

THE INFLUENCE OF SHALLOT SOLUTION ON COLEUS (*Plectranthus scutellarioides* (L.)) SEEDLING

*Pengaruh Larutan Bawang Merah pada Bibit Iler (*Plectranthus scutellarioides* (L.))*

Dian Susanti¹⁾, Prambayu Brenda Herera²⁾, Rissa Tri Ismayanti³⁾, Dyah Subositi¹⁾

¹National Research and Innovation Agency, Tawangmangu, Karanganyar, Indonesia

²Medicinal Plant and Traditional Medicine Research and Development Center, National Institute Health Research and Development, Ministry of Health, Karanganyar, Indonesia

³Agrotechnology, Agriculture Faculty, Universitas Diponegoro, Semarang, Indonesia

*e-mail: dian.ssanti@gmail.com

ABSTRACT

Nurseries are the main critical phase in the cultivation of Coleus (*Plectranthus scutellarioides* (L.) R.Br.), which is useful as a raw material for traditional medicine. The key to producing well-grown, healthy, and uniform seedlings is using exogenous hormones throughout the seedling stage. Shallot has the potential to be used as an exogenous hormone. The study was conducted with shallot concentration treatment (0, 35, 70, and 100%) and soaking time (0, 12, and 24 hours). The results show shallot's activity as an exogenous hormone in coleus seedlings, including increasing the root volume of coleus seedlings through the initiation of root hairs, altering seedling height, and stimulating seedling adventitious shoot growth. Conversely, high concentrations of shallots reduced the number of coleus seedling shoots.

Keywords: Coleus, IAA, seedling, shallot

ABSTRAK

Pembibitan adalah fase utama yang penting dalam budidaya Coleus (*Plectranthus scutellarioides* (L.) R.Br.), yang berguna sebagai bahan baku obat tradisional. Semua usaha, termasuk penggunaan hormon eksogen pada tahap pembibitan, adalah kunci untuk mendapatkan bibit tanaman yang tumbuh baik, sehat, dan seragam. Bawang merah berpotensi digunakan sebagai hormon eksogen. Penelitian dilakukan dengan perlakuan konsentrasi bawang merah (0, 35, 70, dan 100%) dan lama perendaman (0, 12, dan 24 jam). Hasil penelitian menunjukkan bahwa bawang merah memiliki aktivitas sebagai hormon eksogen pada bibit coleus, diantaranya meningkatkan volume akar bibit coleus melalui inisiasi rambut akar, mempengaruhi tinggi bibit, dan pertumbuhan tunas adventif bibit. Namun, bawang merah dalam konsentrasi tinggi menyebabkan penurunan jumlah tunas bibit coleus.

Kata kunci: coleus, IAA, bibit, bawang merah

INTRODUCTION

Coleus (*Plectranthus scutellarioides* (L.) R.Br.) is a medicinal plant from the *Lamiaceae* family. This perennial shrub with a woody stem base is genuine to Southeast Asia to Australia. Plants with heights up to 1-2 meters are better known as ornamental plants because of their beautiful leaf colors (Suva et al., 2015). In Indonesia, this plant called Miana comprises secondary metabolites that are effective for use in traditional medicine. However, not every coleus variety is utilized because the differences in leaf color signify differences in the metabolite compounds

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contained. Coleus variety with brownish-red leaves comprises an average quercetin content of 3.122 mg/g (0.312%) (Moektiwardoyo et al., 2011; Sukmawati et al., 2019). The purple colors of the coleus leaves express the presence of anthocyanin compounds. Anthocyanin of purple coleus leaf that micro-encapsulated is 0.149 mg/g (Puspita et al., 2018). Purple coleus also contains flavonoid compounds of $3.451 \pm 0.57\%$ to $5.848 \pm 0.25\%$ (Roviqowati et al., 2019).

Coleus was developed for hemorrhoid treatments in Indonesia. The evidence bases of coleus usage as a scientific herbal medicine increases public confidence in using herbal medicine for treatment. The higher public trusts indirectly increase the need for quality and quantity of coleus leaves as raw material for herbal medicine (Astana et al., 2017; Sudrajad et al., 2018). Efforts to improve the quality and quantity of medicinal plants as raw material improvement was carried out by ensuring the correctness of types and varieties of raw materials. Raw materials for traditional medicines obtained from wild nature have not been certified for their validity and have not been standardized. Standardization of raw materials for herbal medicines is carried out by cultivating medicinal plants (Subositi and Mujahid, 2019; Suharsanti and Wibowo, 2014; Wang et al., 2020). Nurseries are one of the notable stages in medicinal plant cultivation, which establishes the success of plant growth, maintenance, and production. Internal and external factors of the plant must be considered when propagating medicinal plants. Internal factors are those within the seedling or plant that affect its growth, whereas external factors are those external to the seedling or plant (Putra and Edwin, 2017).

One of the internal factors is the presence of compounds formed naturally or synthetically in the plant body in fewer amounts and affect plant growth, development, or metabolism. These compounds, known as plant growth regulators (PGR) or hormones, have no nutritional value and are not toxic. The various compound types with different effects, namely increasing growth, inhibiting growth, accelerating fruit ripening, and others (Rademacher, 2015). The use of exogenous hormones at the nursery can affect plant growth, development, and resistance (Kosakivska et al., 2022). Shallots can be used as an exogenous hormone in the nursery because, in its application, it gives good results in the percentage of live cuttings, when shoots appear, shoots length, number of leaves, number of roots, root length, and root volume of pepper plants (Tarigan et al., 2017). The growth-regulating compounds contained in young harvested shallot solution in the form of auxin (IAA; 2,4 D; and NAA) and cytokinin (BAP), while old age-harvested shallot solutions contain auxin (IAA) and cytokinin (BAP) (Yunindanova et al., 2018). Manurung et al. (2020) stated that shallot solutions containing auxin (IAA) at 0.0027%, gibberellin (GA3) at 0.0021%, and cytokinin at 0.0022%. Plant nurseries head to obtain healthy and uniform plant seedlings. One of the efforts to achieve this goal is to use hormones on seedling material. However, the use of natural hormones to improve medicinal plant seedlings has not been widely studied. This study intends to explain the effect of shallot solution bioactivities on the growth of the coleus seedling.

METHODS

The research was carried out in 2019 at the Medicinal Plant and Traditional Medicine Research and Development Center (MPTMRDC) Experimental Field in Kalisoro Village, Tawangmangu, Karanganyar. The field is located on the slope of Mount Lawu with an altitude of $\pm 1,200$ m above sea level; have the soil types of Complex Brown Andosol, yellowish Brown Andosol, and Litosol. The average night temperature is around 8-12°C, and 15-30°C average

daytime temperature. Average rainfall is about 2.634 mm on 161 rainy days in a year (Climate Data, 2022; Widiastiti, 2011).

This research employed a randomized block design with two treatment components and three replications, with 10 cutting samples per treatment. First factor was the concentration of shallot phytohormone solution (0, 35, 70, and 100%), and the second was the immersion time (0, 12, and 24 hours). The 0% concentration was using distilled water as a control. Immersion for 0 hours is done by immersing briefly until the lowest stems of the seedlings are wet.

The stock solution (200% w/v solution) was made by crushing 2.000 g of shallots plus 1,000 mL of water until smooth with a blender and straining to filter the liquid. The 100% concentration was made by diluting 250 mL stock solution into a total volume of 250 mL water. At the same time, the 70% v/v solution was diluted with 175 mL stock solution into a total volume of 250 mL water. Next, the 35% concentration was made by diluting 87.5 mL stock solutions into a total volume of 250 mL water.

Planting was carried out in polybags measuring 10 x 10 centimeters with soil media, goat manure, and rice husks in a ratio of 1:1:1. The cutting material was the middle stem of the accession-1 coleus (*Plectranthus scutellarioides* (L.) R. Br) plant with three bud eyes as a fixed factor. Cutting material should be healthy and of good quality, not attacked by pests, not too old, and has a diameter of about 0.75 – 1 centimeter. After being treated, coleus was planted in polybags at 1-2 cm depth and placed under shade. The watering was carried out according to the conditions of the media.

Observations were carried out every 15 days, destructively four times for 45 days from the beginning of the cutting process. Variables observed were the seedling's height, number of shoots, number of leaves, internode length, number of internodes, number of branches, branch length, root length, number of roots, and root volume. The number of roots counted from the main root. Root volume was measured by cutting the roots, putting them in a measuring cup filled with water, and observing the increase in water level. The root volume obtained from the water level difference between the water-filled cup measurement and the cup containing water and root (E1).

$$\text{Root Volume} = V1 - V2 \quad (\text{E1})$$

V1 : The volume of water in the cup before the roots are entered

V2 : The volume of water in the cup after the roots are entered

Data were analyzed descriptively and 5% ANOVA, with further analysis with 5% DMRT and regression using SPSS and Microsoft Excel (E2).

$$\text{Variables confidence level} = 1 - \text{significance value (ANOVA result)} \times 100\% \quad (\text{E2})$$

RESULT AND DISCUSSION

Significance value showed that the difference in the concentration of the shallot solution at the 95% confidence level (5% significance level) affected the coleus seedling height of 0.016, the branch length of the coleus seedling of 0.027, and the root volume of the coleus seedling of 0.028, but not affecting the other variable. However, based on the variable's confidence level, the results of the analysis were able to certify the truth that the concentration of onion solution used affected the number of leaves by 86.3%, the number of inter-nodes by 84%, and root length by 89.5%; it has the potential to be used for further development of these growth variables.

Based on the significance value, the immersion time gives significant differences in the seedling height of 0.012 and the number of shoots of 0.024. In other growth variables, the confidence level showed that immersion time did not differ significantly. Still, the duration of immersion affected inter-node length with a confidence level of 86.2% and root volume with a confidence level of 82.3%. This significant influence provides an opportunity to develop the use of shallot extract on the growth variables of root volume and inter-node length. The interaction of immersion time and concentration of shallot solution significantly affected the cutting height of 0.039 and root length of 0.02. The height of the coleus seedling in the 12 hour immersion treatment was significantly different from the 24 and 0 hour treatments. The 0 and 24 hours immersion treatment gave higher seedlings than the 12 hour treatment. Meanwhile, at the number of shoots, 0 hour immersion gave a higher amount than 12 and 24 hours (Table 1).

Table 1. Effect of immersion time of shallot solution on height and number of shoots of coleus seedling

Immersion time	Height (cm)	Number of shoots
0-hour	23.10 ± 1.98 ^b	1.75 ± 0.79 ^b
12-hour	21.33 ± 1.98 ^a	1.00 ± 0.79 ^a
24-hour	23.02 ± 1.98 ^b	1.00 ± 0.79 ^a

Note: The numbers followed by different letters are significantly different based on DMRT 5%.

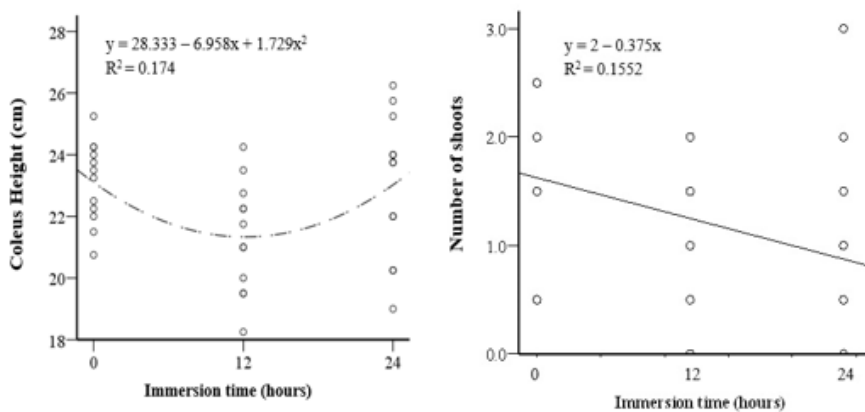


Fig. 1 The height of cuttings and the number of shoots at the immersion time treatment

Increasing the immersion time from 0 to 12 hours caused the coleus seedling's height to decrease by 6.96, and increasing immersion time to 24 hours caused an increase of 1.73. The R^2 value shows how much variability of the observed variables was caused by the independent variables used in the study (Singh et al., 2019). The value of R^2 (0.174) describes that the immersion time as an independent variable included in the regression analysis explained the 17.4% variability in seedling height. The observation showed that the immersion time negatively affects the shoot's number of coleus seedlings by 0.375 and affects the variability of the shoot's number of coleus by 15.52% (R^2 0.155). As much as 82.6% of coleus seedling's height and 84.48% of shoot number variabilities were unexplained. It may be caused by other variables or factors not in the analysis (Singh et al., 2019) (Figure 1).

Table 2 showed that the shallot solution concentrations of 35%, 70%, and 100% had no significant differences in the height of coleus seedlings. The difference in concentration does not give better results than the control treatment (0%). Table 2 also shows that the concentrations of shallot solution did not affect the growth of branch length, but the 70% concentration gave a slightly better effect than the concentration of 35% and 100%. The highest root volume (2.61 mL)

was obtained at 70% shallot solution usage, while the other two concentrations were not significantly different from the control. In general, 70% concentration positively affects coleus seedling height, branch length, and root volume.

Table 2. Effect of shallot solution concentration on coleus seedling height, branch length, and root volume

Solution (%)	Seedling height (cm)	Branch length (cm)	Root volume (mL)
0	23.97 ± 1.98 ^b	5.78 ± 1.62 ^b	1.19 ± 1.09 ^a
35	21.67 ± 1.98 ^a	3.90 ± 1.62 ^a	1.59 ± 1.09 ^a
70	22.11 ± 1.98 ^a	4.34 ± 1.62 ^{a,b}	2.61 ± 1.09 ^b
100	22.19 ± 1.98 ^a	3.58 ± 1.62 ^a	1.89 ± 1.09 ^a

Note: The numbers followed by different letters are significantly different based on DMRT 5%.

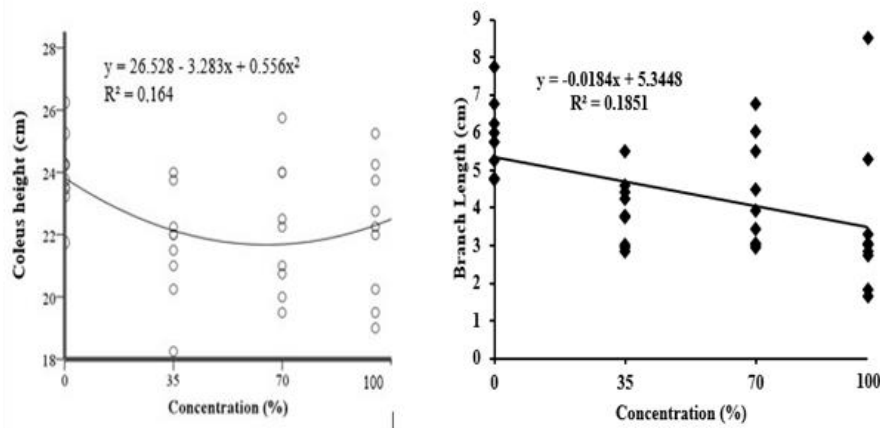


Fig. 2 Coleus seedling height and branch length in shallot solution concentration treatments

The R² value (Figure 2) shows that the concentration of shallot solution affected 16.4% of coleus seedling height and 18.5% diversity of coleus branch length. In line with the effect of soaking time on seedling height, an increase in the concentration of the shallot solution caused a decrease in the 35% and 70% concentration treatments of 3.282 and an increase in the 100% concentration treatment of 0.556. In branch length, there was a decrease of 1.839 for each concentration of shallot solution used.

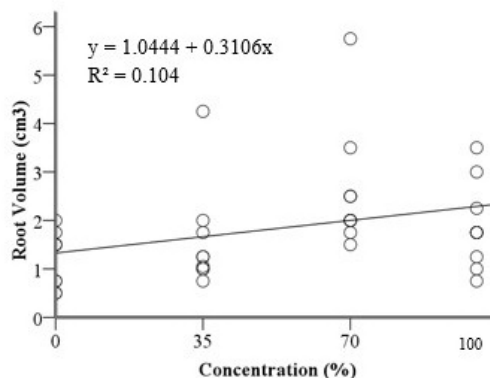


Fig. 3 Root volume of coleus medicinal plant seedlings at various concentrations of shallot solution

The increase in the shallot solution's concentration positively affects the root volume of 0.3106. Coleus seedling root volumes were impacted by shallot solution concentrations (R^2 10.4%) and other factors outside the treatment (89.6%).

Table 3. The interaction effect of immersion time and shallot solution concentration on the coleus seedling height (cm)

Interaction		Concentration (%)			
		0	35	70	100
Immersion time (hours)	0	23.67 ± 1.98 ^{c,d,e}	23.08 ± 1.98 ^{b,c,d,e}	21.83 ± 1.98 ^{a,b,c,d}	23.83 ± 1.98 ^{d,e}
	12	23.17 ± 1.98 ^{b,c,d,e}	20.50 ± 1.98 ^{a,b}	20.17 ± 1.98 ^a	21.50 ± 1.98 ^{a,b,c,d}
	24	25.08 ± 1.98 ^e	21.42 ± 1.98 ^{a,b,c,d}	24.58 ± 1.98 ^e	21.00 ± 1.98 ^{a,b,c}

Note: The numbers followed by different letters are significantly different based on DMRT 5%.

Table 3 shows that the height of coleus plant seedlings treated with immersion for 24 hours using 0% shallot solution was not significantly different from that of 70% shallot solution. The two treatment combinations gave the growth of cuttings height which was not significantly different from the control, but significantly different from the 70% shallot solution treatment with 12 hour immersion time.

The 100% shallot solution with a 24 hour immersion time was not significantly different from the control treatment (0 hours immersion time of cuttings in 0% shallot solution). The treatment of immersion of coleus medicinal plant cuttings with 0% shallot solution for 24 hours gave the best effect on the root length of the coleus by 29.33. On the observed root length variable, the application of 35, 70, and 100% shallot solution had no significant effect on the results of the 0% shallot solution immersion treatment for 24 hours. Of all the treatment combinations used, 0 hours immersion of coleus plant cuttings in 100% shallot solution and immersion of coleus plant cuttings in 70% shallot solution for 24 hours resulted in slightly lower root length than 24 hour immersion in 0% shallot solution, namely of 27.00 and 28.52 (Table 4).

Table 4. The interaction effect of immersion time and the shallot solution concentration on the root length (cm) of the coleus medicinal plant

Interaction		Concentration (%)			
		0	35	70	100
Immersion time (hours)	0	18.25 ± 5.04 ^a	20.92 ± 5.04 ^{a,b}	24.32 ± 5.04 ^{a,b,c}	27.00 ± 5.04 ^{b,c}
	12	25.33 ± 5.04 ^{a,b,c}	20.57 ± 5.04 ^{a,b}	25.42 ± 5.04 ^{a,b,c}	24.62 ± 5.04 ^{a,b,c}
	24	29.33 ± 5.04 ^c	21.67 ± 5.04 ^{a,b,c}	28.52 ± 5.04 ^{b,c}	17.75 ± 5.04 ^a

Note: The numbers followed by different letters are significantly different based on DMRT 5%.

The interaction of the immersion time and shallot solution concentration affects the coleus seedling's branch length and root volume diversity by 19.06% (R^2 0.1906) and 14.31% (R^2 0.1431), respectively. As much as 80.94% of branch length and 85.69% of root volume variabilities may be caused by other variables. The interaction of the two treatments negatively affects the branch length of 0.2027 and positively impacts the root volume of 0.118. The interaction does not affect the leaves. Some treatment, such as 0%-12 hours, 35%-0 hours, 70%-0 hours, 100%-0-hours, and 100%-24 hours have two data result in the same value (Figure 4).

Shallot solution did not influence the height and number of branches of coleus plants, but at 70% concentration, it produced the most significant root volume. Compared to 12 and 24 hours of immersion, the 0% immersion time treatment resulted in the highest seedling height and number of shoots for coleus. Immersion for 24 hours without shallot solution resulted in the highest height and length of the roots. The interaction between 24 hours of immersion in 70%

shallot solution and 24 hours of 0% immersion did not result in a root length that was significantly different from 0% immersion.

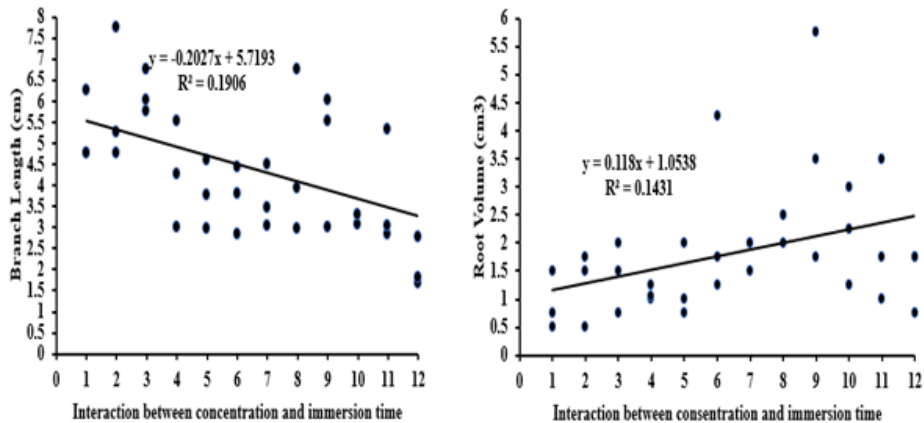


Fig. 4 The interaction effect of immersion time and concentration of shallot solution on branch length and root volume of coleus medicinal plant seedlings

Note: 1= 0%-0 hours; 2= 0%-12 hours; 3= 0%-24 hours; 4= 35%-0 hours; 5= 35%-12 hours; 6= 35%-24 hours; 7= 70%-0 hours; 8= 70%-12 hours; 9= 70%-24 hours; 10= 100%-0 hours; 11= 100%-12 hours; 12= 100%-24 hours

Juiced shallot solution was more effectively used as an exogenous auxin because it affects growth compared to a blender. The shallot solution from the juicer is more effective for melon germination and growth. The shallot solution obtained from the juicer is more concentrated and purer than the blender. In contrast, the solution obtained from the blender is less and mixes with the dregs (Yunindanova et al., 2018). Auxins are critical regulators of almost every aspect of plant growth and development, from embryogenesis to senescence (Li et al., 2016). Auxin plays an essential role in cell division and cell expansion. Plants use several cellular mechanisms to regulate auxin levels and responses (Frick and Strader, 2018). The 100% concentration of shallot filtrate, which was given the addition of root one-f, affected the number of shoots, shoot length, number of leaves, leaf area, number of roots, and root length on jasmine seedlings (Marfirani et al., 2014).

The use of shallot solution in inappropriate concentrations will cause antagonistic activity between the auxins and cytokinins contained (Yunindanova et al., 2018). Kurepa et al. (2019) stated that in low to moderate concentrations, auxins and cytokinins act as antagonists and inhibit each other. Auxins inhibit cytokinins, while cytokinins signal auxin to inhibit cytokinins. The presence of cytokinins triggers auxin signaling and triggers auxin-regulated growth. The increasing anthocyanin content decreased leaf area, and the decreasing lateral root formation is a plant response to the strong auxins due to increased cytokinin signaling. Auxins can quickly regulate root growth by mediating apoplast alkalinization, the cellular mechanism that causes root growth regulation (Frick and Strader, 2018; Li et al., 2021). Endogenous auxin levels of a stem cutting are insufficient to stimulate root growth, so exogenous auxin should be applied. The root growth rate of a cutting was affected by the exogenous auxin levels absorbed by the stem cutting. Various types of exogenous auxin stimulated root growth differently. A balanced combination of auxin sources might provide excellent support for root growth of tea stem cutting at tea stem cutting (Sarjiyah et al., 2020).

Regulation occurs by controlling gene expression through a functionally distinct family of Auxin Response Factor (ARF) DNA-binding. ARF binds to the auxin response DNA element

(AuxRE) at the promoter of the auxin-regulated gene and activates or represses the transcription of this gene depending on the specific domain in the middle of the protein. Protein interactions and the molecular basis of auxin-regulated transcription. ARF and Aux/IAA proteins contain sequences with domains that mediate ARF-ARF, ARF-Aux/IAA, and Aux/IAA-Aux/IAA interactions (Li et al., 2016).

The provision of shallot solution causes the initiation of plant root hairs. The presence of ethylene in plants enhances auxin transport to the roots by increasing the transcription and translation of PIN3 and PIN7 in the central cylinder. The PIN3 and PIN7 transcription and translation activity prevent the local accumulation of auxin required to promote lateral root formation (Qin and Huang, 2018). The content of IAA in shallot solution was slightly higher than cytokinins, thought to be the cause of the low shoot growth and high root volume produced. Gilani et al. (2018) state that high root formation might increase nutrients such as nitrogen and water absorption from media, stimulating vegetative growth. Sevik and Guney (2013) state that all auxins influence plant root growth. The difference in the branch length growth of the coleus seedlings resulted from the presence of Gibberellins (GA3) in the shallots used. GA3 amount contained in the shallot solution treatment did not affect positively at plant height.

In coleus plant propagation by cuttings, there was a decrease in the number of shoots with the increasing concentration of shallot solution used. This condition is in line with Suhono et al. (2017), which stated that low concentrations of 2,4-D auxin could regenerate proliferative callus into shoots, and high concentrations could not give the same effect. The combination of 2,4D with cytokinins cannot initiate shoot formation (Verma et al., 2014). Auxin 2,4-D is more appropriate for tissue culture propagation than conventional cuttings. Cytokinins positively affect axillary bud proliferation. The axillary bud proliferation coefficient was increasing along with the cytokinin concentration increasing. The use of cytokinins and NAA can induce more adventitious shoots and higher somatic embryos than cytokinins stand-alone (Lestari et al., 2019; Liang et al., 2020; Verma et al., 2014).

Growth hormone belongs to plant hormones made in one tissue and cellular regulation in another by linking specific proteins (as receptors) by cellular transduction pathways. At low concentrations, plant hormones react to specific sites in other tissues. Gibberellins are tetracyclic diterpene acids that are biosynthesized through the terpenoid pathway. The presence of cytokinins in plants is generally associated with carotenoid pigments, rubber, abscisic acid, gibberellins, and plant defenses in the form of phytoalexins. ABA (abscisic acid) is a plant hormone formed from the breakdown of the carotenoid pigment violaxanthin, a 40-carbon molecule. The addition of growth hormones such as auxins and cytokinins can stimulate an increase in the production of secondary plant metabolites (Ibrahim, 2022). Wang et al. (2018) stated that auxin is essential for average plant growth and development, especially concerning the ARF-Aux/IAA interaction. In addition to affecting cell division, the presence of auxin also affects the metabolism of secondary metabolites. Further research is needed regarding the mechanism of the effect of shallot solution on the coleus medicinal plants.

CONCLUSION

In plant breeding, shallot solution could serve as an exogenous hormone. The immersion duration of 0 hours resulted in the greatest plant height and number of shoots but not for other variables observed. The 70% shallot solution concentration produced the most significant volume of coleus roots, but not for other variables. The interaction of immersion time for 24 hours with a 70% shallot solution resulted in the most remarkable plant height and root length, whereas other

factors had no effect. The shallot solution may increase the root mass of coleus seedlings by stimulating the growth of root hairs. In the presence of GA₃, shallot solution may affect coleus seedlings. Shallot solution inhibits the development of coleus seedling's lateral shoots. However, large doses of shallot solution reduced the quantity of coleus seedling shoots.

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