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The effect of liquid organic fertilizer on the growth of *Dendrobium* sp. in acclimatization period

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Abstract The application of liquid organic fertilizer (LOF) during the acclimatization of Dendrobium orchids increased plant height and leaf length. The optimal LOF concentration for achieving a plant height of 6.66 cm was is 1 ml/L applied at 2-day intervals, while the optimal leaf width of 5.44 cm was achieved with 2 and 3 ml/L applied at 3-day and 4-day intervals, respectively. LOF concentrations significantly affected leaf width, root number, and root length. The optimal LOF concentration for these variables was 2 ml/L. Additionally, the interval of LOF application significantly affected leaf and shoot numbers, with the optimal interval for these two variables being every 2 days. The research finding provided s sa significant perspective on using liquid organic fertilizer during the acclimatization stage of tissue culture propagation of orchid plants.

Keywords: Application intervals, Fertilizer concentration, Orchid seedlings plantlet acclimatization

Introduction

Dendrobium sp. is one of the commercial orchids with various flower colors, sizes and shapes. *Dendrobium* sp. can be propagated vegetatively by conventional methods or tissue culture techniques. The propagation through the tissue culture is more efficient than the traditional methods so that was often used to produce large quantities of orchid seeds (Bhowmik and Rahman, 2020; Pujasatria *et al.*, 2020). The techniques of seedling culture for the large-scale production of Cypripedium macranthos orchids had developed (Huh, *et al.* 2016).

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Orchid propagation by tissue culture requires a stage for the seedlings to adjust to new environmental circumstances before planting in pots known as an acclimatization stage. This stage is important to maximize the survival rate and stimulate the vigorous growth of seedlings. Transplanting Dendrobium plantlets from the culture room to the acclimatization may desiccate and wither the plantlets due to extreme environmental changes (Teixeira da Silva, *et al.,* 2017). In vitro plantlet leaves generally have weak vascular tissue and have an underdeveloped palisade layer with a small number of mesophyll air spaces, in vitro plantlets cannot close stomata when removed from in vitro culture. This is the cause of excessive water loss in plantlets during acclimatization.

Plant seedlings from tissue culture are still heterotrophs, therefore the acclimatized plantlets require nutrients for growth and development. Fertilization is an important factor for plants to grow well to reach maximum growth. One type of fertilizer that can be used for orchids is liquid organic fertilizer (LOF). Using LOF has not only a positive impact on the plantlet environment, but also it is easy to obtain and not expensive. The management of nutrition in orchids is more complicated than in any other horticultural crop (Biswas *et al.,* 2021). The research reported that N, P, and K are considered the essential elements in orchids' growth. During vegetative plant growth, large quantities of Nitrogen were required. Nutrient solution of NPK plays a vital role in the growth and development of orchids (Wang and Alex-Chang, 2017). The optimum growth and flowering potted of *Dendrobium nobile* required 100, 25, and 100 ppm of N, P, and K solutions, respectively (Bichsel *et al.,* 2008). Several studies have shown that the application of LOF increases the growth of orchid plants. LOF treatments improved the quality by decreasing the stress levels in the petals of snapdragons (*Antirrhinum majus*) (Demirkaya *et al.,* 2016). LOF can be applied by spray or sprinkled directly on leaves or growing media.

Spraying the liquid manure with a higher dilution of 1:20 and 1:30 (v/v) improved the vegetative and reproductive growth of *Cymbidiums* orchids (De *et al.,* 2018). The application of LOF of 0.5 ml/L have a positive response to the leaf length and leaf width of the *Cattleya* sp. (Novianti *et al.,* 2019), while the effective concentration of LOF was 2-2.5 ml/L (Rahmawati *et al.,* 2017; Pratiwi *et al.,* 2019).

Orchid plants can adapt to an inadequate and irregular supply of water and nutrients (Biswas *et al.,* 2021). The right frequency of LOF applications is crucial to get the optimal growth of the seedling. Spraying foliar fertilizers too often can cause toxicity to orchid plants so that the plants will dry out. However, the growth of orchids will be slow and cause stunted plants without LOF. Major difficulties associated with epiphytic orchids are the lack and irregular supply of water and nutrients (Zotz and Hietz, 2021).

The application of LOF "super bionic" at 1 ml/L with interval application of 4 days showed the highest increase in leaf length on the growth of *Cattleya* sp. (Anggoro *et al.,* 2019). The water from washing local brown rice applied every 4 days was the most effective in increasing the growth of *Phalaenopsis sp.* in the acclimatization period (Purnami *et al.,* 2024).

The study was aimed to investigate the effects of concentration and frequency of liquid organic fertilizer (LOF) application on the acclimatization stage of *Dendrobium* sp.

Materials and methods

Experimental conditions

The research was carried out in the screenhouse of the Department of Crop Production, Faculty of Agriculture, University of Bengkulu, Bengkulu. The experiment was arranged in a completely randomized design (CRD), with two experimental factors. The first factor was the concentration of LOF: 1, 2, and 3 mL/L, with a control group without LOF. The second factor was the interval of LOF application: every 2, 3, or 4 days. The study was replicated three times with three pots per treatment. The nutrient content of LOF is presented in Table 1.

Table 1. Nutrient content in liquid organic fertilizer

Macro-nutrients	Concentrations	Micro-nutrients	Concentrations
Organic	9.50 %	Fe	0.58 %
N Total	2.35 %	Mn	0.30 %
P2O5	3.50 %	B	2250.80 ppm
K2O	2.24 %	Mo	0.01 %
CaO	1.10 %	Cu	6.80 ppm
S	1.00 %	Zn	0.20 %
MgO	0.10 %	CI	0.001%

Experimental procedures

The plant materials were derived from tissue culture of *Dendrobium* sp. orchid that had been 6 months maintained in the culture bottle. *Dendrobium* planlets were removed from the bottle using a tweezer 25 cm. The plantlets were washed on tap water to clean the plantlets from agar media. The plantlets were then immersed in a fungicide solution of 2 g/L for 15 minutes and drained on the tissue paper for 10 minutes. The growing media used were prepared from the combination of wood charcoal and sphagnum moss with a ratio of 1:1. Media were sterilized using the pesticide at 2 g/L and the fungicide (1 mL/L) and filled into 300-ml pots. Then, the seedlings were transplanted into the pots and sprayed with LOF according to the treatment. The plants were maintained for 3 months in screenhouse. The LOFs were sprayed to all parts of the plant's surface. The potted plantlets were covered using a cup lid with a small hole for aeration. The humidity of the media was maintained by spraying water using a hand sprayer.

Data collection and analysis

Data were collected as plant height, the number of leaves, leaf width, leaf length, the number of roots, root length, and the number of shoots. Data were collected from selected plants in each unit plot. Data were statistically analyzed with ANOVA at *5%* using SAS program version 9.1, and further tested by a Least Significance Different (LSD) at a 95% confidence level.

Results

During the acclimatization period, 20 percent of plantlets were infected by *Erwinia* sp. The symptoms began as a small water-soaked lesion on the old leaves then enlarged rapidly and finally caused soft rots of the whole plants. The pathogen also infected the wounds of the plants and caused the soft rots which emitted the unpleasant odor. In humic conditions, bacterial can quickly spread which was carried out by water, insects, or equipments. The infections of pathogens were prevented by spraying systemic bactericides and fungicides at 1 g/L every week.

The result showed that the application of LOF had a significant effect on the growth of *Dendrobium* sp. in the acclimatization stage. Plant growth was slower without application of LOF. The plant growth increased due to the LOF application observed on plant height, the number of leaves, leaf width, leaf length, and number of roots (Figure 1).

The responses of *Dendrobium* sp. to the LOF showed a different pattern in plant height, the number of leaves, leaf length, and leaf width (Figure 2). The application of LOF played an important role in the growth of *Dendrobium* sp. in the acclimatization period and the concentrations of 1-3 ml/L significantly increased the plant height, the number of leaves, leaf length, and leaf width from 4 to 10 WAP. Treatment of LOF at 2 ml/L decreased the leaf length sharply at 7 weeks after planting (WAP) and then increased again from 8 to 10 WAP. Increasing the concentration of LOF tends to increase the number of leaves, leaf length, and leaf width each week. Overall, the growth variables of *Dendrobium* sp. were smaller without LOF compared to the variables treated with LOF (Figure 3).

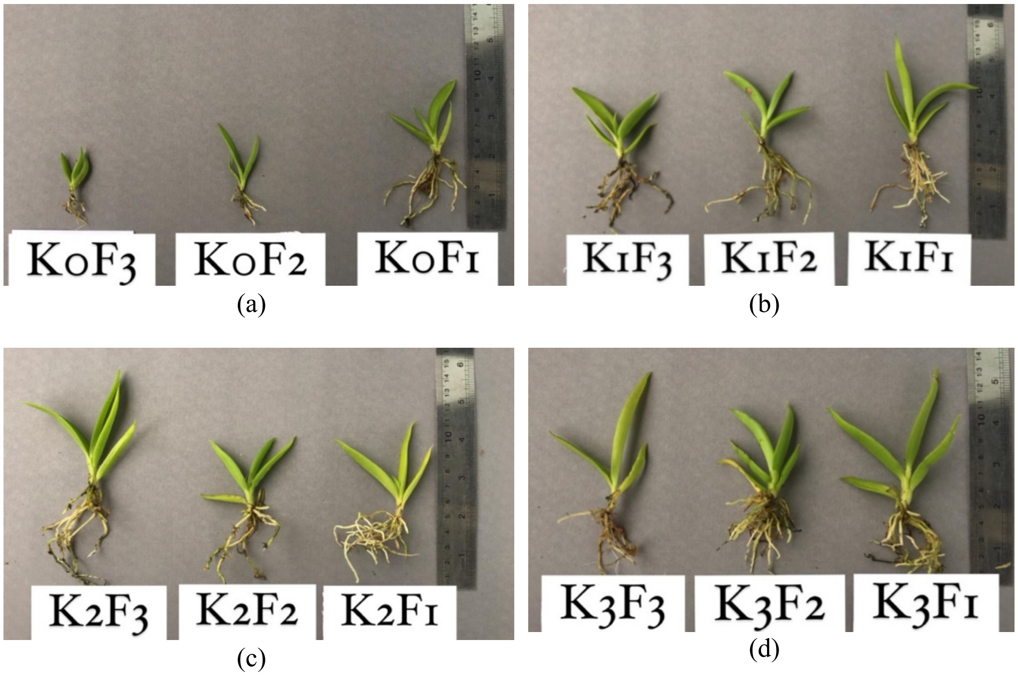

Figure 1. The growth and development of *Dendrobium* sp. (a) K_0 = concentration 0 ml/L (b) K_1 =concentration 1 ml/L, (c) K_2 = concentration of 2 ml/L, (d) K_3 = concentration 3 ml/L, all treatment with interval of every 2 days (F_1), 3 days (F_2), and 4 days (F_3)

The growth of *Dendrobium* sp. plantlets was influenced by the concentration and the application interval of LOF during acclimatization stage. Plant height has increased since 4 WAP. A 2-day fertilization interval gave the highest increases in plant height compared to the 3- and 4-day intervals. The more frequent LOFs were applied the higher the plants growth and this was similar to the pattern of the increase of leaf width and length of *Dendrobium* sp. in 1-10 WAP. These results indicated that the LOF application was important to stimulate the growth of *Dendrobium* sp. in the acclimatization stage.

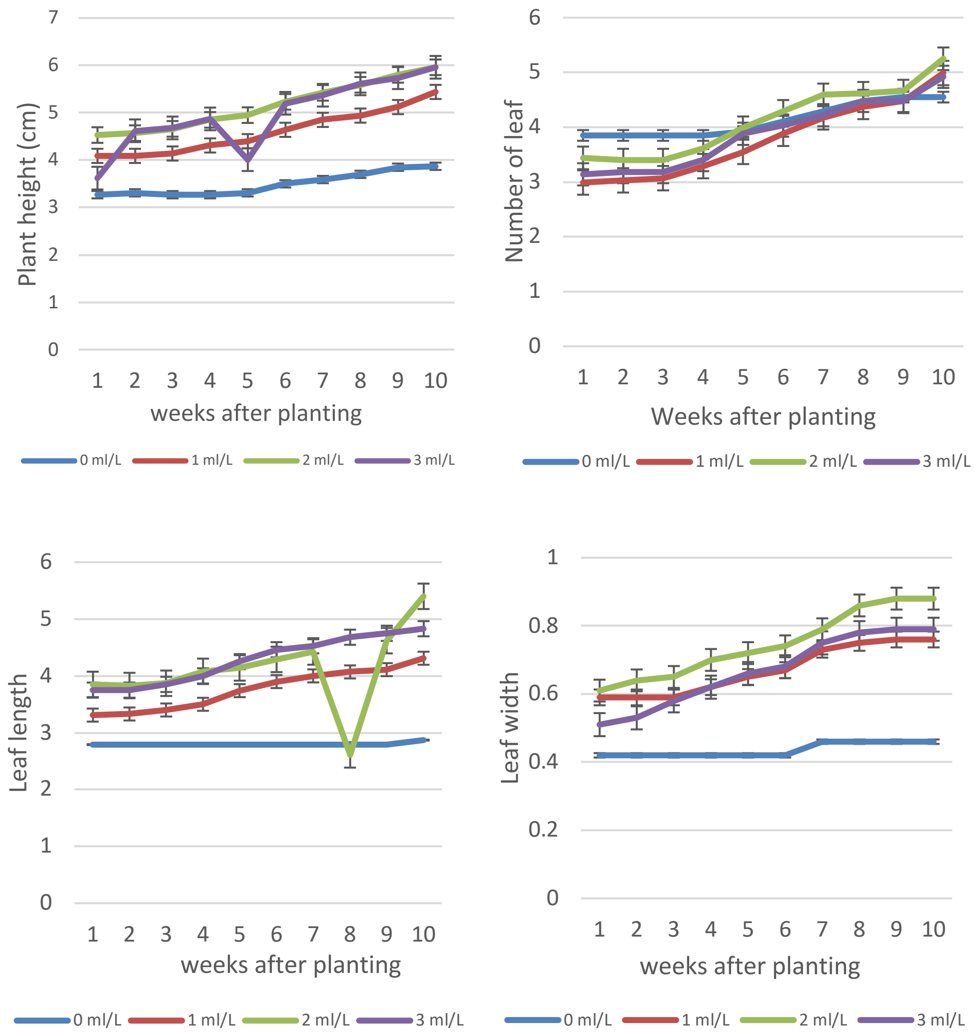

Figure 2. The growth pattern of *Dendrobium* sp. at the acclimatization stage affected by the concentration of liquid organic fertilizers (LOF)

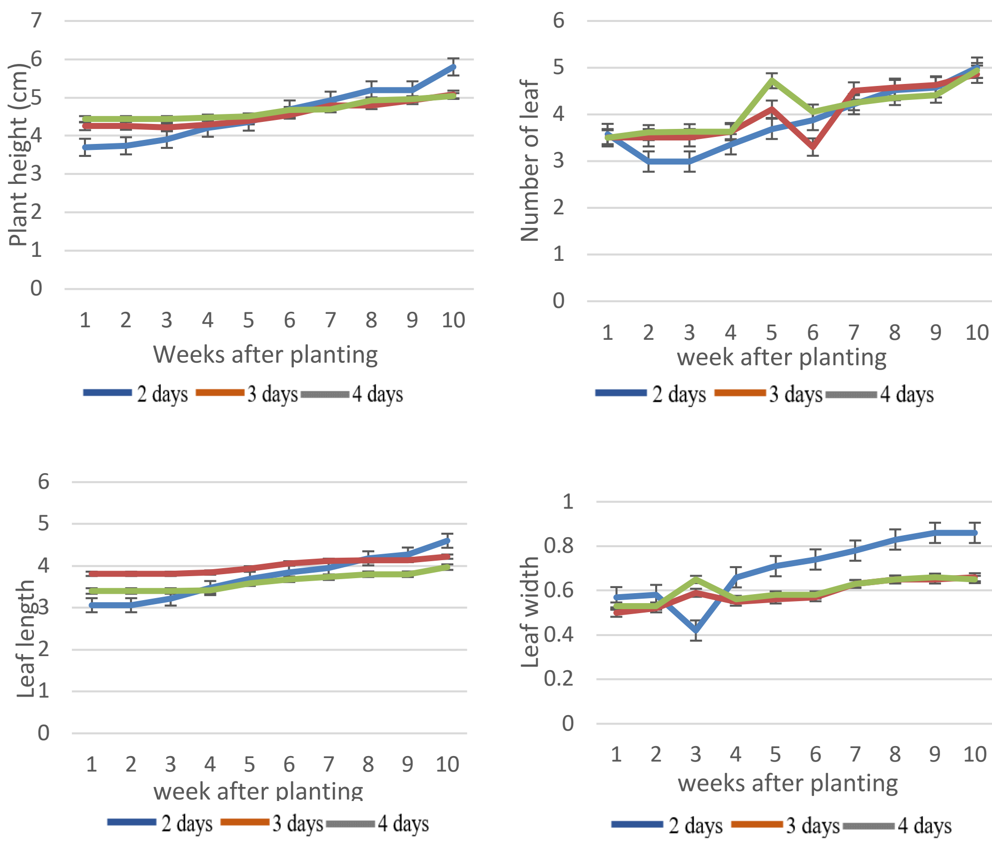

Figure 3. The growth pattern of *Dendrobium* sp. at the acclimatized stage affected by the application interval of liquid organic fertilizers (LOF)

***Interaction of the concentration and interval application of LOF on the growth of Dendrobium* sp.**

The analysis showed a significant interaction between concentrations and application intervals of LOF on the plant height and the leaf length of *Dendrobium* sp. Application of LOF at 1 ml/L with the interval of 2 or 3 days resulted in the highest plant height and it was significantly different from the interval of 4-days (Table 2). The application of LOF at a low concentration and repeated in 2 days stimulated plant growth indicated by the increase in plant height. Increasing the LOF concentration to 2-3 ml/L with the interval of application of 4 days could increase the shoot height of *Dendrobium* sp.

Table 2. The interaction effects of concentration and interval application of liquid organic fertilizer (LOF) on the plant height of *Dendrobium* sp.

Concentration of LOF (ml/L)	Interval of application (days)		
	2	3	4
0	3.83 ± 0.44 a B	4.05 ± 0.82 a B	3.72 ± 0.25 a B
1	6.66 ± 1.30 a A	5.16 ± 0.88 ab AB	4.50 ± 0.92 b B
2	6.44 ± 0.91 a A	4.88 ± 0.34 b B	6.55 ± 0.75 a A
3	6.27 ± 0.35 a A	6.22 ± 0.67 a A	5.38 ±0.50 a AB

Note: Each value is expressed as the mean plus standard deviation. The numbers followed by lowercase letters in the same row are not significantly different in the LSD at 5% level. The numbers followed by capital letters in the same column are not significantly different at the LSD at the 5% level.

The interaction between LOF concentration and the interval of application showed a different pattern of leaf length in each treatment combination (Table 3). The LOF at 1 ml/L with an interval of 2 days was significantly differed with fertilizing every 3 days and every 4 days. Dendrobium was fertilized every 4 days with a concentration of 2 ml/L produced the longest leaf length, but no significant difference was found in the interval of application. Without fertilization (0 ml/L) the length of the orchid leaves was not significantly different at all application intervals. At the LOF at 3 ml/L, the interval of 3 days was not different from 2 days, but both were significantly different from the interval fertilization of 4 days.

Table 3. Effect of concentration and interval of liquid organic fertilizer (LOF) application on leaf length of *Dendrobium* sp.

Concentration of LOF (ml/L)	Interval of application (days)		
	2	3	4
0	2.72 ± 0.69 a B	3.00 ± 1.30 a B	2.88 ± 0.35 a B
1	5.28 ± 1.15 a A	4.11 ± 0.96 b B	3.55 ± 0.42 b B
2	5.34 ± 0.61 a A	4.33 ± 0.33 b AB	5.44 ± 0.35 a A
3	5.05 ± 0.67 ab A	5.44 ± 0.42 a A	4.00 ± 0.29 b B

Note: Each value is expressed as the mean plus standard deviation. The numbers followed by lowercase letters in the same row are not significantly different in the BNT test at the 5% level. The numbers followed by capital letters in the same column are not significantly different at the BNT test at the 5% level.

***The effect of LOF concentration on the growth of Dendrobium* sp.**

The application of several concentrations of LOF to *Dendrobium* sp. showed a significant effect on the leaf width, number of roots, and root length. The LOF at 2 ml/L increased the leaf width of 0.88 cm, but this leaf widt was not significantly different from the concentrations of 1 ml/L and 3 ml/L (Figure 4).

The LOF at 2 ml/L produced the highest number of roots of 9.03 roots, which was not significantly different from the LOF at 3 ml/L. However, plant without organic fertilizer treatment produced the lowest number of roots with an average number of roots of 5.74 roots (Figure 4).

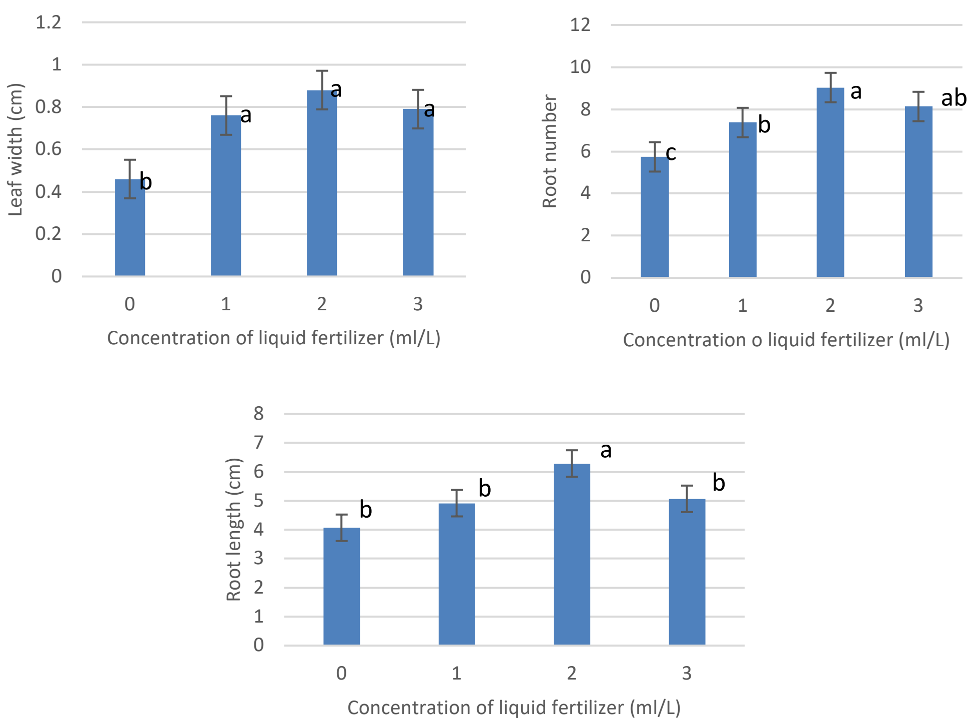

Figure 4. The effect of organic fertilizer concentration (ml/L) on the growth of *Dendrobium* sp. during the acclimatization period. The same lowercase letters in each bar are not significantly different in the BNT test at the 5% level

The application of LOF at 2 ml/L during the plantlet acclimatization of *Dendrobium* sp. produced the longest root of 6.29 cm, which was significantly different from the root applied with LOFs at 1 and 3 ml/L. The lowest mean root length was 4.07 cm observed in the control or without LOF.

***The effect of interval aqpplication of LOF on the vegetative growth of Dendrobium* sp.**

The interval application of LOF had significantly affected on the leaf width and the number of shoots of *Dendrobium* sp. (Table 5). The results showed that applying liquid organic fertilizer at intervals of every 2 days resulted in optimal leaf width and the highest number of shoots.

The number of shoots formed during acclimatization ranged from 0.02-0.19 shoots per plant (Table 5). The application of LOF at the interval of 2 days, the number of shoots was 0.19 which was significantly different from the interval of giving every 3 and 4 days. Applications with 4-day intervals were not able to produce new shoots. Plants that are frequently fertilized will grow better than with less frequent fertilizing intervals.

Table 5. The effect of the interval of application of liquid organic fertilizer (LOF) on the growth of leaf width and number of shoots of *Dendrobium* sp.

Interval application of LOF (days)	Leaf width (cm)	Number of shoots
2	0.86 ± 0.29 a	0.19 ± 0.14 a
3	0.66 ± 0.30 b	0.02 ± 0.96 b
4	0.65 ± 0.33 b	0.00 ± 0.41 b

Note: Each value is expressed as the mean plus standard deviation. The numbers followed by lowercase letters in the same column are not significantly different in the BNT test at the 5% level.

Discussion

The success of plantlets derived from tissue culture techniques is strongly influenced by their ability to survive and adapt in ex vitro environments. This adaptation process is complex because plantlets raised in vitro are accustomed to controlled, aseptic conditions with low light intensity, high humidity, and a consistent supply of nutrients in the culture medium. When these plantlets are transferred to field conditions, they must adjust to fluctuating environmental factors such as light intensity, temperature, and humidity, all of which impose stress that can hinder survival and growth (Sherif *et al.*, 2018; Marlin, 2022). The acclimatization period, therefore, serves as a critical bridge between laboratory conditions and field environments. It is a determining stage that influences whether plantlets can continue to grow normally and eventually enter the reproductive phase. Maitra *et al.* (2020) emphasized that the transition from vegetative to reproductive growth in plants is regulated by both environmental and endogenous signals, highlighting the complexity of this process.

For orchids, particularly *Dendrobium* species, acclimatization is a vital developmental phase. Although normal growth can be achieved, growth is often slow at the beginning of the acclimatization stage due to environmental stress, especially the shift to higher light intensity and lower humidity. During this stage, sunlight acts as the main driver of photosynthesis, which not only supports plantlet growth but also facilitates the accumulation of food reserves necessary for further development. Nasution *et al.* (2025) further explained that environmental conditions such as temperature, humidity, light, and the nutrient content of the growing medium have a profound effect on orchid acclimatization, as these factors determine the plant's ability to survive, adapt, and function optimally in new environments.

Plant growth itself is determined by a combination of internal and external factors, including cultivation methods, genetic composition, and external pressures such as rainfall variability, climate change, and land degradation (Wang *et al.*, 2019; Badiane *et al.*, 2023). Among these, the availability of nutrients is one of the most decisive factors in determining the success of acclimatization. Proper nutrition not only sustains metabolic processes but also provides the energy required for cell division, elongation, and differentiation. Fertilization practices thus play a central role in overcoming nutrient limitations and supporting plant growth during acclimatization. Organic matter, in particular, offers a sustainable source of nutrients, releasing them gradually and continuously in accordance with the plant's physiological demands. Unlike inorganic fertilizers, which may cause nutrient leaching, soil acidification, or environmental contamination, organic fertilizers provide ecological benefits by improving soil structure, enhancing microbial activity, and recycling organic waste materials (Ikoyi *et al.*, 2020; Li *et al.*, 2021).

The benefits of organic fertilizers are well documented. Li *et al.* (2021) reported that organic fertilizer application increased soil nutrient concentrations by 13.8–137.1% while simultaneously reducing soil pH and salinity by 5.6% and 54.7%, respectively. This not only improves nutrient availability but also creates a more favourable environment for root growth and microbial activity. Furthermore, regular application of liquid organic fertilizer (LOF) supplies both macro- and micronutrients, reduces the risk of nutrient leaching, and helps protect crops and soil from long-term degradation (Qaswar *et al.*, 2020). This aligns with findings from Rianawati *et al.* (2022), who reported that while inorganic foliar fertilizers like Hyponex (20:20:20) may show stronger short-term effects on vegetative growth, organic fertilizers contribute to more sustainable plant development and environmental health.

In the present study, the application of LOF had a significant impact on the growth performance of *Dendrobium* sp. during acclimatization. Plants treated with LOF exhibited greater increases in plant height, number of leaves, leaf length, and leaf width compared to untreated plants, particularly between 4 and 10 weeks after planting (WAP). Foliar application proved highly effective, as nutrients dissolved in water were readily absorbed through the stomata and cuticular layers of the leaves. This supports the argument of Setiari *et al.* (2025), who observed that a combined application of 15% LOF and 15 g·plant^{-1} mycorrhizal biofertilizer was the most effective treatment for enhancing leaf growth in *Cymbidium ensifolium*. Similarly, Anggoro *et al.* (2019) showed that applying organic fertilizer at 1 ml/L with 4-day intervals increased leaf length in *Cattleya* sp., reinforcing the importance of foliar nutrient supplementation in orchids.

The physiological basis for these improvements lies primarily in the role of nitrogen. Nitrogen is a vital macronutrient required for the synthesis of amino acids, proteins, and chlorophyll, making it indispensable for photosynthesis and growth (Wang and Alex-Chang, 2017). Phares and Akaba (2022) highlighted that nitrogen contributes directly to chlorophyll formation, thereby enhancing photosynthetic efficiency. Improved chlorophyll content increases the photosynthetic rate, which in turn accelerates the production of assimilates used for the growth of vegetative organs such as stems and leaves. Akhmad *et al.* (2024) reported that LOF application at 4 ml/L increased 100-grain weight in rice, while 6 ml/L produced maximum chlorophyll a and b levels, demonstrating its effectiveness in enhancing both physiological processes and yield components.

In *Dendrobium* sp., nutrient demand during the vegetative stage is particularly high, and the results of this study indicate that LOF supplied these nutrients effectively. Applications at low concentrations every two days promoted growth, as seen in increased plant height, likely due to the steady supply of nutrients necessary for metabolic processes. Essential elements such as N, P, and K contributed directly to cell division, elongation, and overall plant vigor. Increasing the LOF concentration to 2–3 ml/L with four-day intervals further enhanced shoot growth, demonstrating that both dosage and application interval are critical for optimizing plant performance. Without LOF, leaves exhibited the lowest width (0.46 cm), indicating that nutrient deficiency restricts cell expansion and leaf development. Conversely, frequent spraying with LOF produced wider leaves (up to 0.86 cm), as nutrients were efficiently absorbed and utilized.

Overall, these findings highlighted the essential role of liquid organic fertilizers in promoting the successful acclimatization of *Dendrobium* sp. The results showed that appropriate fertilizer doses and application intervals significantly enhance plant growth parameters, including height, leaf number, and leaf dimensions. Beyond immediate growth benefits, organic fertilization also supports long-term plant adaptation by improving soil fertility, enhancing resistance to pests and diseases, and strengthening tolerance to unfavourable climatic conditions.

In conclusion, the application of liquid organic fertilizer plays a decisive role in supporting the successful acclimatization of *Dendrobium* sp. plantlets. By supplying essential macro- and micronutrients in an easily absorbed form, LOF enhances photosynthetic activity, stimulates vegetative growth, and improves plant adaptation to ex vitro environments. The results of this study emphasize that both the concentration and frequency of LOF application are critical for optimizing plant performance, particularly during the sensitive acclimatization stage. From a practical perspective, these findings provide valuable guidance for orchid growers, highlighting LOF as a sustainable alternative to inorganic fertilizers that not only promotes growth but also improves environmental quality. Future research should explore the integration of LOF with other biostimulants, such as mycorrhizal fungi or beneficial microbes, to further strengthen plant resilience and ensure long-term productivity in orchid cultivation.

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Conflicts of interest

The authors declare no conflict of interest.

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