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FOLIAR APPLICATION OF *Moringa oleifera* LEAF EXTRACT IMPROVES MORPHO-PHYSIOLOGICAL TRAITS AND YIELD OF PAKCHOI

SUMMARY

To address the growing global demand for food, the agricultural sector must perform at its highest capacity. *Moringa oleifera* leaf extract (MLE) is a good source of beneficial compounds and nutrients to improve crop yield in an eco-friendly manner. The current study aimed to investigate the effects of different MLE concentrations on the morpho-physiological traits and yield of pakchoi. The designed study followed a randomized complete block design with six replications. Pakchoi cultivar 'Nauli' was foliar applied with MLE solutions at concentrations of 1:40, 1:30, and 1:20. A water spray was used as a control. MLE has altered most parameters observed, except for root fresh weight, root dry weight, and root length. Foliar MLE application at 1:30 was found to be more prominent compared to the control regarding net photosynthesis rate (125.93%), stomatal conductance (53.68%), internal CO_2 (22.15%), and transpiration rate (22.35%). A significant enhancement in plant height (21.78%), leaf number (38.50%), leaf area index (59.91%), and chlorophyll content index (22.35%) validated the efficacious effect of MLE 1:30 over control-treated plants. The increments in shoot fresh weight (36.40%) and shoot dry weight (76.06%) were also observed with a similar treatment. This underscores the potential of MLE foliar application at 1:30 for pakchoi cultivar 'Nauli' under screenhouse conditions.

Keywords: moringa leaf extract, photosynthesis, pakchoi, biostimulant, foliar spray

INTRODUCTION

Pakchoi (*Brassica rapa* subsp. Chinensis) is one of the Brassicaceae vegetables consumed worldwide for many culinary dishes, due to its crisp texture, mild, and peppery flavour (Lin *et al.*, 2024). It is a rich source of minerals, vitamins, and antioxidant compounds (Zou *et al.*, 2021). Further, these

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phytochemicals present in pakchoi may offer protection against cancer and help prevent many chronic diseases (Park *et al.,* 2023).

The global population projected to reach nearly 10 billion by 2050 would create a subsequent increase in food demand (Muñoz-Torres *et al*., 2025). Given these attributes, a short-season vegetable such as pakchoi is widely cultivated with a high input of chemical fertilizer during its growth period (Wang *et al.*, 2022). However, the overuse of synthetic inputs aggravates ecological and environmental damage, which in turn compromises food safety and reduces crop yield (Wu and Ge, 2019). Addressing the cultivation challenges of pakchoi, there is a growing concern to optimize production toward sustainable agricultural practices.

Biostimulant has received extensive attention as a promising strategy to improve plant growth and yield. It refers to formulations derived from biological materials, comprising essential nutrients, phytohormones, plant protective compounds, and plant growth regulators (Yakhin *et al.,* 2017). *Moringa oleifera* leaf extract (MLE) is considered an ecologically inviting and secure biostimulant since it contains numerous useful nutrients (K, P, N, Mg, Ca, Zn, Cu, and Fe), antioxidants (carotenoids, phenols, ascorbic acid, and proline), phytohormones (indole-3-acetic acid, gibberellins, cytokinins), vitamins, soluble sugars, and amino acids (Shalaby, 2024). The availability of chemical components in MLE might be used as a supplement to standard fertilization on plants (Mehmood *et al.,* 2021).

Moringa oleifera is a member of the Moringaceae family, and it is widely known as the horse radish tree or drumstick (Batool *et al.,* 2020). Due to its high adaptability to various climates, the plant has become extensively distributed across tropical and subtropical regions (Kamanga *et al.*, 2025). The application of MLE in agricultural practices showed great potential for improving vegetative growth and yield of many horticultural crops, including lettuce (Admane *et al.*, 2023), cabbage (Yaseen *et al*., 2023), and radish baby leaf (Romano *et al.,* 2022). The improvement in gas exchange traits and photosynthetic pigments was also recorded in MLE-treated plants, contributing to the enhancement in the yield (Khan *et al*., 2022).

One of the most effective ways to provide MLE to plants is through the foliar application method, in which nutrients and beneficial substances from the extract are efficiently adsorbed through the epidermis and stomata, supporting the growth of plants (Karthiga *et al.,* 2022). In addition, the effectiveness of biostimulant is highly dependent on the concentrations applied to the plant, as optimal doses are crucial for maximizing plant growth and yield (Quille *et al.*, 2025). The MLE dilution of approximately 1:30 (v/v) has been frequently adopted in foliar application studies and has proven to be an optimal concentration to improve plant growth performance (Romano *et al*., 2022; Yuniati *et al*., 2023). Aslam *et al.* (2016) found that too high MLE concentrations resulted in no significant improvement in plant growth. Keeping in view the literature reported, there is no information revealing the potential of MLE application in pakchoi. Therefore, the purpose of this study was to investigate the effects of different MLE application concentrations on the morpho-physiological traits and yield of pakchoi. We hypothesized that increasing concentrations of MLE would promote pakchoi growth and yield up to an optimal level, beyond which the stimulatory effect diminishes or remains unchanged.

MATERIAL AND METHODS

Experimental Site

The present experiment was conducted in a screenhouse at the School of Life Sciences and Technology, Institut Teknologi Bandung, Jatinangor, Indonesia (6°55′41.124″S, 107°46′13.62″E) from May to July 2025. The screenhouse temperature and relative humidity were at an average of 22 oC and 70%, respectively. The composition of the experimental soil is shown in Table 1.

Table 1. The characteristics of experimental soil in this study

Soil Characteristics	Value
Total nitrogen	1.01%
Available phosphorus	95.94 ppm
Potassium	67.34 mg 100 g^{-1}
pH	6.3
Sand	50%
Silt	27%
Clay	23%

Experimental Setup

The experiment was laid out and evaluated in a randomized complete block design with six replicates per treatment. Pakchoi (*Brassica rapa* subsp. Chinensis cultivar 'Nauli') plants were foliar sprayed with MLE at different concentrations under the following treatment groups: control (distilled water), MLE (1:40), MLE (1:30), and MLE (1:20).

Pakchoi seeds used in this study were purchased from East West Seed Indonesia company. The sowing was undertaken in a pot tray, filled with a mixture of rice husk charcoal and compost in equal parts. At the four-leaf stage, around 4 weeks after planting, pakchoi seedling was individually transplanted into perforated polybags (20 cm in diameter and 20 cm in height). Each polybag contained 2 kg of soil and chicken manure mixture in a 1:1 ratio. A total of six plots per treatment were arranged in a screenhouse, each containing four plants. Thus, each treatment comprised 24 plant samples, with a planting distance of 20 cm x 20 cm. A water-soluble granular NPK 16:16:16 fertilizer was applied to the plants, with all treatments receiving 250 mL per polybag at a concentration of 5 g L^{-1}. Applications were repeated at 7 d intervals until harvest, beginning at one week after transplanting. After 47 d of growth, plants were harvested to measure both aerial and root parameters.

MLE Preparation and Application

The preparation of MLE followed the procedure described by Yuniati *et al*. (2023). In brief, fresh mature leaves were harvested from moringa plants. A total of 30 g of moringa leaves was mixed with 300 mL of distilled water and crushed using a household blender. The blended leaves were then purified twice using cheesecloth and Whatman No. 1 filter paper. The filtration was then centrifuged at 8000 rpm for 15 m. Finally, the supernatant was diluted with distilled water to 40, 30, and 20 times its original volume, corresponding to the assigned treatments. Fourteen days after transplanting, the plants were foliar-sprayed with the extract to the point of runoff, and reapplication was performed at two-week intervals. The application of MLE in this study was intended solely as a supplementary input to the standard fertilizer. The chemical composition of MLE used is summarized in Table 2.

Table 2. Chemical properties moringa leaf extract used in this experiment

Parameters	Value (mg L^{-1})
P	217.63
K	896.80
Ca	156.80
Mg	912.00
Auxin	26.06
Zeatin	12.00
GA_3	23.00

Photosynthetic Analysis

Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were measured with the help of a photosynthesis system (CI-340; CID, Biosciences, Camas, WA, USA). Chlorophyll Content Index (CCI) was analysed using a non-destructive hand-held chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan). All photosynthetic traits were evaluated 1 d before harvesting on the third fully expanded leaf.

Plant Growth and Yield Analysis

The analysis of plant growth and yield parameters was carried out during the harvest period. Plant height was measured using a steel ruler, and the number of leaves per plant was recorded. Whole plants were then harvested by carefully excavating the experimental soil. Roots were thoroughly rinsed with tap water to remove soil and dirt. The shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight were assessed, along with root length. Later in the experiment, the gravimetric method was used to determine leaf area index (LAI) according to the method explained by Kumar and Salakinkop (2017).

Statistical Analysis

Collected data were analysed with Analysis of Variance (ANOVA) using SPSS v21 software (SPSS Inc., Chicago, IL, USA). The treatment means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSION

This study delineates the dynamics of the plant height of pakchoi as they respond to MLE applications (Figure 1A). Pakchoi supplemented with foliar MLE 1:30 showed the maximum plant height. However, this treatment had no remarkable differences with MLE 1:20. The present results corroborate those of Sardar *et al.* (2021), who reported that MLE application supported the plant height of stevia. Moreover, taller plants were recorded in plots where MLE was sprayed on black cumin during the critical vegetative development phases (Mehmood *et al.*, 2021). This increment might be attributed to the presence of a considerable number of growth-enhancing hormones. Accordingly, auxins and gibberellins present in MLE were likely responsible for promoting cell elongation and positively affected stem growth (El-Serafy *et al.*, 2021).

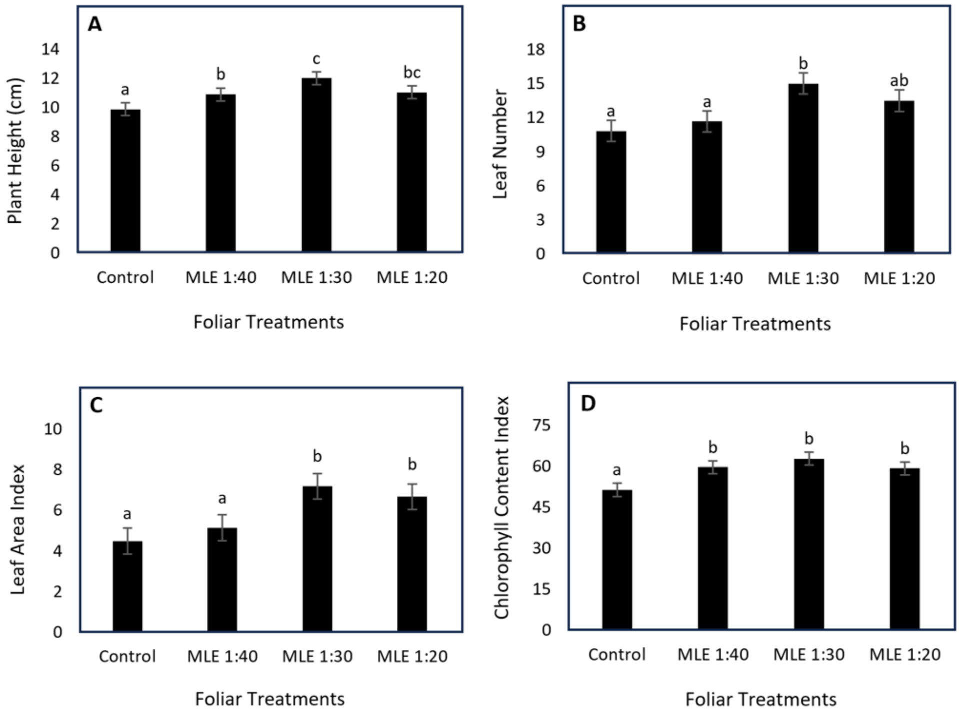

Figure 1. Plant height (A), leaf number (B), leaf area index (C), and chlorophyll content index (D) of pakchoi under different rates of moringa leaf extract foliar application. Different lowercase letters indicate significant differences at P<0.05

Leaf number per plant was significantly affected by treatments, especially when MLE 1:30 was applied (Figure 1B). At this concentration, plants produced an average of 38.50% more leaves than the control, though statistically at par with MLE 1:20. In general, the number of leaves per plant tended to increase as the concentration of MLE was raised (Marie *et al.*, 2025). Exogenous application of MLE showed a significant efficacy on the leaf number of *Silybum marianum* (Yap *et al*., 2021). Prior research on tomato also indicated the advantageous effect of foliar MLE at 1:32 concentrations on the number of leaves both under stress and non-stress conditions (Bello and Ahmed, 2024). The proper concentrations of nutrients and cytokinin found in MLE may have influenced metabolic activity in a way that increased leaf number (Farooq *et al.*, 2023). Cytokinin facilitated fast cell division, cell multiplication, and cell enlargement, which subsequently improved the meristematic activity in the growth zone (Iqbal *et al*., 2020).

As one of the primary growth parameters, leaf area index (LAI) is an important attribute that depicts the economic yield of a plant. LAI was notably influenced by MLE treatments (Figure 1C). Compared to the control, MLE 1:30 and MLE 1:20 generated a similar improvement of up to 59.91%. In accordance with these findings, previous studies also validated MLE effects that consequently developed bigger leaves in common bean (Zaki and Rady, 2015). Romano *et al.* (2022) reported faster growth in radish baby leaves by detecting an increase in leaf area and stem diameter using foliar MLE application at 1:30. The maximum leaf area was noted in the plant treated with foliar spray MLE alone due to the cumulative effect of micro and macro nutrients, as well as growth hormones (Khan *et al*., 2020). The improvement in LAI following MLE treatments also might be associated with the role of phosphorus (P) available in the extract. It was reported that enriching P in plants improved the values of LAI and photosynthetic rate (Zhang *et al*., 2018).

On the other hand, plants treated with foliar MLE showed significantly better enhancement in chlorophyll content index, ranging from 15.33 - 22.35% relative to the control (Figure 1D). Leaf chlorophyll content determines the visual appearance of most leafy vegetables. Various studies revealed a stimulating effect of MLE on chlorophyll concentration of lettuce (Yaseen and Takacz-Hajos, 2022), sweet basil (Alkuwayti *et al.*, 2020), and wheat (Afzal *et al.*, 2020). These findings were possibly linked to magnesium (Mg) content in the extract (El-Serafy *et al.*, 2021). Mg acts as an essential element of chlorophyll, in which nearly 15–35% of the Mg taken up by plants is allocated to this photosynthetic pigment (Ishfaq *et al*., 2022). In addition, MLE comprises a substantial amount of specific phytochromes with proven antioxidant properties, including chlorophyll, which ultimately led to greener leaves (Khan *et al.*, 2023)

Referring to the data, the net photosynthesis rate was progressively enhanced by MLE 1:30, accounting for 125.93% more than the control (Table 3). But this treatment was essentially the same as MLE 1:40. The use of MLE has been discovered to be an excellent biostimulant for improving photosynthesis rate in soybean (Irshad *et al.,* 2025). The observed increase in photosynthetic rate is likely linked to the cytokinin-driven stimulation of chloroplast development and maintenance of chlorophyll content (Howladar, 2020). Likewise, the increase in chlorophyll concentration can explain the higher photosynthetic rate and better plant performance due to MLE application in sword lily (Zulfiqar *et al.,* 2020).

Foliar spraying with MLE displayed greater stomatal conductance compared to the control (Table 3). In this regard, the greatest improvement was recorded in MLE 1:30 treatment, which was statistically equivalent to MLE 1:40. The results from this study are in accordance with those claimed by Lalarukh *et al.* (2022) and Khan *et al.* (2023), who concluded that MLE application stimulated stomatal conductance in wheat and rice, respectively. Potassium (K) content in MLE might be recognized for its crucial role in stomatal regulation. According to Azizah *et al.* (2022), K serves as a key factor in modulating guard cell turgor during stomata movement. They explained that increasing the K+ concentration in guard cells elevates the osmotic pressure, which then magnifies guard cell turgidity and results in stomata opening to ensure smooth CO_2 transport.

Additionally, lower stomatal conductance and net photosynthesis rate observed in MLE 1:20 compared to MLE 1:30 in this study suggested that this concentration is still capable of stimulating stomatal opening and photosynthesis more than the control. However, this stimulatory effect appears to diminish due to physiological limitations or saturation of the plant response. This is supported by Ramamoorthy and Munisamy (2025), who stated that biostimulant works at low concentrations and is involved in many plant physiological systems.

Table 3. Net photosynthesis rate, stomatal conductance, internal CO_2, and transpiration rate of pakchoi under different rates of moringa leaf extract foliar application

Treatments	Net Photosynthesis Rate (μmol m^{-2} s^{-1})	Stomatal Conductance (mmol m^{-2} s^{-1})	Internal CO_2 (ppm)	Transpiration Rate (mmol m^{-2} s^{-1})
Control	0.81 a	13.71 a	412.23 a	0.18 a
MLE 1:40	1.42 ab	20.08 bc	489.48 b	0.34 b
MLE 1:30	1.83 b	21.07 c	503.55 b	0.36 b
MLE 1:20	1.17 a	17.14 b	488.76 b	0.33 b

Note: Different lowercase letters on the same column indicate significant differences at $P<0.05$

For internal CO_2 concentration, the measurements showed that plants exposed to MLE had a higher value than the control by up to 22.15% (Table 3). This aligns with a study showing that MLE enhanced internal CO_2 levels in rice under both stress and non-stress conditions (Saleh *et al*., 2025). It was reported that cytokinin found in MLE increased biosynthesis of photosynthetic enzymes such as Rubisco, thereby improving carbon assimilation (Irshad *et al.,* 2025). Additionally, the improvement may also be attributed to a higher chlorophyll accumulation, which translated to an increase in chemical activity in the leaves (Dalorima *et al.,* 2019).

A comparable pattern appeared on the transpiration rate of pakchoi (Table 3). All MLE treatments were more pronounced compared to the control, reaching an increase of up to 22.35%. Toscano *et al.* (2023) reported that in the case of petunia plants grown under normal conditions, the transpiration rate in leaves exhibited a greater value following foliar MLE treatment. Foliar application of MLE in combination with chemical fertilizer also improved this parameter in radish (Raza *et al*., 2022). The elevated transpiration rate observed under biostimulant treatment was closely associated with the improvement in stomatal conductance (de Farias *et al*., 2024). In lettuce, a higher transpiration rate was observed in plants receiving biostimulant, but it did not represent a significant decrease in water use efficiency (Atero-Calvo *et al*., 2024).

Figure 2. Pakchoi under different concentration application of MLE (A) and moringa leaf extract used in the study (B)

Foliar treatment of MLE caused a significant variability in shoot fresh weight (Table 4 and Figure 2A). Pakchoi treated with MLE 1:30 was characterized by the highest shoot fresh weight, although the difference was not significant compared to MLE 1:20. Applying MLE at 1:20 enhanced biomass yield by 34.77% over the untreated plants (Ali *et al.,* 2018). Based on the findings by Noreen *et al.* (2024), a substantial increase in shoot fresh weight was recorded in pea plants following foliar MLE application under non-stress conditions. They stated that plant extracts (fresh and/or dry) have stimulatory impacts by upregulating enzyme activity and metabolic contents. The improvement in mineral content and photosynthetic activity due to MLE application also contributed to increased vegetative growth and biomass production (Hafeez *et al.,* 2022). The MLE used in this study (Figure 2B) is rich in important minerals and growth-promoting compounds like auxin, gibberellin, and cytokinin. These compounds may stimulate the growth of plants by enhancing cell elongation and cell division during the development of overall biomass (Nazir *et al.,* 2025).

Table 4. Shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and root length of pakchoi under different rates of moringa leaf extract foliar application

Treatments	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	Root Length (cm)
Control	17.36 a	1.42 a	1.25 a	0.19 a	10.25 a
MLE 1:40	18.81 a	1.89 ab	1.27 a	0.20 a	12.12 a
MLE 1:30	23.68 b	2.50 c	1.39 a	0.29 a	14.05 a
MLE 1:20	20.30 ab	2.07 bc	1.49 a	0.28 a	11.10 a

Note: Different lowercase letters on the same column indicate significant differences at $P<0.05$

Amongst all the treatments, the heaviest shoot dry weight was noted in plant foliar-sprayed with MLE 1:30 (Table 4). However, it was statistically equal to MLE 1:20. The presence of nutrients, such as Ca, K, Mg, Mn, Fe, and Zn in MLE might also be involved in biomass increment of treated plants (Hafeez *et al.,* 2022). Afzal *et al.* (2020) revealed that supplementing plants with MLE improved assimilation of photosynthates as a result of extended green leaf area. In a previous study by Jain *et al*. (2020), the exogenously applied MLEs effectively increased zeatin (a cytokinin-related hormone) content in stevia plants, which can be linked to the increment in isopentyl amino purine (precursor of zeatin) content. These reports were presumed to be the underlying factor contributing to the increase in shoot dry weight in plants treated with MLE. Further research is needed to quantify endogenous cytokinin and related hormone dynamics in the plant tissues of pakchoi after foliar spraying with MLE.

As shown in Table 4, there was no significant variation in root fresh weight, root dry weight, and root length regarding foliar MLE application. The root fresh weight values of all treatments ranged from 1.25 to 1.49 g, while 0.19-0.29 g for root dry weight. The data pertaining to the root length showed the values ranged from 10.25 to 14.05 cm.

Consistent with previous investigations, the exogenous application of MLE at the vegetative stage did not considerably improve root fresh weight and root dry weight of chickpea (Nazir *et al.*, 2025). In purple nutsedge, no MLE-induced enhancement effect was detected in both the fresh weight and the dry weight of the root. In harmony with the present study, a response of sweet basil’s root length to different foliar MLE levels was non-significant (Alkuwayti *et al.,* 2020). Similarly, the root length of tamarind was not notably affected by biostimulant treatment (Dantas *et al*., 2012). A study comparing foliar and root-based application of biostimulant has shown that root-based application often produces stronger effects on root growth than the foliar method due to direct contact with root tissues, thereby improving nutrient and water uptake and activating root plasma membrane ATPase activity (Atero-Calvo *et al*., 2024). Therefore, this difference in mode of action likely explains the limited response found in root traits in the present study.

CONCLUSIONS

According to the current study's findings, the treatments had no significant impact on root growth traits. Meanwhile, foliar application of MLE at 1:30 had a substantial improvement on photosynthesis rate (125.93%), stomatal conductance (53.68%), internal CO_2 (22.15%), and transpiration rate (22.35%). This experiment also confirmed that plants sprayed with MLE 1:30 showed better results for all growth and yield attributes as compared to the control, including plant height (21.78%), leaf number (38.50%), leaf area index (59.91%), chlorophyll content index (22.35%), shoot fresh weight (36.40%), and shoot dry weight (76.06%). From a practical agronomic perspective, the application of MLE at 1:30 can serve as an effective supplementary biostimulant to standard chemical fertilization for the pakchoi cultivar 'Nauli' under screenhouse conditions. Moreover, identifying an optimal MLE concentration in this study may help improve the overall efficiency of crop production and avoid unnecessary biostimulant overuse, thereby offering potential economic benefits. Further studies involving different cultivars and open-field conditions are recommended to assess the consistency of the observed responses.

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