Este trabalho teve como objetivo avaliar o processo de desenvolvimento radicular e produção de rizomas por meio de estacas foliares de zamioculcas. O experimento foi conduzido como delineamento completamente casualizado em esquema fatorial com três repetições. Os tratamentos foram compostos pela aplicação de quitosana em três níveis (0, 250 e 500 mg L^{-1}) e a posição de retirada das estacas foliares (apical, médio e basal). Após a aplicação dos tratamentos, as estacas foram submetidas a um período de enraizamento de quatro meses em substrato composto por perlita e fibra de coco (1:1). Os resultados foram significativos para a aplicação de quitosana em vários parâmetros, incluindo número de rizomas, largura do rizoma, número de raízes e qualidade da folha principal. Além disso, o tipo de estaca demonstrou influência significativa na largura e no número de rizomas. Esses resultados demonstraram que a aplicação de quitosana nas concentrações de 250 e 500 mg L^{-1} teve efeito positivo e resultou em aumento do número de rizomas, largura do rizoma e número de raízes. No geral, pode-se concluir que a quitosana pode promover o crescimento e desenvolvimento de zamioculca estimulando a produção de rizomas e melhorando a proliferação de raízes.

Palavras-chave: enraizamento, folha principal, rizoma, substrato, tipo de estaca.

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Introduction

Zamiifolia (*Zamioculcas zamiifolia*) is a monocotyledonous plant that is highly regarded for its ornamental attributes. It belongs to the Araceae family and is native to South Africa (Lopez et al., 2009). This tropical perennial kind has gained worldwide recognition as a recent addition to potted plants (Seneviratne et al., 2020; Mayers, 2023). The propagation of zamiifolia has been inhibited by its inherent characteristic of a slow growth rate (Seneviratne et al., 2013; Sayadi Nejad and Sadeghi, 2019). Numerous studies demonstrate the ability of zamiifolia to thrive over several years. It shows adaptability to different environmental conditions, robust defense mechanisms against pathogens and resistance to climatic changes (Seneviratne et al., 2020). *Z. zamiifolia* was discovered in 1905, leading to the identification of this plant as an indoor ornamental foliage pot plant (Sastry et al., 2019). In addition, Zamiifolia has demonstrated its efficacy in phytoremediation of ozone, as highlighted in a study by Pheomphun et al. (2019) and Ullah et al. (2021). In other words, it is described as a "unique" indoor foliage plant (Pourhassan et al., 2023).

This ornamental plant has dark green leaves that are directly attached to underground tuber-like rhizomes (Chen and Henny, 2003; Mayers, 2023). Rhizome division and leaf cuttings (specifically leaflet cuttings) are the primary methods utilized for the asexual propagation of this plant species (Pourhassan et al., 2023; Mayers, 2023). During the propagation process, a small rhizome emerges at the distal end of the propagules. Then, adventitious roots emerge from the newly developed rhizome. As the plant progresses, shoot production occurs, leading to the growth and development of above-ground foliage (Chen and Henny, 2003). Few studies have been conducted on this plant, especially on its vegetative propagation.

Using different cultivation beds, Seneviratne et al. (2013) investigated propagation methods of *Z. zamiifolia* with leaf cuttings. According to the results, basal leaflets without petioles were the most effective propagules for propagation. Moreover, Lopez et al. (2009) investigated the possibility of asexual propagation of *Z. zamiifolia* by single, apical, and basal leaflets, or axial propagules. It was found that the apical leaflet propagules treatments under a 16-hour photoperiod at 29-32 °C reduced the reproduction and production time of this plant.

Furthermore, the effect of leaflet position and size on rooting and rhizome formation in *Z. zamiifolia* was studied. A comparison was made between the rooting and rhizome formation of leaflet propagules from the apical, middle, and basal parts of the petiole in plants. The results of the study showed that leaves collected from different segments along the petiole of zamiifolia had good performance in both root initiation and rhizome formation (Thongkham and Phavaphutanon, 2018).

Chitosan is a biological biopolymer derived from chitin and used in pharmaceutical, food, agricultural, biotechnology, health, cosmetic, and other fields (Nourafcan, 2017; Solgi, 2018). Chitosan is used in agriculture for coating seeds, leaves and fruits and to control the release of chemicals compouds to protect plants from microorganisms and to stimulate seed germination and plant growth (Solgi, 2018; Malekpoor et al., 2017). To date, chitosan has not been reported to affect various developmental and physiological aspects of *Z. zamiifolia*, particularly the reproductive stage. Gornik et al. (2008) reported that the application of chitosan improved root development of cuttings, increased the number of new formed canes and their length. Furthermore, this compound enhanced the number of internodes and chlorophyll content in the leaves of grapevine stem cuttings. Besides, Rahman et al. (2021) indicated that the combination of chitosan, rice husk, peat moss and oil palm EFB improved plant height and leaf length of *Dendrobium* Shavin White orchid. On the other hand, oil palm EFB and chitosan combination as a growth medium enhanced the leaf number. Accordingly, this study was conducted to evaluate the effects of chitosan treatment and positioning of leaflet cuttings along the main axis of the mother leaf (apical, middle, and basal) on stimulating root and rhizome formation in *Z. zamiifolia*.

Materials and Methods

This study was conducted in the laboratory of the Horticultural Science and Engineering Department, Faculty of Agriculture and Environment Science, Arak University over 2020-2021.

Plant Materials

Z. zamiifolia pots were purchased from the producer of indoor plants in Mahalat, Iran. Plants were selected based on their age (2 years-old), size (20 cm pot), and leaf number (15-20). The used mother leaf cuttings with the apical (juvenile leaves), middle and basal leaves (mature leaves) were separated from the mother plants. Subsequently, they were cultivated following the application of the respective treatments (Figure 1).

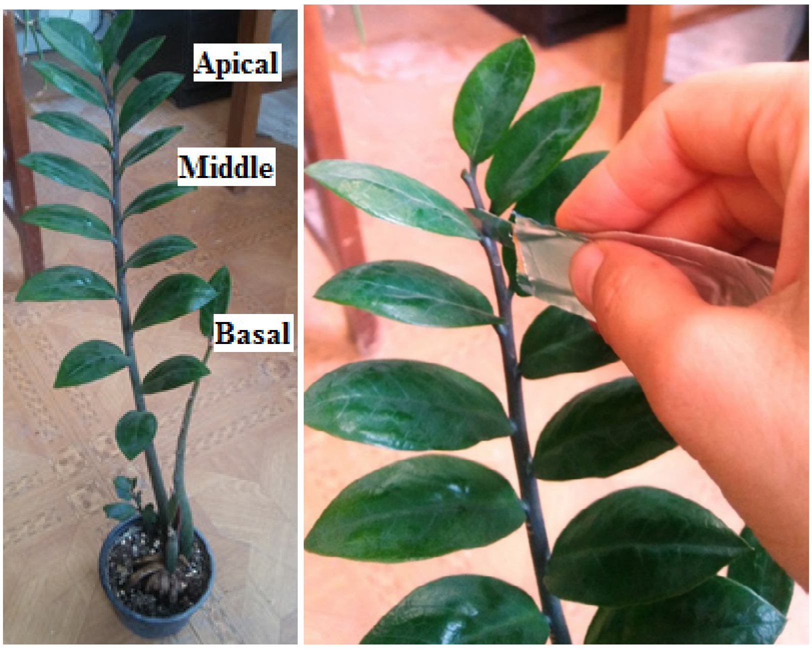

Figure 1. Sample of zamiifolia and types of leaflets which are used in this research

Treatments

Chitosan was purchased from Merck company (Germany; purity > 98%). It was treated at three concentrations (0, 250 and 500 mg L^{-1}) on three type of leaflet cuttings on the main mother leaf axis (apical, middle and basal). The experiment was conducted as a factorial arrangement (3 × 3) based on a completely randomized design with three replicates. Therefore, two concentrations of chitosan were prepared and leaf propagules were immersed in the chitosan solution for 5 minutes. Subsequently, the treated propagules were planted in uniform pots (8 cm) containing a substrate of 50:50% cocopeat and perlite (v/v). Distilled water was used as a control (Figure 2). Chitosan solutions were prepared by accurately weighing 250 and 500 mg L^{-1} chitosan and subsequently dissolving it in 1% acetic acid (v v^{-1}). Distilled water was then added to achieve the desired volume of the solution.

Figure 2. Dipping of samples in chitosan treatments (left)
and planting the treatments in pot with cocopeat and perlite (right)

Measurements

Four months after propagation, leaf cuttings were removed from the substrate to investigate their morphological characteristics. The rhizomes were cleaned and measured without damaging the roots. Several traits were evaluated including of rhizome numbers, length and width of rhizomes, root numbers, the length of the longest root, and the quality of the mother leaf. Length and width of the rhizomes and the root length were measured by using 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) (Thongkham and Phavaphutanon, 2018) (Figure 3).

Figure 3. Quality of mother leaf cuttings by senescence scored visually (1: full yellow color or dry, 2: 25% green, 3: 50% green, 4: 75% green, and 5: full green**)**

Propagation Conditions

The light intensity in the laboratory was 10 µmol $m^{-2}s^{-1}$ which was provided by cool-white fluorescent lamps. The temperature of the laboratory was 25±2 °C and the relative humidity was 60%-70%.

Experimental Design and Data Analysis

SAS software was used to analyze the data using ANOVA statistical analysis. Duncan's multiple range test (DMRT) was performed to determine the significance of the statistical difference at the 1% and 5% probability levels. Tables were drawn using Excel software.

Results and Discussion

Based on the variance analysis, the statistical evaluation revealed that there was a significant interaction between chitosan concentrations and the types of leaflet cutting in zamiifolia, specifically in relation to root number. Furthermore, different levels of chitosan significantly affected rhizome number, rhizome width, number of roots, and the mother leaf quality. Rhizome width and number were also significantly affected by leaflet cutting types.

Rhizome parameters

The comparison between chitosan levels showed that 500 mg L^{-1} chitosan produced the greatest rhizome number with an average of 2.33 rhizomes in each treatment. This treatment performed significantly superior to others which produced one rhizome per pot (Figure 4).

According to Figure 5, the concentration of 250 mg L^{-1} chitosan exhibited the maximum rhizome width (5.5 mm), which was significantly superior than other treatments (2.7 mm). As the formation of rhizome (tuber-like) is the first step in vegetative propagation of zamiifolia by leaf cutting. Therefore, any substance and/or condition could assist this development process. One of the potential compounds that can be utilized is the eco-friendly chitosan compound. Our research results confirm that the application of chitosan resulted in improved rhizome production. It is worth noting that there are few studies investigating the effects of chitosan on vegetative propagation of ornamental plants.Similar to our results, Asghari-Zakaria et al. (2008) identified that the concentration of 500 mg L^{-1} of chitosan increased the number of tubers in potatoes. It has been proposed that the increasing formation and growth effect of chitosan could be attributed the presence of the nitrogen element within its chemical structure (Malekpoor et al., 2017).

According to the data in Figure 6, the apical leaf cuttings had the largest width of the rhizome (5.73 mm), which was significantly larger than the other treatments. However the basal leaflets showed the lowest rhizome width (2.66 mm). Furthermore, the middle leaflets exhibited an average diameter of 4.26 mm, which was not statistically different from the basal leaflets.

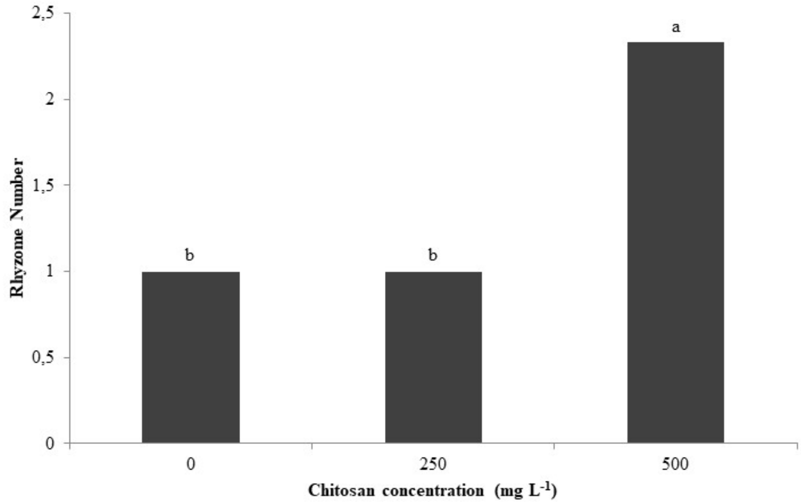

Figure 4. Effect of three concentrations of chitosan on rhizome number of zamiifolia after four months

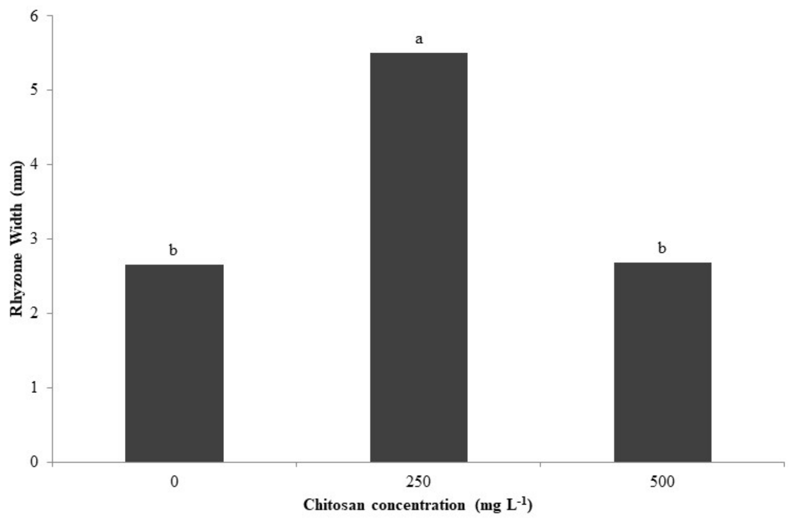

Figure 5. Effect of three concentrations of chitosan on rhizome width of zamiifolia after four months

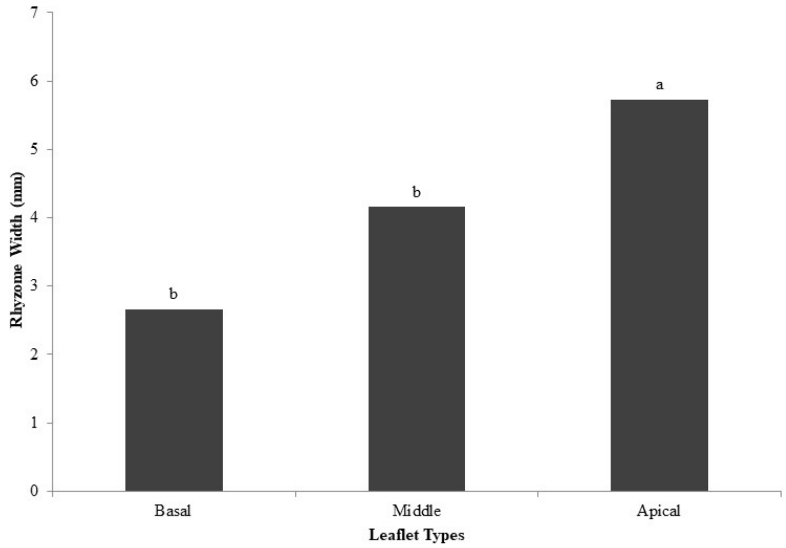

Figure 6. Effect of different types of leaflet on rhizome width of zamiifolia after four months

Similarly, Lopez et al. (2009) found that the use of propagation material with apical leaflets reduced the reproduction and production time of this plant. This is because the apical leaflets are younger than the other leaflets. It seems that they produce more plant growth regulators to improve rhizome growth.

Root paramaters

Mean comparison results revealed that the highest number of roots was obtained with a concentration of 250 mg L^{-1} of chitosan application. Interaction effects also showed that the same concentration and the apical leaflet cutting exhibited the maximum number of roots (4 roots per rhizome). In contrast, the lowest number of roots was produced without using of chitosan (control) with the basal leaflet types and also 500 mg L^{-1} chitosan plus middle leaflet treatments (one root per rhyzome). Since, apical leaflets were rotted by using 500 mg L^{-1} chitosan, therefore, no roots were produced in this treatment (Figure 7).

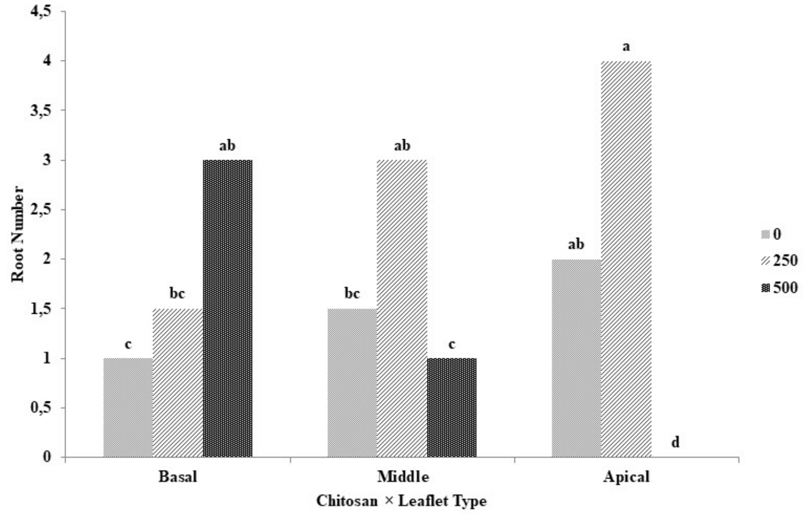

Figure 7. Effect of of three concentrations of chitosan and three types of leaflet on root number of zamiifolia after four months

It has been proposed that chitosan has the ability to regulate plant growth and development by influencing signaling pathways that ultimately stimulate auxin biosynthesis (Malekpoor et al., 2017). Auxins are important signaling molecules that play a pivotal role in regulating various developmental processes at all stages of plant ontogeny. These processes include root formation and elongation (Zhang et al., 2022). It has been confirmed that the cell division of the first initiatied roots is depend on either internal or external auxin (Solgi and Sahraei, 2022). The highest number of roots was observed in the middle and apical leaves when a chitosan concentration of 250 mg L^{-1} was applied. Increasing chitosan concentration was found to promote root development specifically in basal leaves. The higher chitosan concentrations resulted in increased number of roots and increased root length in lemon verbena (Nourafcan, 2017) and grapes (Gornik et al., 2008). Chitosan has been shown to enhance the uptake and transfer of auxin by inhibiting the enzymes involved in oxidation and producing a synergistic effect that ultimately affects root formation (Gornik et al., 2008).

Mother leaf parameters

As shown in Figure 8, the results revealed that the best quality of mother leaves was achieved with 250 mg L^{-1} chitosan (4.12). Although the quality of the mother leaf was found to be significantly lower in the control treatment (2), there was no significant difference observed when compared to the treatment with 500 mg L^{-1} chitosan (2.6) (Figure 8).

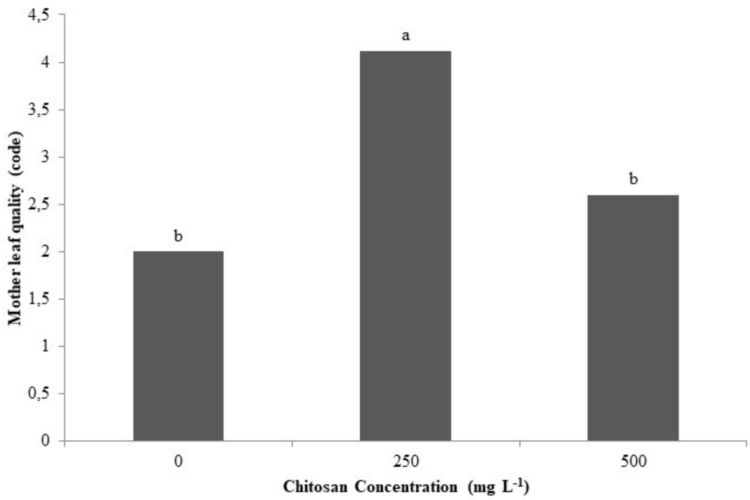

Figure 8. Effect of of three concentrations of chitosan on mother leaf quality of zamiifolia after four months

The results indicate that higher concentrations of chitosan do not seem to be effective in maintaining the quality of the mother leaves. Therefore, the concentration of chitosan plays a crucial role in maintaining the quality of the mother leaf. In other words, the protective effect of chitosan on mother leaf quality is concentration dependent, suggesting that an optimal chitosan concentration must be determined to achieve the desired results. Maintaining healthy mother leaves has a significant impact on rhizome formation and subsequent root formation and growth. This is because healthy mother leaves contribute to the production of growth-promoting substances and photosynthetic assimilates, which are essential for the overall development of the plant. According to the study by Mahdavi et al. (2014), chitosan demonstrated a beneficial effect, especially at low concentrations. This positive effect can be attributed to the ability of chitosan to act as a barrier and reduce water evaporation from plant leaves. Additionally, chitosan can involve in other physiological functions such as modulating the abscisic acid synthesis pathways. Subsequently it can cause stomata closure take places and reduced transpiration. Finally, the retention of moisture in the plant, facilitated by the application of chitosan, has been found to have a positive effect on its longevity (Noorafcan, 2017). Furthermore, it can be used as antitranspirant compound through foliar application in many plants (Hidangmayum et al., 2019). Consequently, chitosan can decrease negative effects of environmental coditions by its specific properties (Gornik et al., 2008; Solgi, 2018). In other words, chitosan improves the physiological response of plants and helps mitigate the adverse effects of abiotic stresses. It achieves this by acting through stress transduction pathways, which involve the use of secondary messengers (Hidangmayum et al., 2019).

Conclusions

Our results revealed that chitosan (250 and 500 mg L^{-1}) increased rhizome development (formation, and width), rooting in zamiifolia plants compared to the control. Furthermore, chitosan treatment at 250 mg L^{-1} produced the best mother-leaf quality. Overall, it can be concluded that the effectiveness of chitosan in promoting desirable effects depends on its concentration.

Author Contribution

MS: supervision, corresponding author, experimental set up procedures. **FB:** experimental procedures, data acquisition and elaboration, draft preparation. **MT:** experimental set up advisor, experimental procedures, draft preparation. **AA:** experimental set up advisor, experimental procedures, draft preparation.

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