LenteraBio
Berkala Ilmiah Biologi

LenteraBio, 2026; Volume 15, Issue 1: 64-70

p-ISSN: 2252-3979 e-ISSN: 2685-7871
https://journal.unesa.ac.id/index.php/lenterabio/index

Effect of Shallot Filtrate in Murashige and Skoog (MS) Medium for the Growth of Acehnese Patchouli (*Pogostemon cablin*)

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Article History:
Received:
29-July-2025
Revised:
20-January-2026
Available online:
31-January-2026
Published regularly:
31-January-2026

Abstract
Acehnese patchouli (*Pogostemon cablin*) is an important export commodity known for producing high-quality essential oil. However, its propagation through stem cuttings is insufficient, which can be overcome through tissue culture techniques. This study aimed to examine the effect of various concentrations of shallot filtrate in Murashige and Skoog (MS) medium on the growth of Acehnese patchouli (*Pogostemon cablin*) plantlets. A completely randomized design with shallot filtrate (15 g/L, 25 g/L, and 35 g/L) as treatment factor was used, with a control group (BAP 1 mg/L + NAA 2 mg/L). The study consisted of 5 replications, resulting in 20 experimental units. Data on the average height, root length, and leaf number were analyzed quantitatively using normality and homogeneity tests, one-way ANOVA and Duncan's test (p=5%). Data that did not show a significant difference were analyzed descriptively. The results showed that the addition of shallot filtrate at various concentrations to MS medium significantly affected the growth of nilam plantlets. Filtrate at 25 g/L concentration gave the best overall results, with an average height increase of 0.62 ± 0.25 cm, root length 0.28 ± 0.15 cm, and 5.50 ± 1.78 leaves.

Keywords: murashige and skoog; Acehnese patchouli; shallot; tissue culture; agriculture

How to Cite: Levinawati A & Ratnasari E, 2026. Effect of Shallot Filtrate in Murashige and Skoog (MS) Medium for the Growth of Acehnese Patchouli (*Pogostemon cablin*). LenteraBio; 15(1): 64-70.

DOI: https://doi.org/10.26740/lenterabio.v15n1.p64-70

INTRODUCTION

Acehnese patchouli or known locally as *nilam Aceh* (*Pogostemon cablin*) is a plant with high economic value and an important export commodity for Indonesia. According to the Balai Informasi Standar Instrumen Pertanian (2023), patchouli oil is exported in 1,200-1,500 tons per year to various countries, including the United States, the United Kingdom, France, Singapore, Spain, and Switzerland. Indonesia meets as much as 90% of the world's patchouli oil demand, making it a foreign-exchange-earning commodity with good development prospects (Siregar and Hasibuan, 2022). There are three types of patchouli in Indonesia: *nilam sabun* (*Pogostemon hortensis* Backer.), *nilam Aceh* (*Pogostemon cablin* Benth.), and *nilam Jawa* (*Pogostemon heyneanus* Benth.). Acehnese patchouli has higher oil content and quality than other nilam varieties, making it the most widely cultivated (Husein *et al*., 2019). The various benefits from Acehnese patchouli as essential oils must also be balanced with optimal cultivation (Sahwalita and Herdiana, 2016).

Acehnese patchouli rarely or never flowers. Therefore, they propagated vegetatively using cuttings (Wudianto, 1998). However, this method is constrained by the risk of pathogen transmission from the parent plant. The cutting method also cannot meet the high demand for healthy seedlings because it requires large amounts of plant material (Dinas Perkebunan Provinsi Jawa Timur, 2013). Therefore, an alternative method is needed. Tissue culture is a method for growing plants by isolating cells or tissues under aseptic conditions (Harahap *et al*., 2019). This method can produce large numbers of new individuals in a short time while ensuring high seedling quality (Yuliarti, 2010). The success of tissue culture is influenced by the growth regulators and the growing medium (Wardani, 2020).

Plant growth regulators are non-nutrient organic compounds that can affect plant growth and development even at low concentrations (Wattimena, 1991). Synthetic plant growth regulators used in tissue culture are relatively expensive. This encourages the search for alternative sources that are more affordable and equally effective (Kartika and Supriyanto, 2020). Shallots (*Allium cepa* L. var. *aggregatum*) are an organic material that can replace synthetic plant growth regulators due to their lower cost (Asmarani *et al*., 2024). Shallot filtrate contains hormones such as auxin, cytokinin, and gibberellin, which synergize to promote plant growth and development (Widasari *et al*., 2021).

The combination of 1 mg/L BAP and 2 mg/L NAA had a positive effect on the shoot growth time, shoot height, and number of leaves in the tissue culture of Acehnese patchouli plantlets (Maulia and Basyah, 2021). In another case, 20 g/L of shallot filtrate and 50 ml/L of coconut water provides the best results for plantlet height and increases root length in Moon Orchid (*Phalaenopsis amabilis*) plantlets (Ilham *et al*., 2024). The use of shallot filtrate in tissue culture has been reported, but further research is needed to determine its effects and the optimal concentration that yields the best results for the growth of Acehnese patchouli plantlets. This study was conducted to examine whether shallot filtrate could replace synthetic growth regulators for in vitro propagation of Acehnese patchouli.

MATERIALS AND METHODS

This research was conducted for 3 months from April to June 2025 at the Tissue Culture Laboratory of PT. Pawitra Jaya Sakti Biotek. This research was an experimental study with a completely randomized design, with one factor consisting of 4 treatments: concentrations of shallot filtrate (15 g/L, 25 g/L, and 35 g/L) and the control (BAP 1 mg/L + NAA 2 mg/L). This study had 5 replications, resulting in 20 experimental media.

This research consists of 4 stages: preparation, implementation, maintenance, and observation. The preparation stage began with the sterilization of culture bottles, Petri dishes, and tweezers in an autoclave at 121^{o}C under 1 atm for 15 minutes. NAA and BAP were each weighed at 0.01 g, then added separately using a few drops of 1 M NaOH for auxin and 1 M HCl for cytokinin (Setiawati *et al*., 2019). Distilled water was added until 100 mL, then mixed until homogeneous to obtain stock solutions of NAA (100 mg/L) and BAP (100 mg/L). Shallot filtrate concentrations of 15 g/L, 25 g/L, and 35 g/L were made by weighing 15 g, 25 g, and 35 g of shallots, which was the washed, blended, filtered, and added distilled water to a final volume of 1 L. About 41.71 grams of instant MS media was weighed, then added 2 mg/L NAA + 1 mg/L BAP to the medium mixture, while shallot filtrate was given according to the treatment (15 g/L, 25 g/L, and 35 g/L). The medium was homogenized using a hot plate, then the pH was adjusted to 5.6-5.8 (Asmarani *et al*., 2024). Medium mixture was heated to boiling, then poured 10 ml into the culture bottle, covered with PP plastic, and finally sterilized for 20 minutes.

The implementation stage began with the sterilization of the Bio Safety Cabinet using 96% alcohol and UV light for 1 hour. Plantlet were taken using sterile tweezers and cut to a size of ± 0.5 cm. A pair of plantlets was put into the treatment medium, before culture bottle was sealed with plastic wrap, and labelled accordingly. All inoculation procedures were carried out aseptically. The maintenance was carried out by placing the culture bottles on a storage rack under 40 watts fluorescent lighting at 28^{o}C. The observation stage lasted 7 weeks and included monitoring plantlet height increase, root length, and number of leaves.

Data on height increase, root length, and number of leaves were collected before inoculation and on the last day of observation. The data were analyzed quantitatively using SPSS 25, including normality test, homogeneity test, and a one-way ANOVA with Duncan's test at the 5% significance level. Data that did not show significant differences were further analyzed descriptively.

RESULTS

This study aimed to determine the effect of shallot filtrate at various concentrations in MS medium on the growth of Acehnese Patchouli (*Pogostemon cablin*) plantlets. The parameters of height increase, root length, and number of leaves were analyzed statistically. Based on the average value, it is known that the BAP 1 mg/L + NAA 2 mg/L treatment resulted in highest increase oh height at 0.78 cm, followed by 25 g/L shallot filtrate at 0.62 cm, 15 g/L shallot filtrate at 0.51 cm, and 35 g/L shallot filtrate at 0.48 cm. These results indicate that among the three concentrations of shallot filtrate, 25 g/L provides the best response in height increase of patchouli plantlets (Table 1 and Figure 1).

Table 1. The effect of shallot filtrate concentration on the height increase of Acehnese Patchouli (*Pogostemon cablin*)

Treatment	Height Increase (cm)*
BAP 1 mg/L + NAA 2 mg/L	0,78 ± 0,30^{b}
Shallot filtrate 15 g/L	0,51 ± 0,24^{a}
Shallot filtrate 25 g/L	0,62 ± 0,25^{ab}
Shallot filtrate 35 g/L	0,48 ± 0,20^{a}

Notes: *)The same superscript notation indicates no significant difference, while different superscript indicate a significant difference at the 5% level (DMRT test). All treatments used MS (Murashige and Skoog) media with a concentration of 100%.

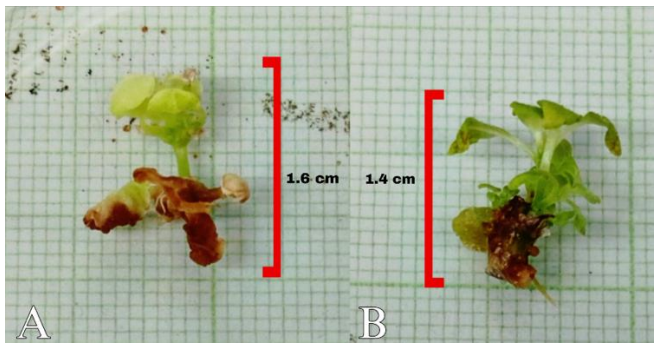

Figure 1. Height increase of Acehnese Patchouli (*Pogostemon cablin*). A = BAP 1 mg/L + NAA 2 mg/L; B = Shallot filtrate 25 g/L

Table 2 shows that there were differences in the average root length of Acehnese Patchouli plantlets across all treatments. However, the results of the one-way ANOVA test indicated no significant effect of the various shallot filtrate concentrations. The highest root length was observed in the treatment with 1 mg/L BAP + 2 mg/L NAA at 0.34 cm, followed by 25 g/L shallot filtrate at 0.28 cm, 15 g/L shallot filtrate at 0.25 cm, and 35 g/L shallot filtrate at 0.18 cm. These results suggest that among the three concentrations of shallot filtrate, 25 g/L produced the best response in terms of root length of Acehnese Patchouli plantlets (Figure 2.).

Table 2. The effect of shallot filtrate concentration on the root length of Acehnese Patchouli (*Pogostemon cablin*)

Treatment	Root Length (cm)
BAP 1 mg/L + NAA 2 mg/L	0,34 ± 0,12
Shallot filtrate 15 g/L	0,25 ± 0,14
Shallot filtrate 25 g/L	0,28 ± 0,15
Shallot filtrate 35 g/L	0,18 ± 0,08

Notes: All treatments used MS (Murashige and Skoog) media with a concentration of 100%.

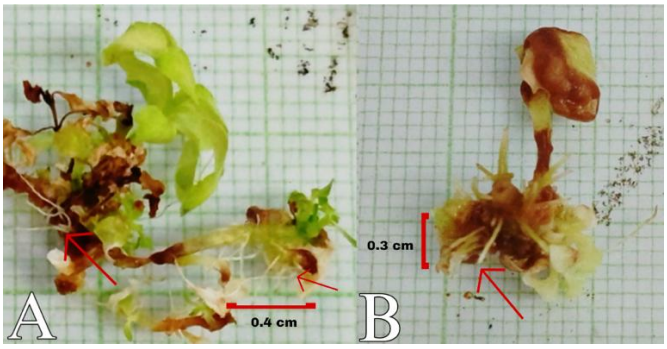

Figure 2. The root length of Acehnese Patchouli (*Pogostemon cablin*); A = BAP 1 mg/L + NAA 2 mg/L; B = Shallot filtrate 25 g/L.

Table 3 shows that there were differences in the average number of leaves of the Acehnese Patchouli plantlets across all treatments. The greatest number of leaves was found in the treatment with 1 mg/L BAP + 2 mg/L NAA at 6.60 leaves, followed by 25 g/L shallot filtrate at 5.50 leaves, 15 g/L shallot filtrate at 4.70 leaves, and 35 g/L shallot filtrate at 4.00 leaves. These results indicate that among the three shallot filtrate concentrations, 25 g/L gave the best response in number of leaves of Acehnese Patchouli plantlets (Figure 3.).

Table 3. The effect of shallot filtrate concentration on number of leaves of Acehnese Patchouli (*Pogostemon cablin*)

Treatment	Number of Leaves*
BAP 1 mg/L + NAA 2 mg/L	$6,60 \pm 1,34^{c}$
Shallot filtrate 15 g/L	$4,70 \pm 1,06^{ab}$
Shallot filtrate 25 g/L	$5,50 \pm 1,78^{bc}$
Shallot filtrate 35 g/L	$4,00 \pm 1,05^{a}$

Notes: *)The same superscript notation indicates no significant difference, while different superscript indicate a significant difference at the 5% level (DMRT test). All treatments used MS (Murashige and Skoog) media with a concentration of 100%.

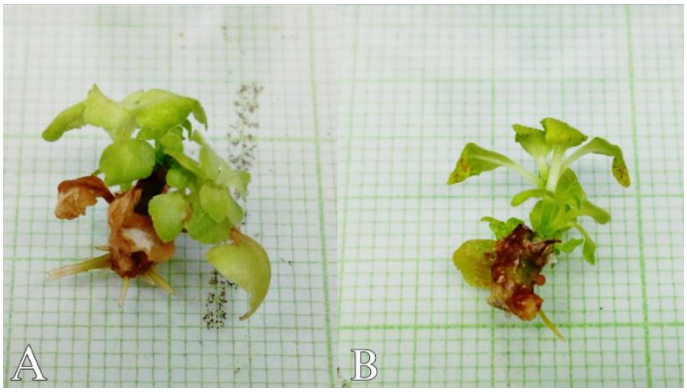

Figure 3. Number of leaves of Acehnese Patchouli (*Pogostemon cablin*); A = BAP 1 mg/L + NAA 2 mg/L; B = Shallot filtrate 25 g/L

DISCUSSION

The results presented in Table 1 indicate differences in the average height increase of Acehnese Patchouli (*Pogostemon cablin*) plantlets across treatments. The results suggest that the control treatment (2 mg/L NAA + 1 mg/L BAP) remained the most effective treatment for stimulating plantlet height growth compared to shallot filtrate at various concentrations. However, the application of 25 g/L shallot filtrate at resulted in the greatest increase in plantlet height closest to control group compared to other concentrations. These results align with research by Julaiha *et al*. (2025), which found that 3ml/L shallot filtrate produced the tallest Barangan banana plantlets compared to 2 ml/L and 4 ml/L.

Hormones such as auxins, cytokinin, and gibberellins present in shallot filtrate play a role in vegetative growth, including stem elongation. Auxin functions by stimulating the pumping of H^+ ions into the cell wall, which lowers the pH and activates the expansin enzyme. This enzyme breaks the hydrogen bonds between cellulose fibers then increasing cell wall plasticity. As a result, water enters the cell by osmosis, allowing cell elongation (Campbell *et al*., 2003). The absorption of auxin by plant tissues promotes cell division and elongation, thus increasing plantlet height (Pamungkas and Puspitasari, 2019). Gibberellins also contribute to height increase by promoting cell division and elongation in meristematic tissues, enhancing cambial activity, RNA synthesis, and protein formation (Heddy, 1990). Furthermore, shallots also contain vitamins, minerals, and antioxidants that support plant metabolism. These compounds enhance photosynthetic efficiency as well as nutrient absorption and transport, which can influence plant height growth (Julaiha *et al*., 2025). The balance between exogenous and endogenous hormones is a key factor in determining plantlet height. The results of this study indicate that 25 g/L shallot filtrate provides an optimal hormonal balance for the growth of Acehnese patchouli plantlet.

Root length is one of the parameters that can be observed to determine plantlet growth. The results in Table 2 shows differences in the root length of the patchouli plantlets across treatments. These results indicate that the control treatment was most effective in stimulating root growth in Acehnese Patchouli plantlets. However, the addition of shallot filtrate at 25 g/L provided the closest performance compared to other concentrations. Similar results were also reported in previous research by Numba *et al*. (2024), in which 40% concentration of red onion filtrate produced the highest root length in potato explants compared to a 60% concentration.

Shallot filtrate contains compounds that have the potential to increase fertility and accelerate plant organ development. Thiamine, found in the shallot filtrate, plays a crucial role in providing energy for plantlets during root growth. Root length is influenced by auxin absorption because it regulates cell division and elongation (Pamungkas and Puspitasari, 2019). The presence of hormones similar to endogenous auxin can aid root cell elongation and differentiation, thereby increasing root length (Idly *et al*., 2023). The application of exogenous auxin interacts with endogenous auxin to trigger cell differentiation and root formation (Zuhroh *et al*., 2018). In this case, auxin works by pumping H+ ions into the cell wall, lowering the pH and activating the expansin enzyme, which increases the plasticity of the cell wall, allowing water to enter by osmosis and promoting cell elongation (Campbell *et al*., 2003). In addition to auxin, cytokinin also regulates root growth. High cytokinin levels can suppress auxin from acting on root initiation and elongation (Lutfiani *et al*., 2022). The appropriate interaction between endogenous and exogenous hormones significantly influences root formation and elongation. Although

roots were formed at all treatment groups, the 25 g/L concentration resulted in the greatest average root length, indicating its potential as the optimal concentration for promoting root elongation.

The number of leaves can serve as an indicator of the growth success of cultured plantlets (Arafah *et al*., 2021). The results in Table 3 show a pattern similar to the previous two parameters, in which increasing the concentration of shallot filtrate does not always correlate directly with an increase in number of leaves. The control treatment was the most effective for increasing the number of leaves in the patchouli plantlets. However, the application of 25 g/L shallot filtrate yielded the best results among the filtrate treatments in supporting leaf growth. This result is consistent with previous research, namely that administering shallot filtrate at a concentration of 3 ml/L produced the highest number of leaves compared to 4 ml/L in Barangan variety banana plantlets (Julaiha *et al*., 2025).

To support plantlet growth, a sufficient supply of energy and essential compounds is required. Shallot filtrate contains growth-promoting substances such as auxin and riboflavin, which stimulate the formation of new leaves (Idly *et al*., 2023). When fresh shallots are crushed, they produce thiosulfate (allicin), a compound derived from allin with the help of the enzyme alliinase. Allithiamine is formed when a chemical bond forms between allicin and thiamine. The presence of allithiamine can facilitate the metabolism and transport of food by influencing the respiration process, namely the decarboxylation of pyruvate oxidation. This compound undergoes phosphorylation to form thiamine pyrophosphate, a cofactor for cell formation that supports energy (ATP) production in tissues (Marfirani *et al*., 2014). Shallot filtrate also contains cytokinin, which play a role in the leaf differentiation process (Pradita *et al*., 2022). The application of exogenous cytokinin through the filtrate can influence the endogenous cytokinin ratio in plantlets, which then interacts with auxin to regulate leaf differentiation (Nurkapita *et al*., 2021). When auxin levels exceed those of cytokinin, leaf development becomes suboptimal, as meristem cells tend to divide more frequently rather than differentiate into shoots or leaves (Pamungkas and Puspitasari, 2019). Furthermore, high auxin concentrations may inhibit plantlet growth by disrupting cell division (Illahi *et al*., 2022). In addition to hormonal factors, leaf development is also influenced by the composition of the growth medium and light intensity during cultivation (Lutfiani *et al*., 2022).

Shallot filtrate at a concentration of 15 g/L is presumed to be insufficient to meet the needs for plantlet growth, indicating the need for a higher concentration. However, increasing the concentration of shallot filtrate does not always correlate directly with plant growth. This suggests that there is a limit to the effectiveness of shallot filtrate concentration on plant growth. This is evidenced by the administration of 35 g/L shallot filtrate resulted in the lowest results across all growth parameters, as excessively high concentrations can potentially cause stress to the plants (Julaiha *et al*., 2025). Based on the results and discussion above, the addition of shallot filtrate at a concentration of 25 g/L to MS medium was effective for the growth of Acehnese patchouli (*Pogostemon cablin*) plantlets. This concentration produced results closest to the control across all observed parameters compared to other concentrations. The nutrient and hormone content at this is considered sufficient to meet optimal requirements for plantlet growth, suggesting its potential as an alternative to synthetic growth regulators.

CONCLUSION

Based on the results and discussion, it can be concluded that varying concentrations of shallots added to MS media (Murashige and Skoog) affected the growth of Acehnese patchouli plantlets (*Pogostemon cablin*). The most effective concentration for promoting plantlet growth was 25 g/L. The shallot filtrate concentration of 25 g/L consistently provides results closest to the control for each parameter, with an average increase in height 0.62 ± 0.25 cm, root length 0.28 ± 0.15 cm, and leaves 5.50 ± 1.78.

ACKNOWLEDGEMENTS

The author would like to express sincere gratitude all staff of Tissue Culture Laboratory of PT. Pawitra Jaya Sakti Biotek and reviewers for their valuable feedback and suggestions.

CONFLICT OF INTEREST

There is no conflict of interest.

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