Effect of Mycorrhizae and Liquid Organic Fertilizer on the Leaf Growth of *Cymbidium ensifolium* (L.) Sw

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Summary

People popularly cultivate *Cymbidium ensifolium* (L.) Sw as an ornamental plant because it is a terrestrial orchid. The high desirability of the *C. ensifolium* orchid leads to its exploitation in nature, raising concerns about its potential endangerment. Therefore, to address this issue, *ex situ* conservation methods are necessary, such as cultivating the orchid. One supporting factor for orchid cultivation is the application of fertilizer, especially using natural fertilizers that can be used as an alternative to inorganic fertilizers that have a negative impact on the environment. Natural fertilizers that are commonly used are liquid organic fertilizers (LOF) prepared from coconut water and washed rice water. In addition, there are also other natural fertilizers that are eco-friendly, namely, mycorrhizal biofertilizers. The aim of this study is to determine the effect of LOF and mycorrhizal biofertilizer on the development of *C. ensifolium* orchids. The research was conducted for 3 months in the experimental garden with two treatment factors. The first factor is LOF with levels of 0%, 15%, and 30%. The second factor is mycorrhizal biofertilizer with levels of 0, 5, 10, and 15 g·plant^{-1}. The results showed that the application of 15% LOF and 15 g·plant^{-1} mycorrhizal biofertilizer was the optimum treatment in increasing the leaf growth of *Cymbidium ensifolium* orchid in the parameters of the number of shoots, shoot length, number of leaves, leaf length and width and leaf area.

Key words

Liquid Organic Fertilizers (LOF), mycorrhizal biofertilizer, *Cymbidium ensifolium* (L.) Sw, leaf growth

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Introduction

Cymbidium ensifolium (L.) Sw, also known as the four-season orchid, is one of the orchid species in Indonesia. The species belongs to a group of terrestrial orchids that prefer warm and humid climates with uniform sunlight (Jiang et al., 2019). Orchids in the genus *Cymbidium* are economically valuable in China, Japan, Korea, and Southeast Asia because of their beautiful, fragrant flowers, and they flower more than twice a year (Wei et al., 2020). Meanwhile, in Indonesia this orchid has an export value of 176 million US dollars along with *Paphiopedilum* orchids (Litbang Pertanian, 2020). *Cymbidium* orchids are in high demand because they cannot only be used as ornamental plants but also as genetic resources for orchid hybridization (Singh and De, 2018). The extreme beauty of *C. ensifolium* orchids causes the availability to be exploited, so there is a fear that it can be jeopardized. One of the ways to maintain its sustainability is through *ex situ* cultivation. Optimal production can be pursued by filling the nutritional requirements of plants that can boost development, one of which is fertilization. Organic fertilizers can be an alternative to minimize the use of inorganic fertilizers that can have a negative impact on the soil and the environment (Hartanti, 2014).

Liquid organic fertilizer (LOF) is a type of organic fertilizer composed of natural ingredients. The basic ingredients commonly used to make LOF are coconut water and washed rice water. Coconut water contains primary macronutrients such as nitrogen (N), phosphorus (P) and potassium (K), along with secondary macronutrients such as calcium (Ca) and magnesium (Mg). In addition, there are micronutrients in coconut water, namely, iron (Fe) and chlorine (Cl) (Priya and Ramaswamy, 2014). Coconut water also contains organic substances, such as sugar, protein and natural hormones such as auxins and cytokinins that are involved in cell division (Tiwery, 2014). Another natural material that can be used as liquid organic fertilizer is washed rice water (WRW). WRW contains protein, phosphorus and vitamin B1 which are important in plant metabolism (Rahmadsyah, 2015). Research by Emilda et al. (2020) using coconut water as LOF on ginger plants shows that it can have a positive effect on plant height, width and number of leaves at a concentration of 15 mL·pot^{-1}.

Besides LOF, another kind of natural fertilizer that can be used is biofertilizer. Biofertilizers are fertilizers that contain live microorganisms to increase nutrient binding by plants from the soil or air, for example, mycorrhiza (Wardhani et al., 2014). Mycorrhiza is a form of mutualism symbiosis between fungi and plant roots. The benefits of fungi in plants are to increase the absorption of water and mineral nutrients (Goh et al., 2019; Salmeron-Santiago et al., 2022). Fungi in this association will obtain carbon which amounts to one-fifth of the photosynthetic products of the host plant, and in return the fungus will increase the plant's ability to take water and minerals which will affect the increase in plant growth and reproduction (Goh et al., 2019; Yeh et al., 2019). Mycorrhiza can increase root uptake due to the presence of external hyphae that can penetrate soil pores. P elements are absorbed more by mycorrhiza up to 10%–27%, while nonmycorrhizal plants are only 0.4%–13%. Mycorrhiza can also increase N, K, Mg, Cu, and Zn in host plants (Bhat et al., 2017). N and P elements absorbed by mycorrhiza will be given to the host plant so that it is not excessive and does not interfere with the growth of mycorrhiza. Mycorrhiza is indicated by vesicles, arbuscules, or hyphae which are signatures of mycorrhizal infections. Arbuscular mycorrhizal fungi are a symbiosis found between fungi (zygomycetes) and roots. These fungi form vesicles and arbusculars in the plant cortex. Vesicles are spherical hyphal ends, serving as storage organs, while arbuscular are hyphae with the same shape and function as haustoria and located in plant cells (Basri, 2018). The results of research by Herliana et al. (2018) showed that the application of mycorrhizal biofertilizer at 20 g·pot^{-1} caused the highest increase in the number of leaves on *Dendrobium* sp., while the highest increase in leaf area was produced in the 10 g·pot^{-1} treatment. In the research of Trizayuni et al. (2021), giving mycorrhiza 15 g·pot^{-1} is the best treatment in increasing the leaf width of watermelon.

Research on the effects of LOF and mycorrhizal biofertilizer is typically conducted independently. In addition, there has been no similar research applied to orchids *in vivo*. Therefore, this research was conducted to determine the effect of LOF and mycorrhizal biofertilizer and the combination of both on the growth of *C. ensifolium* orchids *in vivo*.

Material and Methods

Time and Place of Research

The study was conducted in an experimental garden in Tembalang District, Semarang, Central Java, Indonesia, from November 2021 to February 2022. The research was conducted during a rainy season with an average temperature of 26 °C.

Preparation of *Cymbidium ensifolium* Orchid Plants

The plants used as research samples were *Cymbidium ensifolium* orchids obtained from forest orchid collectors in Trenggalek, East Java, Indonesia. *Cymbidium ensifolium* used had passed the selection process with the criteria that the selected plants were mature and uniform in size, had never flowered or had not developed flower buds, and looked fresh with bright leaf color. The orchids were then cleaned by washing the roots in running water and then placed into polybags containing planting media such as soil/husk/hairy husk in a ratio of 1:1:1 without sterilization. After acclimatizing for 1 month, the process continued with mycorrhiza and LOF treatments.

Mycorrhiza and LOF Treatment Procedures

The mycorrhiza used was from a mycorrhizal fertilizer sold on the market under the brand name "MycoGrow." The application of mycorrhiza fertilizer was done by scattering mycorrhiza according to the treatment around the roots (Hardiatmi, 2008). Furthermore, orchid roots were covered with planting media. The LOF used was made with a composition of 2 L of coconut water, 1 L of first WRW, 20 gr of brown sugar, and 4 tsp of EM4. The application of LOF was carried out by watering as much as 20 mL until it reached the leaves and planting media so that it could be absorbed by the roots. According to Rahmayanti et al. (2019), nutrient absorption occurs through roots and leaves so that it will be more effective if applying LOF to these parts.

Plant Care

Watering was done every 2 days using well water, as much as ± 500ml until all parts of the plant were wet. Furthermore, maintenance was carried out by spraying 0.2% vitamin B1 as much as 20 mL per plant twice a week. Growmore fertilizer was applied once a week at the acclimatization stage. After being treated with mycorrhizal biofertilizer, the application of Growmore fertilizer was stopped. Weeding was done manually by pulling from the plant area.

Observation

Parameters measured included shoot and leaf emergence time, leaf growth (leaf length and width, leaf area and number of leaves), and leaf chlorophyll amount. Shoot and leaf emergence time was observed from the first time new shoots and leaves appeared. Shoot length was measured from the ground surface to the tip. Length, width, and leaf area were measured using YMJ B. Leaf Area Meter. The leaves measured were the new leaves or the youngest leaves. The measurement of chlorophyll content was done by cutting the middle part of orchid leaves of the same color index weighing 0.5g and then extracting with 10 mL of 80% acetone. The extract solution was then analyzed using a UV-vis spectrophotometer with wavelengths of 646 and 663 nm to determine the content of photosynthetic pigments (Hendry & Grime, 1993). Chlorophyll content was calculated based on the formula of Wellburn (1994):

Chlorophyll *a* (Ca) = 12.21 (A663) − 2.81 (a646)

Chlorophyll *b* (Cb) = 20.13 (A646) − 5.03 (A663)

Total chlorophyll = Ca + Cb (g·mL^{-1})

Percentage Mycorrhizal Infection

The percentage of mycorrhizal infection was determined by taking five root samples from three plant samples in each treatment. Observations were performed using an OLYMPUS CX23 microscope with semipermanent preparations (Brundrett et al., (1996) modified), and the roots were cleaned and cut ± 1 cm into five pieces. The roots were put in 10% KOH and kept for ± 24 hours and then washed using distilled water five times. Furthermore, coloring was carried out by soaking in 5% ink–vinegar (1 mL of ink and 19 mL of 5% vinegar) for ± 12 hours, and then the roots were soaked with 5% vinegar for ± 30 minutes so that the roots were not overstaining. Mycorrhizal infection is indicated by the presence of vesicles, arbuscules, or hyphae on plant roots. The root infection rate was calculated based on Schenck's formula (1982):

$$Percent\ of\ root\ infections = \frac{Number\ of\ infected\ roots}{Number\ of\ root\ pieces\ observed} \times 100\%$$

Experiment Design and Data Analysis

The research was conducted experimentally using a completely randomized design factorial with 2 factors, namely, the concentration of liquid organic fertilizer (LOF) and mycorrhizal biofertilizer consisting of 12 treatment levels (Table 1).

Each treatment was given 5 replicates so that there were 60 experimental samples. The data obtained were assessed using analysis of variance with multivariance analysis at the 95% significance level. If there was a real effect on each treatment, then further test analysis was carried out by Duncan's multiple range test (DMRT) at the 5% level.

Table 1. The concentrations of liquid organic fertilizer (LOF) and mycorrhizal biofertilizer used in the experiments

Treatment		Mycorrhizae (g·pot^{-1})			
		0	5	10	15
LOF (%)	0	M0P0	M5P0	M10P0	M15P0
	15	M0P15	M5P15	M10P15	M15P15
	30	M0P30	M5P30	M10P30	M15P30

Results and Discussion

Leaf and Shoot Emerge Time

The results showed that there was an interaction between mycorrhiza and LOF in affecting the time of leaf and shoot emergence of *C. ensifolium*. Mycorrhiza treatment of 5 g·pot^{-1} and 15% LOF caused new leaves and shoots to appear faster than other treatments (Table 2).

Table 2. Data analysis for leaf and shoot emerge time parameters of *C. ensifolium* after application of mycorrhizae (g·pot^{-1}) and liquid organic fertilizer (%) at 12 weeks after planting

	Leaf emerge time (day)	Shoot emerge time (day)
M0P0	46.00 ± 6.93^{abc}	16.00 ± 6.93^{a}
M5P0	54.67 ± 9.24^{bcd}	14.67 ± 8.33^{a}
M10P0	48.00 ± 22.27^{abc}	31.20 ± 16.10^{ab}
M15P0	44.00 ± 11.78^{abc}	17.60 ± 8.30^{a}
M0P15	65.33 ± 20.13^{cd}	57.33 ± 36.07^{b}
M5P15	30.00 ± 16.49^{a}	11.20 ± 7.16^{a}
M10P15	36.33 ± 14.15^{ab}	44.00 ± 27.71^{ab}
M15P15	40.00 ± 10.58^{ab}	17.00 ± 7.57^{a}
M0P30	44.80 ± 13.08^{abc}	28.00 ± 22.63^{ab}
M5P30	72.00 ± 6.53^{d}	45.33 ± 36.95^{ab}
M10P30	34.67 ± 16.17^{ab}	36.00 ± 31.24^{ab}
M15P30	40.8 ± 7.69^{ab}	21.00 ± 10.52^{a}

Note: The numbers in the same column and row followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at the $P < 0.05$ significance level

This is because the addition of mycorrhiza and LOF increases the production of the hormone cytokinin in plants. Coconut water used as an ingredient for making LOF naturally contains cytokinins that can encourage shoot growth (Hilmy et al., 2022). In addition, the results of research by Yurkov et al. (2017) reported that cytokinin levels in *Medicago lupulina* L. plants treated with mycorrhiza were higher than in control plants. Cytokinin hormone plays an important role in the process of cell division and cell differentiation through the process of protein synthesis. Cells that grow and differentiate will develop into the tissues that make up the shoots (Pamungkas and Adiguna, 2020). According to Mahadi (2016), primordial shoots formed are indicated by the presence of protrusions or nodules which will subsequently form new shoots. The appearance of nodules is caused by high concentrations of endogenous cytokinins so that differentiation and morphogenesis occur in stem tissues that increasingly develop into shoots.

Plants containing mycorrhiza accumulate more cytokinin than plants without mycorrhiza, both in shoots and roots. The accumulation of cytokinin in plants containing mycorrhiza depends on the fungal species, the availability of phosphorus in the soil, plant tissue, and the developmental stage of the plant (Bhat et al., 2017; Goh et al., 2019). Coconut water used as the primary constituent of LOF is also known to contain cytokinins that will promote cell differentiation. The cytokinin translocation process is through transpiration with the help of xylem vessels to the leaves and then circulation to young tissues that are still actively dividing. Cytokinin increases the transition from the G2 phase to the mitotic phase so as to accelerate the occurrence of cytokinesis (Lestari et al., 2013). The process is related to the increased rate of protein synthesis. This results in the formation of new organs such as new shoots and leaves. After leaf primordia are formed, differentiation occurs into perfect leaf organs which include several processes, such as cell enlargement, cytoplasmic growth and cell differentiation into stomata and transport bundles (Kalve et al., 2014).

Besides increasing cytokinins, the addition of mycorrhiza also increases auxin and gibberellin hormones which affect cell elongation, which results in accelerated shoot and leaf formation (Eliyani et al., 2022). This is supported by Pons et al. (2020) who state that mycorrhizal colonization of tomato roots has been shown to increase auxin concentrations. Shoot commencement and development are regulated by the cooperation of auxin and cytokinin growth regulators. The right concentration ratio will increase cell division and differentiation. A higher concentration of auxin than cytokinin has the opposite effect on shoot growth. Meanwhile, when the concentration of cytokinin is higher, it will influence the cells to divide rapidly to develop into shoots, stems and leaves.

Shoot Growth (Shoot Number and Length)

The results showed that there was an interaction between mycorrhiza and LOF in affecting shoot growth of *C. ensifolium* orchid. The treatment with mycorrhiza 15 g·pot^{-1} and 15% LOF caused the best shoot growth (shoot number and length) (Table 3 and Fig. 1). This is thought to be due to the balanced treatment of mycorrhiza, so LOF can help fulfill the nutritional needs of orchids to emerge new shoots and their development.

Table 3. Data analysis for shoot growth (shoot number and length) parameters of *C. ensifolium* after application of mycorrhizae (g·pot^{-1}) and liquid organic fertilizer (%) at 12 weeks after planting

	Shoot number	Shoot length (mm)
M0P0	0.33 ± 0.58^{b}	31.67 ± 54.85^{ab}
M5P0	0.67 ± 0.58^{ab}	52.00 ± 53.03^{ab}
M10P0	1.33 ± 0.58^{ab}	38.00 ± 19.67^{ab}
M15P0	1.00 ± 1.00^{ab}	44.00 ± 46.13^{ab}
M0P15	1.00 ± 1.00^{ab}	37.33 ± 40.27^{ab}
M5P15	1.33 ± 0.58^{ab}	80.00 ± 24.27^{a}
M10P15	1.33 ± 0.58^{ab}	57.00 ± 50.86^{ab}
M15P15	1.67 ± 0.58^{a}	96.00 ± 21.28a
M0P30	1.00 ± 0.00^{ab}	87.33 ± 3.51^{a}
M5P30	0.67 ± 0.58^{ab}	10.00 ± 8.66^{b}
M10P30	1.33 ± 0.58^{ab}	83.00 ± 9.85^{a}
M15P30	1.00 ± 0.00^{ab}	69.00 ± 25.51^{ab}

Note: The numbers in the same column and row followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at the $P < 0.05$ significance level

Mycorrhizae strengthen the root structure so that the plant's absorption of nutrients needed for shoot growth is improved (Begum et al., 2019). Mycorrhizae produce extensive underground extraradical mycelia ranging from the roots to the surrounding rhizosphere, thus helping in enhancing nutrient uptake, especially nitrogen (Battini et al., 2017).

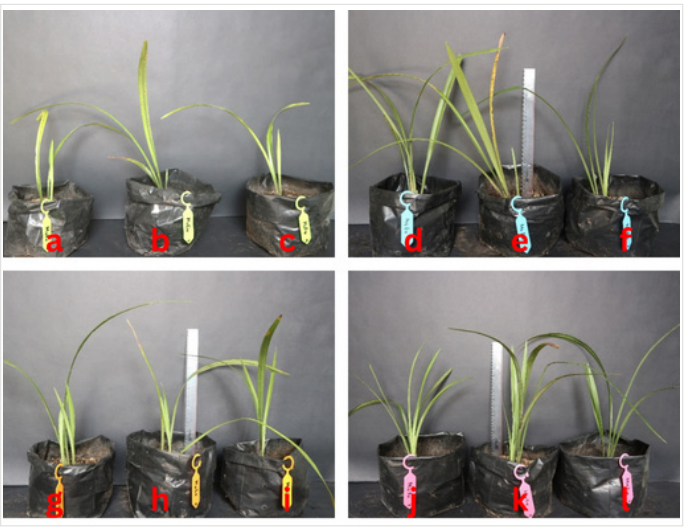

Figure 1. Habitus of *C. ensifolium* orchid at 3 months after treatment. A. M0P0 (control); b. M0P15; c. M0P30; d. M5P0; e. M5P15; f. M5P30; g. M10P0; h. M10P15; i. M10P30; j. M15P0; k. M15P15; l. M15P30.

The N element is the raw material for the formation of amino acids that will be used for protein synthesis. Proteins that act as enzymes are useful for metabolism that occurs in plants. One of them is the enzyme that causes hormone synthesis which will affect the number and length of the shoots.

Meanwhile, LOF treatment made from coconut water and WRW naturally contains cytokinins which can stimulate shoot growth. Purniawati et al. (2015) explain that the content of coconut water as a growth regulator is cytokinin which can stimulate shoot growth. Sitohang et al. (2023) mention that WRW contains N, P, and K, with increased nitrogen quantities. Higher nitrogen content will cause vegetative growth (shoots) which is more dominant than generative growth (Pamungkas and Supijatno, 2017).

According to Purniawati et al. (2015), shoot growth is in relation to root growth. If the roots have formed and developed well, the shoots will also be formed (Tamba et al., 2020). This is thought to be related to cytokinin synthesis that occurs in the roots, so that it will affect shoot growth. Mycorrhiza causes improvement in root structure (Begum et al., 2019), so it is thought to be supporting the work of LOF in promoting shoot growth by optimizing root growth as a source of endogenous cytokinin synthesis.

Leaf Growth

The results showed that there was an interaction between mycorrhiza and LOF in affecting leaf growth of *C. ensifolium* orchid. The treatment with mycorrhiza 15 g·pot^{-1} and 15% LOF produced the greatest leaf growth (leaf length and width, leaf area, number of leaves) (Table 4 and Fig. 2).

This is because mycorrhiza and LOF provide nutrients including N, P, K, Mg, and Zn. The nitrogen content in WRW in LOF can help leaf formation (Wijiyanti et al., 2019) and leaf growth (Nuraida et al., 2021). Fertilizers that cause optimal leaf length are fertilizers with higher nitrogen content compared to phosphorus and potassium elements. Nitrogen is a component composed of proteins and enzymes as essential plant compounds. High nitrogen content can increase the process of leaf elongation due to cell growth (Ayuningtyas et al., 2020). In addition, nitrogen plays a role in the photosynthesis process which will produce energy so that it can increase the process of cell division in plant tissues. This process will cause the growth in plants to increase so that the size of leaf organs will increase (Irawan et al., 2016).

Mycorrhiza can improve the uptake of nutrients near the roots of *C. ensifolium*. The external hyphal tissues of mycorrhiza will produce intensive hyphal braiding that expands the field of water and nutrient uptake with hyphae sizes that are finer than root hairs, allowing hyphae to infiltrate the smallest soil pores to absorb water in very low soil water conditions (Widiati et al., 2015). Phosphate elements are absorbed more by mycorrhiza up to 10%–27%, while nonmycorrhizal plants are only 0.4%–13%. Mycorrhiza can also increase N, K, Mg, Cu, and Zn in host plants (Bhat et al., 2017). The elements of N and P absorbed by mycorrhiza will be given to the host plant so that it is not excessive and does not interfere with the growth of mycorrhiza.

Optimal leaf growth in *C. ensifolium* orchid plants treated with mycorrhiza and LOF is also due to an increase in cytokinin and auxin in these plants. As in shoot growth, LOF contains cytokinin which can spur the growth of *C. ensifolium* through cell division at the base of the leaf.

Table 4. Data analysis for leaf growth (leaf width, leaf lenght, leaf number, and leaf area) parameters of *C. ensifolium* after application of mycorrhizae (g·pot^{-1}) and liquid organic fertilizer (%) at 12 weeks after planting

	Leaf width (mm)	Leaf length (mm)	Leaf number	Leaf area (mm^2)
M0P0	14.99 ± 0.42^{abc}	150.1 ± 6.11^{cd}	2.50 ± 0.58^{ab}	1669.96 ± 200.74^{def}
M5P0	12.51 ± 2.01^{de}	148.79 ± 50.18^{cd}	3.00 ± 1.73^{ab}	1605.64 ± 879.87^{def}
M10P0	11.86 ± 1.19^{e}	126.1 ± 30.98^{d}	3.00 ± 2.00^{ab}	1196.61 ± 411.84^{f}
M15P0	15.24 ± 1.02^{abc}	186.76 ± 78.54^{bcd}	2.50 ± 0.58^{ab}	2320.19 ± 1294.49^{bcde}
M0P15	14.31 ± 0.76^{bcd}	143.12 ± 17.45^{d}	1.67 ± 0.58^{b}	1644.22 ± 330.73^{def}
M5P15	15.16 ± 1.27^{abc}	218.61 ± 14.10^{ab}	2.60 ± 1.34^{ab}	2770.58 ± 434.19^{abc}
M10P15	13.13 ± 0.43^{cde}	181.96 ± 38.83^{bcd}	3.00 ± 0^{ab}	1907.93 ± 424.25^{cdef}
M15P15	16.94 ± 0.68^{a}	259.62 ± 11.78^{a}	4.33 ± 2.52^{a}	3589.14 ± 278.72^{a}
M0P30	17.28 ± 1.86^{a}	216.86 ± 17.89^{ab}	2.00 ± 0.71^{b}	3025.64 ± 525.63^{ab}
M5P30	14.91 ± 1.87^{abc}	133.96 ± 0.43^{d}	1.33 ± 0.58^{b}	1463.00 ± 78.84^{ef}
M10P30	16.34 ± 1.44^{ab}	227.77 ± 8.73^{ab}	3.00 ± 1.41^{ab}	3001.99 ± 382.47^{ab}
M15P30	15.08 ± 1.19^{abc}	206.82 ± 12.21^{abc}	2.33 ± 0.82^{ab}	2568.91 ± 37.72^{abcd}

Note: The numbers in the same column and row followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at the $P < 0.05$ significance level

Figure 2. Leaves of *C. ensifolium* orchid at 12 weeks after liquid organic fertilizer and mycorrhiza treatment. a. M0P0; b. M0P15; c. M0P30; d. M5P0; e. M5P15; f. M5P30; g. M10P0; h. M10P15; i. M10P30; j. M15P0; k. M15P15; l. M15P30. Bar: 0.2 cm.

Meanwhile, the auxin content plays a role in spurring leaf expansion through cell osmosis in the process of cell elongation in the center of the growing leaf (Hidayati and Subroto, 2018).

Cytokinin and auxin content will also rise during mycorrhizal association with plants. Increased auxin accumulation is indicated by the presence of the auxin-responsive reporter gene DR5-GUS which is specifically induced in the arbuscular of *Medicago truncatula* Gaertn., tomato and rice. The DR5-GUS gene is an auxin-responsive promoter system used to monitor auxin responses in leaves, roots and stems (Chen et al., 2013; Das and Gutjahr, 2020). An increase in this hormone can affect the growth of the host plant.

Leaf Chlorophyll Content

The results showed that there was an interaction between mycorrhiza and LOF in affecting chlorophyll A and B and total chlorophyll content of *C. ensifolium* orchid leaves. The highest chlorophyll A and B and total contents were found in orchid leaves treated with 15 g·pot^{-1} mycorrhiza and 30% LOF (Table 5). This is because mycorrhiza can increase the available N content in the soil by converting unavailable N content into available. According to Marzukah et al. (2023), the increase in leaf chlorophyll indicates the effectiveness of mycorrhizal hyphae in the root in increasing nutrient uptake, especially N and Mg elements that play a role in the formation of chlorophyll synthesis. The presence of mycorrhizal hyphae, in addition to increasing P and N nutrients, also helps increase other nutrients including Mg, Zn, Cu, K, Mo, and B. Rupaedah et al. (2015) state that the chlorophyll content of sweet sorghum leaves with mycorrhiza treatment combined with fertilizer was significantly increased.

Furthermore, WRW content in LOF also contains nitrogen. Nitrogen is the raw material for chlorophyll synthesis. LOF also contains coconut water, which contains the hormone cytokinin. The hormone stimulates chlorophyll formation by spurring chloroplast creation, which forms thylakoid. Cytokinin also affects the direction of transport of organic metabolites and minerals and accumulation of photosynthesis products in leaf cells, increases RNA synthesis associated with chlorophyll synthesis and mobilizes and takes nutrients, so that nutrients are used for chlorophyll synthesis. In chlorophyll synthesis, cytokinins trigger the activation of NADH protochlorophyllide reductase, which is a key enzyme in the chlorophyll biosynthetic pathway (Ghorbanpour and Hatami, 2015). Furthermore, cytokinin can stop the breakdown of RNA so that it has an effect in preventing chlorophyll degradation.

Table 5. Data analysis for chlorophyll content parameters of *C. ensifolium* after application of mycorrhizae (g·pot^{-1}) and liquid organic fertilizer (%) at 12 weeks after planting.

	Chlorophyll *a* (µg·ml^{-1})	Chlorophyll *b* (µg·ml^{-1})	Total Chlorophyll (µg·ml^{-1})
M0P0	12.14 ± 4.63^{d}	6.11 ± 2.57^{d}	118.82 ± 45.33^{d}
M5P0	12.09± 0.72^{d}	5.69 ± 0.51^{d}	118.21 ± 7.1^{d}
M10P0	12.85 ± 1.68^{d}	6.11 ± 1.09^{d}	125.7 ± 16.5^{d}
M15P0	14.47 ± 0.30^{bcd}	7.63 ± 0.39^{bcd}	141.68 ± 2.98^{bcd}
M0P15	17.49 ± 1.40^{b}	9.60 ± 1.05^{b}	171.24 ± 13.77^{b}
M5P15	17.21 ± 0.80^{bc}	9.04 ± 0.06^{bc}	168.47 ± 7.76^{bc}
M10P15	12.91 ± 2.41^{d}	6.61 ± 1.34^{d}	126.29 ± 23.61^{d}
M15P15	14.74 ± 1.45^{bcd}	7.46 ± 0.97^{bcd}	144.25 ± 14.25^{bcd}
M0P30	18.20 ± 2.96^{b}	8.85 ± 1.24^{bc}	178.00 ± 28.94^{b}
M5P30	15.25 ± 1.37^{bcd}	7.57 ± 1.03^{bcd}	149.15 ± 13.55^{bcd}
M10P30	13.74 ± 0.84^{cd}	7.23 ± 0.57^{cd}	134.53 ± 8.34^{cd}
M15P30	21.62 ± 0.26^{a}	13.54 ± 1.28^{a}	211.99 ± 2.85^{a}

Note: The numbers in the same column and row followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at the $P < 0.05$ significance level

Percentage of Mycorrhizal Infection

The findings demonstrated that there was an interaction between mycorrhiza and LOF influencing the proportion of mycorrhizal infection. Treatment with a high concentration of mycorrhiza (15 g·pot^{-1}) combined with 0% and 15% LOF yielded the highest percentage of mycorrhizal infection, compared with other concentrations of mycorrhiza treatment paired with the same concentration of LOF. Nevertheless, in the treatment with 30% LOF, the combination of mycorrhiza with the highest concentration produced the lowest proportion of infection (Table 6). This is believed to be because the treatment with low concentrations of LOF (0% and 15%) has not been able to meet the high nutrient requirements so that mycorrhiza plays a part by aiding in increasing nutrient absorption. In the mycorrhiza treatment with a combination of 30% LOF, it is thought that the nutrient needs have been provided by LOF so that mycorrhiza does not play a role in increasing the rate of infection.

Table 6. Data analysis for mycorrhizal infection parameters of *C. ensifolium* after application of mycorrhizae (g·pot^{-1}) and liquid organic fertilizer (%) at 12 weeks after planting

	Mycorrhizae infection (%)
M0P0	0.5 ± 0.5^{ab}
M5P0	0.71 ± 0.11^{a}
M10P0	0.79 ± 0.04^{a}
M15P0	0.91 ± 0.08^{a}
M0P15	0.25 ± 0.25^{b}
M5P15	0.75 ± 0.25^{a}
M10P15	0.7 ± 0.10^{a}
M15P15	0.91 ± 0.08^{a}
M0P30	0.25 ± 0.25^{b}
M5P30	0.75 ± 0.0^{a}
M10P30	0.8 ± 0.2^{a}
M15P30	0.62 ± 0.37^{ab}

Note: The numbers in the same column and row followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at the $P < 0.05$ significance level

The findings of this study support the idea that *C. ensifolium* orchids are symbiotic with mycorrhiza because hyphae grow on the roots. Sasmita et al. (2019) state that the percentage of root infection indicates that mycorrhiza can coexist symbiotically with plant roots and is proven by the presence of hyphae, vesicles, or arbuscules. The higher the mycorrhizal infection, the higher the contact with plant roots. When the percentage of mycorrhizal infection is higher, it can indicate that mycorrhiza is more active in infecting and expanding the region of root absorption so that the quantity of water and nutrient uptake by plants is increased (Basri, 2018; Musafa et al., 2015).

The presence of mycorrhizal infection in the roots of plants that are not given mycorrhizal fertilizer treatment is believed to be due to natural infection by mycorrhiza in the soil. This could happen because the planting medium used in this study has not been sanitized. However, identification has not been done so that it is unknown precisely which species of mycorrhiza infect the control plants. Similar findings were reported by Herliana et al. (2018) demonstrating that *Dendrobium* sp. without mycorrhiza treatment had mycorrhiza infection of 11.11%. This is explained by Marwani et al. (2013) that the association of mycorrhiza with plant roots can occur naturally.

Conclusion

The data showed that the mixture of 15% LOF and 15 g·pot^{-1} mycorrhizal biofertilizer was the optimum treatment in boosting the growth of *C. ensifolium* orchid leaves in the parameters of quantity of shoot, shoot length, number of leaves, leaf length and width and leaf area.

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CRediT Authorship Contribution Statement

Nintya Setiari: formulating of overarching research goals and aims, producing metadata and maintaining research data, analyzing the data, acquisition of the financial support for the project leading to this publication, conducting a research and investigation process, designing the method and writing draft. **Yulita Nurchayati:** Management and coordination responsibility for the research activity planning and execution, provision of study materials, programming and software development, supervising the research activity, verification of the overall of results, writing original draft. **Sri Widodo Agung Suedy:** Preparation, creation and visualization of data presentation, writing draft-review and editing including pre- or postpublication stages.

Declaration of Competing Interest

We (the authors) declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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