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Moringa (*Moringa oleifera Lam.*) leaf extract as biostimulant to enhance growth and yield of bitter gourd (*Momordica charantia L.*)

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ABSTRACT

Overreliance on synthetic agricultural inputs, such as inorganic fertilizers and pesticides that pose health and environmental risks has led to a continuous quest for more sustainable and ecologically sound approaches to crop production. This study explored using Moringa Leaf Extract (MLE) as a natural plant-based biostimulant to enhance the productivity of bitter gourd. It investigated the effects of foliar MLE application on the growth, compared to water and ethanol as solvents. The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications and five treatments: T1- 0 Application; T2- AVRDC Recommended Rate of Fertilizer (RRF) + Tap Water Spray; T3- RRF + Tap Water Spray; T4- RRF + MLE (water solvent); and T5- RRF + MLE (ethanol solvent). Data on vegetative growth, flowering, yield, and yield components were analyzed using ANOVA, while the Duncan Multiple Range Test (DMRT) was used to compare differences in treatment means. Results showed that except for vine and fruit length, MLE application significantly improved most attributes of bitter gourd, including days to 50% flowering, number of marketable fruits and fruits per plant, and average fruit weight and yield. Ethanol as a solvent for MLE yielded the best results, although statistical analyses revealed no significant differences between the two extracting solvents. The application of MLE, regardless of the solvents used, proved to be an effective biostimulant offering an alternative and environmentally friendly approach to enhance the productivity of bitter gourd.

Keywords: Environmentally friendly, plant resilience, sustainable agriculture

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INTRODUCTION

Continuous and excessive application of chemical inputs leads to crop stress, which affects plants' biochemical processes, morphology, physiology, and gene regulation, impacting visible and internal plant characteristics (Zhang et al., 2023). Therefore, one of the challenges in agricultural crop production is to develop strategies that boost plant resilience while harboring no adverse environmental impact.

The use of plant bio-stimulants (PBs) has emerged as a new area of study, attracting attention in recent years due to its potential to improve crop production sustainably and eco-friendly. Although similarly encouraging plant development, PBs differ from traditional fertilizers and soil amendments because they are not intended to be applied in large quantities but act as targeted metabolic enhancers (Zhang and Schmidt, 1997). They are neither pesticides that inhibit the pest population nor plant growth hormones that change or control one or more specific physiological processes within a plant (Lemaux, 1999). Plant biostimulants are bioactive compounds extracted from raw organic materials that, when applied to plants in precise formulations, can alter the physiological actions of plants, enhancing nutrient uptake efficiency and promoting growth, development, and tolerance to abiotic stresses (Du Jardin, 2012; Posmyk and Szafrańska, 2016). Their targeted approach strengthens the plant's natural defenses to improve plant health (Rouphael and Colla, 2020). Biostimulants are categorized according to their substances and components. Specifically, biostimulants that are products of plant origin or extracted from natural plant components like roots, leaves, and seeds are referred to as plant-based biostimulants (PBBs) or plant-derived biostimulants (PDBs) (Cavani et al., 2006; du Jardin, 2015; Kopta et al., 2018; Madende and Hayes, 2020).

Moringa leaf extract (MLE) is one of the most notable PDBs (Elzaawely et al., 2017). *Moringa oleifera* leaves are excellent sources of minerals like calcium, potassium, iron, phosphorus, proteins, carbohydrates, fiber, beta-carotene, and vitamin C, and possess antioxidant properties (Anwar et al., 2005; Kashyap et al., 2022). The available information from recent literature on the effect of MLE on crops indicated enhanced growth and yield of vegetables such as chili (Weerasingha, 2020), tomato and Indian spinach (Hoque et al., 2022), cucumber (Ahmed et al., 2020), lady's finger (Kanchani and Harris, 2019), pepper (Matthew, 2016), and lettuce (Yaseen and Takacs-Hajos, 2022).

In addition, since the nutrient content from MLE is extracted particularly from the leaves, the appropriate extraction procedure, techniques, and variables, including the extracting solvents used to separate different types and amounts of bioactive components from the crude extracts, are also carefully considered to ensure optimal extraction efficiency (Hayat et al., 2009). The biological activity of the extract is caused by its active components and their concentrations can change depending on the extraction technique and solvent employed (Foidl et al., 2001; Mehmood et al., 2022; Ngcobo and Bertling, 2021; Nasir et al., 2020; Phiri and Mbewe, 2010; Khan et al., 2021; Olvera-Aguirre et al., 2022; and Simon et al., 2023). Several studies have highlighted this variability and suggested specific solvents and concentrations effective for extracting antioxidant compounds from Moringa leaves. The most commonly used solvents for extracting bioactive compounds from Moringa leaves include methanol (80%) and ethanol (70%) and their combinations Siddhuraju and Becker, 2003; Batool et al., 2020). Moreover, Vongsak et al. (2013) reported that using 70% concentration of ethanol as extracting solvent yielded the highest amount of total polyphenols, total flavonoids, and ferric-reducing power from Moringa leaves.

Hence, the current study evaluated the effects of MLE as a plant-based biostimulant in enhancing the growth and yield potential of bitter gourd. The effect of using ethanol and water as extraction solvents was also investigated. The specific objectives were: a) to assess the growth and yield performance of bitter gourd applied with MLE as a crop bio-stimulant and b) to compare the effect of using water and ethanol as extracting solvents in MLE in terms of growth and yield performance of the bitter gourd.

MATERIALS AND METHODS

Time and location of the study

The study was conducted at the Davao Oriental State University (DORSU) Demonstration Farm in Sitio Marfori, Barangay Don Enrique Lopez, City of Mati, Davao Oriental. The research duration was from June 2022 to May 2023.

Research design

The study employed five treatments, which include: T1- 0 Application; T2- AVRDC Recommended Rate of Fertilizer + Tap Water Spray; T3- Recommended Rate of Fertilizer + Tap Water Spray; T4- Recommended Rate of Fertilizer + MLE (water as a solvent) and; T5- Recommended Rate of Fertilizer + MLE (alcohol as a solvent).

The first treatment, T1, was neither applied with any fertilizers nor MLE extract and was considered the negative control. T2 was applied with fertilizer following the recommended rate prescribed by the Asian Vegetable Research Center (AVRDC) f or bitter gourd (Palada and Chang, 2003). However, it was not applied with MLE and thus was considered a positive control. For T3, T4, and T5, random soil samples were collected before land preparation for a soil analysis to determine the recommended fertilizer rate. This was the basis for applying fertilizer for T3, although no MLE was applied, thus, regarding it as another positive control. T4 and T5 were applied with MLE using water and alcohol as the extraction solvents, respectively.

The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replications. A total of 15- unit plots measuring 4 m x 4.5 m were made and each plot contained 28 plants at the spacing of 1 m (row to row) x 0.5 m (hill to hill). The total land area was 456 m2 (16 m x 28.5 m).

Preparation of moringa leaf extract

The moringa used in the experiment came from the same variety located in the research area. Fresh moringa leaves were collected from a mature moringa tree, and the juice was extracted using the method of Foidl et al. (2001). Moringa young leaves were ground using a blender mixed with either one liter of water or 70% ethanol (1 L/10 kg fresh material). The extract was then filtered twice with cheesecloth. About 40 ml of the extract was diluted with 960 ml of distilled water to get the 4% concentration (Figure 1).

Land preparation and cultural management

The area was harrowed, and weeds were manually removed. Plots were prepared with spades and covered with plastic mulch. Seeds were sown in seedling trays with a sterilized potting mix of garden soil and humus in a ratio of 1:1. Transplanting was done 15 days after germination. Lean-to-A-shaped trellises, 2 m in height, were used to hold up the lateral stems and climbing vines with side shoot pruning. Irrigation was provided as and when needed. Infestation of insects was controlled by bagging fruits of 7 cm or 2.5 inches in size. Pesticides were applied as required. Harvesting and picking fruits were based on horticultural maturity, size, color, and age.

Rate and period of treatment application

The application of MLE treatments as foliar sprays started three weeks after the transplanting date and was repeated twice at 15-day intervals: 15 (1L of MLE), 30 (2L of MLE), and 45 (3.5L of MLE) days after transplanting (DAT). The AVRDC recommended fertilizer rate included 61.33 kg N, 112 kg P, and 41.33 kg K per hectare, while the recommended fertilizer rate based on soil analysis was 118.04 kg of N, 559 kg P, and 30 kg K per hectare.

Data gathered and analysis

Five plants per plot were randomly selected 22 days after transplanting to measure the vine length, taken from the base to the tip of the main vine. The number of days from transplanting to 50% flowering was also recorded. Furthermore, ten fruit samples were randomly collected to measure fruit length and compute other fruit and yield parameters such as the number of marketable fruits, average number of fruits per plant, average fruit weight, and fruit yield. The gathered data were analyzed using Analysis of Variance (ANOVA), subsequently followed by the Duncan Multiple Range Test (DMRT) to assess disparities between treatment means with significance levels set at $p \leq 0.05$.

Figure 1. The process of preparation of moringa leaf extract: A. Collection of moringa leaves; B. Removal of stems; C Rinsing with clean water; D. Addition of 1L extraction solvent (water or ethanol); E. Mixing using the blender; F. Filtration using a cheesing cloth; G. Dilution of 40 ml extract with 960 ml water; H. Labeling of MLE

Results and Discussion

Vegetative growth
A. Vine length

The application of MLE at a 4% concentration had no significant effect on the vegetative growth of bitter gourd. Nevertheless, notable increases in vine length were observed with the use of ethanol solvent (by 46.94 cm) in T5 and water solvent (by 27.30 cm) in T4, compared to the control treatments (Table 1). In addition, plants treated solely with inorganic fertilizer demonstrated considerable increments in vine length as observed in T3 (Recommended fertilizer rate + Tapwater spray). This phenomenon can be attributed to the rapid release of macroelements from inorganic fertilizers, promoting accelerated vegetative growth (Sureshkumar and Kuruppaiah, 2008). In contrast, T2 (AVRDC Recommended Fertilizer Rate + Tapwater spray)plants exhibited marginal differences in vine length compared to T1 plants, suggesting that the AVRDC fertilizer recommendation rate might not be adequate to provide the nutrient requirement of the plants. Furthermore, combining the recommended

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dosage of inorganic fertilizer with organic supplements such as MLE enhanced nutrient availability, resulting in improved crop growth. This finding supports previous studies highlighting the synergistic effect of inorganic fertilizers and organic supplements in augmenting nutrient availability and fostering enhanced plant development (Akande et al., 2010; Priyadharshini, 2022; Sani et al., 2020).

Table 1. Effect of foliar application of moringa leaf extract (MLE) with different solvents at 4% concentration on the mean vine length (cm) of bitter gourd.

Treatment	Mean vine length (cm)
T1 - No Application (- Control)	81.67
T2 - AVRDC Recommended Fertilizer Rate + Tapwater spray (+Control)	85.34
T3 - Recommended Fertilizer Rate + Tapwater spray (+Control)	110.00
T4 - Recommended Fertilizer Rate+ MLE (Water Solvent)	108.72
T5 - Recommended Fertilizer Rate + MLE (Ethanol Solvent)	128.61
***p*-value**	0.31 ns
CV	21.88%

ns = not significant

Number of days to flowering

As depicted in Table 2, foliar application of MLE enhanced the reproductive performance of bitter gourd, irrespective of the solvent used, and significantly promoted early flowering in the plants ($p = 0.04$). Among all treatments, the earliest onset of flowering occurred in T5 (Recommended Fertilizer Rate + MLE with Ethanol as solvent) at 37 days, while T1 (No application) exhibited the latest attainment of 50% flowering at 40 days. This result aligns with the findings of Biswas et al. (2020), which reported a significant reduction in the number of days to flowering (by 36.90%) with MLE application on tomatoes compared to the control (no application). Fuglie (2000) explained the underlying mechanism behind this phenomenon, suggesting that the phosphorous content in MLE facilitates root development and enhances nutrient absorption and supply through the roots, thereby fostering increased assimilate synthesis, ultimately leading to a higher number of floral branches and early flowering and fruiting (Arif et al., 2023; Basra et al., 2011; Yasmeen et al., 2014).

Table 2. Effect of foliar application of moringa leaf extract (MLE) with different solvents at 4% concentration on total days to flowering of bitter gourd.

Treatment	Days to flowering
T1- No Application (- Control)	40.00[b]
T2- AVRDC Recommended Fertilizer Rate + Tapwater spray (+Control)	39.00[ab]
T3- Recommended Fertilizer Rate + Tapwater spray (+Control)	38.33[ab]
T4- Recommended Fertilizer Rate+ MLE (Water Solvent)	37.67[a]
T5- Recommended Fertilizer Rate + MLE (Ethanol Solvent)	37.00[a]
***p*-value**	0.04*
CV	3.81%

Means with the same letters are not significantly different from each other;
* Significant

Fruit and yield components

1. Fruit length

The foliar application of Moringa leaf extract (MLE) using different solvents had no significant effects on the fruit length of bitter gourd. However, plants treated with MLE using ethanol as a solvent (T5) displayed the longest fruits with a mean length of 28.23 cm, while those in the control group (T1 -No application) obtained the shortest mean fruit length of 22.22 cm (Table 3). Despite the lack of statistically significant results, the application of MLE yielded the highest fruit lengths observed in the plants. This observation may be attributed to the high content of zeatin present in MLE, a natural cytokinin known for its role in promoting cell division and elongation of fruit cell walls, consequently contributing to an i crease in fruit length (Siddharaju and Becker, 2003; Anwar et al., 2007).

Table 3. Effect of foliar application of Moringa leaf extract (MLE) with different solvents at 4% concentration on fruit length (cm) of bitter gourd.

Treatment	Fruit length (cm)
T1- No Application (- Control)	22.22
T2 - AVRDC Recommended Fertilizer Rate + Tapwater spray (+Control)	25.90
T3- Recommended Fertilizer Rate + Tapwater spray (+Control)	24.52
T4 - Recommended Fertilizer Rate+ MLE (Water Solvent)	26.08
T5- Recommended Fertilizer Rate + MLE (Ethanol Solvent)	28.23
***p*-value**	0.16 ns
CV	11.57%

ns = not significant

b. Number of marketable fruits and average Number of fruits per plant

Bitter gourd plants applied with MLE at a 4% concentration yielded a significantly higher number of marketable fruits (p=0.01) and a more significant Number of fruits per plant (p = 0.02) compared to control treatments. Further analysis using DMRT revealed that using ethanol as a solvent for MLE extraction (T5) significantly led to producing more marketable fruits than water as a solvent (T4). However, the choice of solvent for MLE extraction did not show a statistically significant difference in the average number of fruits per plant (Table 4).

Various studies support the positive influence of MLE on fruit production in plants. Talon and Zeevart (1992) demonstrated that MLE can enhance the number of fruits per plant, possibly due to its rich composition of beneficial components such as zeatin, minerals (calcium, magnesium, potassium, phosphorus, and iron), phenolics, vitamins (A, B1, B2, B3, C, and E), sugars, and various hormones involved in regulating fruit production and fruit set. In addition, Sivakumar and Ponnusami (2011) observed that foliar spraying of MLE, combined with farmyard manure (FYM) soil application, increased the nitrogen, phosphorus, and potassium content of *Solanum nigrum*. Hafez and El-Metwally (2007) explained that the nutrient contents of MLE, particularly potassium and zinc, can enhance nutrient uptake and photo synthetic activity in the leaves, thereby increasing the number of fruits and fruit sets. The increase in the number of fruits per plant is likely associated with the rise in the number of flower clusters per plant, similar to the result of Mehdawe et al. (2023) on pepper.

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Table 4. Effect of foliar application of Moringa leaf extract (MLE) with different solvents on number of marketable fruits and average number of fruits per plant of bitter gourd.

Treatments	Number of marketable fruits/456m^2	Average number of fruits per plant
T1- No Application (- Control)	199^b	8.39^b
T2- AVRDC Recommended Fertilizer Rate + Tapwater spray (+ Control)	202^b	11.22^{ab}
T3- Recommended Fertilizer Rate + Tapwater spray (+ Control)	207^b	11.50^{ab}
T4 - Recommended Fertilizer Rate+ MLE (Water Solvent)	241^b	13.38^a
T5- Recommended Fertilizer Rate + MLE (Ethanol Solvent)	300^a	16.66^a
***p*-value**	0.01**	0.02*
CV	19.98%	27.90%

Means with the same letters are not significantly different from each other;
* Significant
** Highly significant

Moreover, a consistent increase in the quantity of harvested fruits was observed from the second to the fourth harvests (67 DAT), with a drastic increase during the fifth harvest (71 DAT) followed by a swift decline in the sixth and seventh harvests (Figure 2). The observed reduction in harvests can be attributed to leaf yellowing as a result of leaf aging, reduced floral productivity, and increased pest infestations, particularly by the corn earworm (*Helicoverpa zea*).

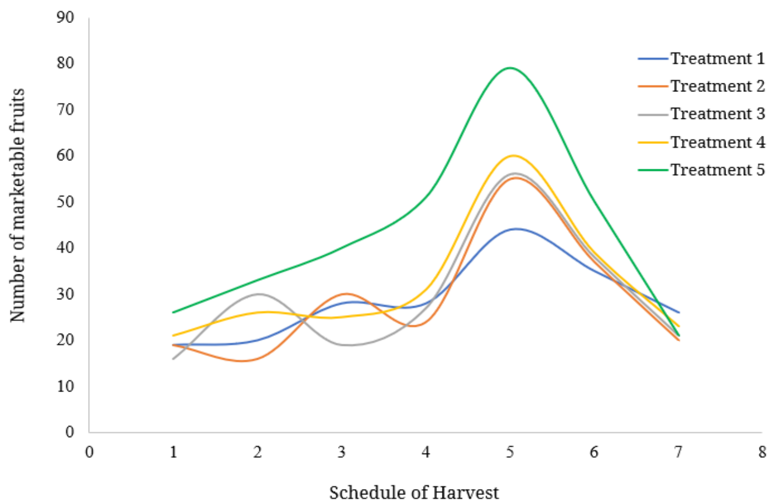

Figure 2. Total number of marketable fruits harvested from first to seventh harvests.

c. Average fruit weight

Table 5 showed that the application of MLE resulted in a highly significant increase (p=0.00) in the average fruit weight of bitter gourd. Utilizing ethanol as a solvent for MLE (T5) resulted in the highest average fruit weight among all treatments. However, further DMRT analysis revealed that this value is statistically comparable to T3

(Recommended Fertilizer Rate + Tapwater spray) and T4 (Recommended Fertilizer Rate + MLE with water as solvent). This indicates that the fruit weight of bitter gourd is notably augmented with the application of sufficient fertilizers, irrespective of whether MLE was applied or not.

On the other hand, for T2 (AVRDC Recommended Fertilizer Rate + Tapwater spray), it appears that the fertilizer rate applied may not be adequate to meet the nutritional requirement necessary for optimal fruit development to significantly enhance fruit weight in bitter gourd.

Table 5. Effect of foliar application of Moringa leaf extract (MLE) with different solvents at 4% concentration on average fruit weight of bitter gourd.

Treatments	Average fruit weight (kg)
T1- No Application (- Control)	0.93^{b}
T2- AVRDC Recommended Fertilizer Rate + Tapwater spray (+Control)	1.06^{b}
T3- Recommended Fertilizer Rate + Tapwater spray (+Control)	1.39^{a}
T4 - Recommended Fertilizer Rate+ MLE (Water Solvent)	1.37^{a}
T5- Recommended Fertilizer Rate + MLE (Ethanol Solvent)	1.45^{a}
***p*-value**	0.00**
CV	19.09%

Note. Means with same letters are not significantly different from each other
** Highly Significant

d. Fruit yield

The different solvents used for Moringa leaf extract revealed a highly significant influence (p = 0.00) on the fruit yield of bitter gourd, as presented in Table 6. The highest fruit yield was observed in T5 (Recommended Fertilizer Rate + MLE with ethanol as solvent) with a mean value of 14.05 t/ha, while the lowest mean yield of 6.17 t/ha was recorded in T1 (No application).

These findings were consistent with the previous results of Palada (1996), who demonstrated that foliar application of MLE significantly increased yields of peanut, soybean, sorghum, and tomato. A study by Foildl et al. (2001) also reported that MLE application can increase yields by 25 to 30% across a wide range of crops, with potential increases of up to 35%, alongside sugar and mineral levels enhancements. Moreover, Phiri and Mbewe (2010) highlighted the role of MLE, similar to a plant growth hormone, in improving seed germination and overall plant yield.

The DMRT further validated the efficacy of T5, which uses ethanol as a solvent for MLE extraction over other treatments, in enhancing bitter gourd yield. This also confirmed the results of Ismail and Granzour (2021), claiming that foliar application of MLE at 4% concentration improved strawberries yields even when planted in sandy soil.

Table 6. Effect of foliar application of Moringa leaf extract (MLE) with different solvents at 4% concentration on the fruit yield (t/ha) of bitter gourd.

Treatments	Total fruit yield (t/ha)
T1- No Application (- Control)	6.17^{b}
T2- AVRDC Recommended Fertilizer Rate + Tapwater spray (+Control)	9.39^{ab}
T3- Recommended Fertilizer Rate + Tapwater spray (+Control)	9.41^{ab}
T4 - Recommended Fertilizer Rate+ MLE (Water Solvent)	10.59^{ab}
T5- Recommended Fertilizer Rate + MLE (Ethanol Solvent)	14.05^{a}
p-value 0.00**	
CV 28.90%	

Note. Means with same letters are not significantly different from each other;
** Highly Significant

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CONCLUSION AND RECOMMENDATION

Foliar application of MLE enhanced the growth and yield performance of bitter gourd. Regarding vegetative growth, the application of MLE led to notable increases in the vine length of the plants despite the lack of statistically significant results. In addition, we utilized MLE as a crop bio-stimulant to significantly accelerate flowering and improve yield and yield components, including an increased number of marketable fruits and fruits per plant, average fruit weight, and fruit yield of bitter gourd.

No significant differences were observed in most studied parameters when comparing water and ethanol as solvents for MLE extraction. This suggests that water and ethanol can be used to extract MLE solvents at 4% concentration without compromising efficacy. This also implies flexibility to farmers based on solvent availability and preference. Thus, applying MLE is a potential alternative to conventional synthetic plant growth stimulators and offers a promising and eco-friendly approach to sustainable vegetable crop production.

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