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ORIGINAL RESEARCH

The difference in leaves production, protein and calcium of *Moringa oleifera* under modification planting media, application of PGR and nitrogen

RINI SULISTIANI[1*], MUKHTAR YUSUF[1], SYAIFUL AMRI SARAGIH[1]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia

*Corresponding Author:
rinisulistiani@umsu.ac.id

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Abstract. Moringa has many ingredients of nutrients that are beneficial for food sources and nutrients that have not been widely cultivated. The nutritional content, benefits and high demand for Moringa abroad will open large opportunities for exporting Moringa flour. Foods full of nutrition will support the maintenance of good public health. For this reason, it is necessary to study and research cultivation techniques that produce high Moringa leaves and can be available sustainably. Production of Moringa leaves as a source of secondary metabolites can be increased by modifying the planting media and applying Plant Growth Regulator (PGR) and Nitrogen. The study used Split Split Plot Design with the main plot immersion by PGR, consisting of 3 types, namely: G_1 (Fresh water), G_2 (Coconut water), and G_3 (GA_3). The subplot was the treatment of planting media with two types: M_1 (soil: sand: manure = 1:1:2); M_2 (soil: sand: manure = 1:2:1). The sub subplots were N (urea) fertilizer, with four levels: N_0 (0 g/plant); N_1 (5 g/plant); N_2 (10 g/plant); and N_3 (15 g/plant). Each treatment combination goes over three times. The agronomic parameters observed were plant height, the number of leaves, fresh crop weight, and root volume, and the biochemical parameters observed were chlorophyll, protein, and calcium levels. The composition of the planting media caused significant differences in plant height at 4, 6, and 10 weeks after planting (WAP), the number of leaves at 4 WAP, and root length at 10 WAP. Growth Regulators significantly affected plant height at 4, 6, and 10 WAP, the number of leaves at 4 WAP, and root length at harvest. Nitrogen fertilization caused significant differences in plant height at 4, 6, and 10 WAP, volume, and root length at harvest (10 WAP). The combination of Planting media, PGR, and Nitrogen treatments caused significant differences in plant height at 4, 6, and 10 WAP and the number of leaves at 6 WAP. Laboratory analysis in this study showed high calcium and protein in Moringa leaves.

Keywords: antioxidants, bioactive compounds, calcium, fertilizer, growth

INTRODUCTION

Moringa oleifera, including the Moringaceae, is a plant that grows [1], has a long life, flowers all year [2], and can resist extreme heat conditions. Moringa is an original plant in tropical and subtropical regions of South Asia. The Moringa, starting from the leaves, bark, fruit, and seeds, has been implemented since the early 1980s. In Ethiopia, Somalia, and Sudan, for a long time, growing Moringa has been a tradition of the people in their daily life as vegetables, medicinal raw materials, and trading materials. In Indonesia, Moringa plants are commonly used as food and medicine.

Moringa can also be used as plants for landslide resistance, soil conservation, and terracing. The Moringa root system is sufficiently tight to prevent landslides so that in the rainy season, even in the most minimal quantity, the Moringa root system can handle it. In the dry season, the water savings around the Moringa roots become a source of water for other plants [3].

Moringa contains very high antioxidants [4] and is very good for diseases related to digestive problems, such as intestinal ulcers and gastric ulcers. Any piece used is safe if you pay attention to the method. Moringa leaf decoction should be drunk while the water is warm because the antioxidant effect is stronger in warm conditions. The contents of anti-nutritional components in its leaves such as tannins, lecithins, and protease. Moringa leaves contain [5] a balanced profile of essential amino acids and are an important source of vitamins A, C and antioxidants.

The problems need to be studied because Moringa is widely used as a hedge or protective plant in fields and

gardens. Moringa has not been widely cultivated. At the same time, it had the potential and benefits as a functional food ingredient to support health. Cultivation has not been encouraged, especially in areas with large, unused, or marginal land. Based on analysis of the highest protein and calcium content (1.956%) in Moringa leaves PT. Socfindo obtained 1.956% using the Dry ashing-HCl analysis method with AAS in North Sumatra, especially Deli Serdang [6].

The specific objectives of the study were to obtain an immersing solution that supports good germination, with the right composition of planting media and efficient nitrogen fertilization to produce maximum leaves. The formulation of the research problem was based on the previous analysis of the highest protein and calcium content of Moringa leaves in the Deli Serdang area, so Moringa seeds were collected from several locations in Sumatera Utara. Further research was conducted on immersing seeds with PGR to get the right solution to stimulate seed germination. The second treatment is the difference in the composition of the planting media because determining the appropriate planting media will support optimal growth. Also, the third treatment with nitrogen fertilization with the right dose will promote maximum and efficient leaf growth.

METHODOLOGY

The research was conducted from March to June 2022 at the research land of the Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara. The altitude is 27 meters above sea level with an average daily temperature of 29.5 ºC, 85% humidity, and an average rainfall of 50-75 mm/month.

The materials used are Moringa seeds (*Moringa oleifera*), urea fertilizer, water, and manure. The tools used are Atomic Absorption Spectrophotometer (AAS, heater, glass funnel, 50 ml and 1000 ml volumetric flask, 50 ml measuring cup, 100 ml beaker, dropper pipette, 1.0 ml volumetric pipette; 1.5 ml and 2.0 ml, measuring pipette 5 ml, watch glass, filter, scale, and desiccator.

Moringa leaves (*Moringa oleifera*), standard solution of calcium, Whatman 40 filter paper, distilled water, tissue, label, concentrated nitric acid (HNO_3), and 37% hydrochloric acid (HCl) were made into HCl (1+1).

The research uses Split Split Plot Design [7]. Where the Main Plot is immersion with PGR consisting of 3 types, namely: G_1 (Fresh water), G_2 (Coconut water), and G_3 (Gibberellins/GA_3). The subplot factor was the treatment of planting media with 2 types: M_1 (Soil: sand: manure = 1:1:2); M_2 (Soil: sand: manure = 1:2:1). The factor of the sub-sub-plots was N (urea) fertilizer, with 4 levels: N_0 (0 kg/ha); N_1 (50 kg/ha); N_2 (100 kg/ha); and N_3 (150 kg/ha). Each treatment combination was repeated three times.

The linear design model is as follows [8]:

$$Y_{ijkl} = \mu + \rho_l + \alpha_i + \gamma_{ij} + \beta_j + (\alpha\beta)_{ij} + \delta_{ijl} + c_k + (\alpha c)_{ik} + (\beta c)_{jk} + (\alpha\beta c)_{ijk} + \varepsilon_{ijkl}$$

Remarks:

Y_{ijkl} = Observed value of the level l experimental unit that received a combination of treatment level i from factor α, level j from factor β, and level k from factor c.
μ = Mean population value.
ρ_l = The main effect of group level l.
α_i = The main effect of factor α level i.
γ_{ij} = The effect of the main plot error.
β_j = The main effect of factor β level j.
$(\alpha\beta)_{ij}$ = The effect of the interaction component of factor α level i and factor β level j.
δ_{ijl} = The effect of the subplot error.
c_k = The main effect of factor c level k.
$(\alpha c)_{ik}$ = The effect of the interaction component of factor α level i and factor c level k.
$(\beta c)_{jk}$ = The effect of the interaction component of factor β level j and factor c level k.
$(\alpha\beta c)_{ijk}$ = The effect of the interaction component of factor α level i, factor β level j and factor c level k.
ε_{ijkl} = The effect of the sub-sub plot error.

The number of samples is 4 of 6 plants per plot, totaling 432 plants.

Implementation of research

Before land preparation, the first activity was to look for materials in the form of seeds taken from the parent tree of Moringa. Land preparation started by measuring the area of the land used in the study; after that, it was cleaned of weeds and plant debris. Cleaning is done manually.

Application of PGR and Nitrogen fertilizers

Seeds immersed in fresh water, coconut water, and GA_3 were applied for 1 hour at the same time before the seeds were germinated. After the seeds are immersed and drained, they are sown in a tray for seven days. Seedlings were transferred from the nursery by watering tray until wet, then planted in polybags. Planting was undertaken in the morning. Urea (N) fertilizer is given to Moringa at 2, 4, and 6 WAP with a predetermined dose concentration.

Maintenance of plant

Plants are watered twice daily in the morning and evening. Plants that died or were damaged were substituted with seedlings of the same age that had been prepared at 5, 7, and 12 DAP (days after planting). Insertion plants were done until 2 WAP. Removing weeds around and in polybags manually and at intervals of once a week to free weeds and keep soil friable. Pest control has done manually by directly taking out pests found in plants. If the pests and diseases exceeded the threshold, chemical control was done. The agronomic parameters observed were plant height, stem diameter, number of leaves, fresh crop weight, and biochemical parameters: protein and calcium content.

Table 1. Moringa plant height by treatment Planting media and Nitrogen (M×N) at 4 WAP

Treatment	Planting media (soil:sand:manure)		Average N
Nitrogen (kg/ha)	M_1 (1:1:2)	M_2 (1:2:1)	
N_0 (0)	19.92[a]	15.93[b]	17.93[a]
N_1 (50)	20.07[a]	15.34[b]	17.71[a]
N_2 (100)	20.00[a]	14.76[b]	17.38[a]
N_3 (150)	22.82[a]	14.56[b]	18.69[a]
Average M	20.70[a]	15.15[b]	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

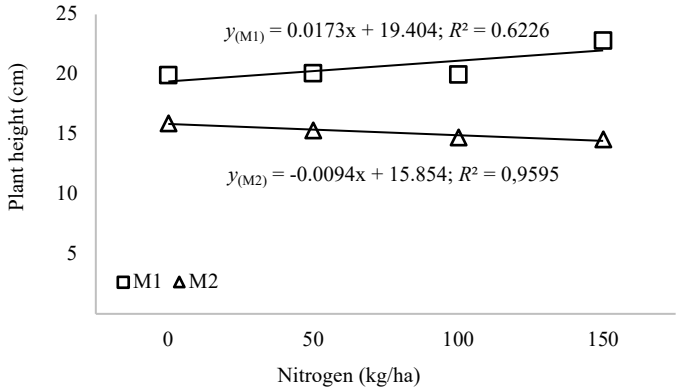

Figure 1. The relationship between plant height with interaction of Planting media and Nitrogen at 4 WAP. Note: M_1 (soil: sand: manure = 1:1:2); M_2 (soil: sand: manure = 1:2:1)

The procedure of protein analysis

Each sample of Moringa leaves was weighed 100 grams and put in a beaker, adding 1000 ml of distilled water. Puree with a blender, then filter with filter paper. Protein content was measured by taking 0.9 ml of protein sample and precipitating it by adding ammonium sulfate. The precipitated protein was centrifuged for 10 minutes and separated from the clear part (supernatant). The precipitate, protein, is then Re-dissolved with acetic acid buffer pH 5 to 10 ml. In each test tube, 0.9 ml of the sample was added, 0.8 ml of Biuret reagent was added, and 1.3 ml of acetic acid buffer solution was added. Let stand for 10 minutes,and read the absorbance at the maximum wavelength.

The Procedure of calcium analysis

Preparation of samples

The sample was crushed in a mortar until homogeneous, then 5 grams of the sample was weighed using a Petri dish. Furthermore, the Petri dish was put into the furnace and heated at 600 °C until all the carbon was gray. Furthermore, the Petri dish was taken from the furnace and cooled, then the sample was put into a beaker glass and 20 ml of HCl (1+1) was added. After that, the beaker containing the sample was heated to boiling for 10 minutes and covered with a watch glass. Then the beaker glass is taken from the heater and cooled, then 20 ml of distilled water is added to the beaker. The solution was filtered using filter paper, then the filtration results were put into a 50 ml volumetric flask and the residue left in the cup was rinsed 2 times with distilled water. The residue left on the filter paper is washed using distilled water and diluted to the mark using distilled water (9).

Preparation of calcium (Ca) standard solution

Pipette 5 ml of 1000 mg/L calcium liquor and put the solution into a volumetric flask, then add 2% HNO_3 diluent solution to get 50 ml and homogenize to obtain a standard calcium solution of 100 mg/L.

Preparation of calcium solution

Pipette 0.5; 1.0 ml; 1.5 ml; and 2.0 ml of 100 mg/L calcium standard solution each into a 50 ml volumetric flask and then add a 2% HNO_3 diluent solution until the tera mark is right then homogenized to obtain a calcium content of 1.0 mg/L; 2.0 mg/L; 3.0 mg/L and 4.0 mg/L.

$$Ca = \frac{Conc - Bl \times Vol \times fp}{Sample\ weight}$$

Remarks:

Ca = levels obtained from measurement results (µg/g)
Conc = concentration (µg/ml)
Bl = Blanko (µg/ml) (if the blanko level used is minus (-), then the blanko level is considered zero)
fp = dilution factor
Vol = volume of sample solution (ml) Weight of sample (g)

Procedure

Optimizing the Atomic Absorption Spectrophotometer (AAS) according to the instructions for using the tool, then measuring the absorption of 2% HNO_3 as a blanko, measuring the absorption of each working solution that has been made at a wavelength of 422.7 nm and continuing with the measurement of the test samples that have been prepared.

Table 2. Plant height (cm) of Moringa due to interaction of Planting media, PGR, and Nitrogen fertilizer (M×G×N) at 4 WAP

Treatment	Nitrogen (N)				Average M×G
Planting media × PGR	N_0	N_1	N_2	N_3	
M_1G_1	22.25^{a-d}	24.42^{a}	21.67^{a-e}	23.92^{ab}	23.06^{a}
M_1G_2	22.58^{abc}	22.53^{abc}	22.17^{a-d}	23.17^{abc}	22.61^{a}
M_1G_3	14.92^{gh}	13.25^{gh}	16.17^{e-h}	21.38^{a-f}	16.43^{b}
M_2G_1	14.50^{gh}	16.47^{d-h}	15.70^{fgh}	18.00^{c-g}	16.17^{b}
M_2G_2	15.00^{gh}	15.42^{gh}	14.38^{gh}	11.92^{h}	14.18^{b}
M_2G_3	18.30^{b-g}	14.15^{gh}	14.18^{gh}	13.77^{gh}	15.10^{b}
Average N	17.93^{a}	17.71^{a}	17.38^{a}	18.69^{a}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Table 3. The Moringa height caused Planting media and PGR immersion (M×G) at 10 WAP.

Plant Growth Regulator	Planting media (soil:sand:manure)		Average G
	M_1 (1:1:2)	M_2 (1:2:1)	
G_1 (Fresh water)	67.58^{ab}	58.67^{bc}	63.13^{a}
G_2 (Coconut water)	69.33^{a}	56.63^{c}	62.98^{a}
G_3 (Gibberellins/GA_3)	60.50^{abc}	62.00^{abc}	61.25^{a}
Average M	65.81^{a}	59.10^{a}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Analysis of data

Analysis of agronomic data from observations with analysis of variance (F test) continued with the DMRT (Duncan's Multiple Range Test). Data were analyzed with SPSS ver. 24 and continued by the regression.

RESULTS AND DISCUSSION

Vegetative component

Plant media caused differences in the Moringa plant height at 4 WAP. Meanwhile, nitrogen fertilization had no significant effect. However, the combination of planting media and nitrogen (M×N) caused interactions, therefore to occur the differences in plant height (Table 1).

Based on Table 1, planting media M_1 showed better fertility, where the rate for Moringa growth was faster than the composition of M_2. The composition of soil:sand:manure media with a ratio of 1:1:2 (M_1) produced significantly different plant heights compared to the combination of soil:sand:manure media with a composition ratio of 1:2:1 (M_2) for all doses of nitrogen. But the application of nitrogen singly showed no difference in plant height. The difference in plant height in both compositions of planting media can be seen in Figure 1.

The difference in plant growth (Figure 1) shows the interaction of planting media (M) with nitrogen causing different growth patterns. The combination of M_1 planting media with nitrogen tends to increase plant height along with a rising nitrogen supply. Meanwhile, the combination of M_2 with nitrogen tends to decrease plant height as the amount of fertilizer applied increases. Plant height growth is quite closely related to any interaction of planting media and nitrogen.

The interaction of three combinations of planting media, PGR and Nitrogen (M×G×N) caused significant differences in plant height at 4 WAP. The difference in plant height is due to the three treatments (Table 2).

The combination of $M_1G_1N_1$ treatments resulted in the highest plant Moringa, namely a combination of seed-immersed treatment with fresh water that was planted on M1 media and fertilized 50 kg/ha N. It was significantly different in height with $M_1G_3N_{0\text{-}2}$, $M_2G_1N_{1\text{-}3}$, $M_2G_2N_{0\text{-}3}$, and $M_2G_3N_{0\text{-}3}$. However, it was not significantly different from $M_1G_1N_{0,2,3}$, $M_1G_2N_{0\text{-}3}$, and $M_1G_3N_3$.

At 10 WAP, the interaction of plant media and PGR caused of significant effect on the height of Moringa. However, each treatment of plant media and PGR affected no significance in plant height (Table 3).

The interaction resulting from the combination of plant media and PGR (M×G) caused the plant height to be significantly different at 10 WAP. The combination of treatments M_1G_2 was significantly different in plant height compared to M_2G_1 and M_2G_2. PGR and Nitrogen treatment caused differences in plant height at 10 WAP (Table 4).

The interaction between PGR and Nitrogen had a significantly different effect on plant height, where immersing the seeds with coconut oil without nitrogen fertilization was significantly higher than other treatments.

The relationship of plant height due to the interaction of PGR and nitrogen fertilization at 10 WAP can be seen in Figure 2.

Based on Figure 2, plant height growth increased in seeds immersed in fresh water and GA_3 as the nitrogen fertilizer dose increased, although the relationship was weak. However, immersing the seeds in coconut solution and increasing the nitrogen dose will reduce plant height with a relatively strong interplay. Based on the quadratic equation $y_{(G2)} = 0.0012x^2 - 0.2873x + 108.43$, a minimum plant height of 91.23 cm is acquired by applying 119.71 kg/ha of Nitrogen in G_2 immersion. While in the G_1 and G_3 immersion, the requirement for Nitrogen tends to increase (linear equation).

Table 4. Moringa plant height that received PGR and Nitrogen (G×N) treatment at 10 WAP

Treatment Nitrogen (kg/ha)	Plant Growth Regulator			Average N
	G_1 (Fresh water)	G_2 (Coconut water)	G_3 (GA_3)	
N_0 (0)	96.83^{ab}	107.50^{a}	97.75^{ab}	100.69^{a}
N_1 (50)	98.08^{ab}	99.92^{ab}	96.58^{ab}	98.19^{a}
N_2 (100)	101.08^{ab}	89.25^{b}	99.58^{ab}	96.64^{a}
N_3 (150)	100.58^{ab}	94.00^{ab}	98.67^{ab}	97.75^{a}
Average G	99.15^{a}	97.67^{a}	98.15^{a}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

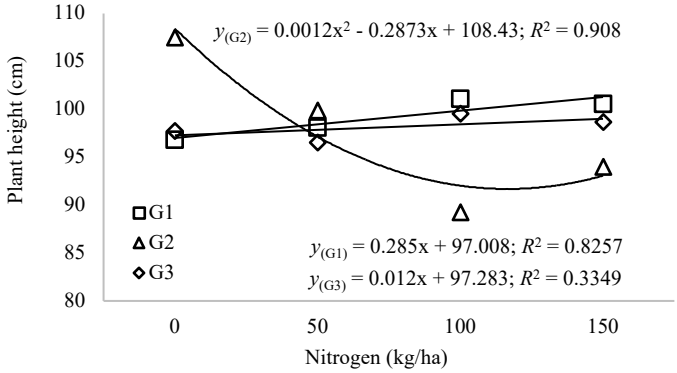

Figure 2. Relationship of plant height due to the interaction of PGR and nitrogen fertilization at 10 WAP

Table 5. The stem diameter (mm) of Moringa received Planting media and PGR (M×G) treatment at 4 WAP

Plant Growth Regulator	Planting media (soil:sand:manure)		Average G
	M_1 (1:1:2)	M_2 (1:2:1)	
G_1 (Fresh water)	3.00^{a}	1.96^{a}	2.48^{a}
G_2 (Coconut water)	2.48^{a}	1.58^{a}	2.03^{a}
G_3 (Gibberellins/GA_3)	1.78^{a}	1.80^{a}	1.79^{a}
Average M	2.42^{a}	1.78^{b}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Observation of stem diameter at 4 weeks after planting (WAP) showed that the effect of planting media and nitrogen fertilization was not significantly different. The average plant height data can be seen in Table 5.

Based on Table 5, the application of planting media resulted in differences in plant diameters, where Moringa planted on M_1 (soil:sand:manure ratio 1:1:2) was significantly larger in diameter than plants grown on M_2 (sand:soil:manure) with a ratio of 1:2:1. The PGR independently and the interaction between the planting media and PGR gave a more large diameter that was not significantly different. The combination of PGR, planting media, and nitrogen fertilizer (M×G×N) resulted in differences in stem diameter (Table 6).

Table 6 shows the combination of planting media, PGR, and nitrogen fertilization (M×G×N) treatments that resulted in differences in stem diameter. The treatment combination $M_1G_1N_0$ gave the largest diameter and significantly differed from $M_1G_3N_{0\text{-}3}$, $M_2G_1N_{0\text{-}3}$, $M_2G_2N_{0\text{-}3}$, and $M_2G_3N_{0\text{-}3}$. M×G×N had the same effect on the diameter for the rest of the treatment combinations compared to $M_1G_1N_0$. The effect of planting media and PGR to stem diameter causing differences at 8 WAP can be seen in Table 7.

The treatment of M_1 planting media resulted in significantly different stem diameters compared to plants grown on M_2. The various PGR resulted in the same effect of diameter. The interactions that occurred due to the combination of planting media and PGR resulted in significant differences in stem diameter at 8 WAP. Table 7 shows that plants on M1 planting media combined with fresh water and coconut water treatments (M_1G_1, M_1G_2) produced significantly different stem diameters compared to M_2G_2.

The number of petioles significantly differed due to the independent treatment of the planting media and PGR. While the treatment combination of both causes a difference in the number of petioles at 4 WAP (Table 8).

Based on Table 8, the M_1 planting medium has significantly more petioles than M_2. Treatment of seeds with fresh water (G_1) and coconut water (G_2) had the same effect on the number of petioles, but both had significantly different numbers of petioles compared to plant seeds immersion with GA_3 (G_3).

Based on Table 9, the combination of planting media, plant growth regulators, and nitrogen (M×G×N) caused differences in stem diameter. The largest diameter was found in the $M_2G_2N_1$ treatment and significantly different from $M_2G_3N_2$ and $M_1G_3N_0$ but not significantly different from other treatment combinations.

Fresh crop weight was not significantly affected. The treatment of planting media and the interaction of

Table 6. The Moringa's stem diameter (mm) is due to the interaction of PGR, Planting media, and Nitrogen fertilizer (M×G×N) at 6 WAP

Treatment	Nitrogen (N)				Average M×G
Planting media × PGR	N_0	N_1	N_2	N_3	
M_1G_1	7.98^{a}	6.33^{a-e}	6.75^{ab}	6.28^{a-f}	6.84^{a}
M_1G_2	6.40^{abc}	6.40^{abc}	6.05^{a-h}	6.08^{a-g}	6.23^{a}
M_1G_3	3.22^{ij}	3.68^{ghi}	4.13^{c-j}	4.68^{b-j}	3.93^{a}
M_2G_1	4.30^{b-j}	5.10^{b-j}	4.98^{b-j}	5.17^{b-i}	4.89^{a}
M_2G_2	4.40^{b-j}	4.47^{b-j}	3.45^{ij}	4.00^{c-j}	4.08^{a}
M_2G_3	4.92^{b-j}	4.88^{b-j}	3.62^{ghi}	3.75^{ghi}	4.29^{a}
Average M	5.20^{a}	5.14^{a}	4.83^{a}	4.99^{a}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Table 7. The stem diameter (mm) of Moringa in Planting media and PGR (M×G) treatment at 8 WAP

Plant Growth Regulator	Planting media (soil:sand:manure)		Average G
	M_1 (1:1:2)	M_2 (1:2:1)	
G_1 (Fresh water)	10.92^{a}	9.18^{ab}	10.05^{a}
G_2 (Coconut water)	10.97^{a}	8.55^{b}	9.76^{a}
G_3 (Gibberellins/GA_3)	9.13^{ab}	9.50^{ab}	9.32^{a}
Average M	10.34^{a}	9.08^{b}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Table 8. The number of petioles (stalks) of Moringa caused the interaction of Planting media and PGR (M×G) at 4 WAP

Plant Growth Regulator	Planting media (soil:sand:manure)		Average G
	M_1 (1:1:2)	M_2 (1:2:1)	
G_1 (Fresh water)	6.50^{a}	5.92^{ab}	6.21^{a}
G_2 (Coconut water)	6.63^{a}	5.42^{bc}	6.02^{a}
G_3 (Gibberellins/GA_3)	4.88^{c}	5.58^{abc}	5.23^{b}
Average M	6.00^{a}	5.64^{b}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

planting media and nitrogen fertilizer (M×N) did not significantly affect fresh crop weight at 10 WAP (Table 10).

Although Nitrogen and planting media did not significantly affect fresh weight, applied Nitrogen 150 tons/ha (N_3) resulted in a fresh weight of 205.42g. Cultivated Moringa at media M_1 obtained the largest fresh weight of 235.29g.

Analysis of biochemical compounds

The results of laboratory analysis of protein content were significantly different due to the treatment of planting media and immersing the seeds with growth regulators (Table 11).

The treatment of planting media caused no significant difference in the protein content of Moringa leaves at harvest. The application of growth regulators caused differences in protein content, where immersion in fresh water resulted in the highest protein content and significantly differed from seeds immersed in coconut water. Still, the protein content was not significantly different from the GA_3 treatment.

The combination of planting media and PGR resulted in differences in protein content. The highest protein content was found in the group of plants treated with M_2G_1, which immersed the seeds in fresh water and planted in soil:sand:manure (1:2:1), which was significantly different from the combination of M_1G_2, M_1G_3, and M_2G_2 treatments.

The preliminary test of the calcium content of Moringa leaves as a composite and without treatment was 1.956% using the Dry Ashing-HCl with AAS analysis method. The results of laboratory analysis of calcium content by treatment of planting media and Nitrogen at 10 WAP showed results that were not significantly different (Table 12).

Although the results of the analysis of calcium levels due to planting media and Nitrogen at 10 WAP were not significantly different. But, the results of the analysis of calcium levels were higher with an average of 2.13% after getting treatment than before treatment at 1.956%.

Planting media treatment affected the vegetative growth of plants during the nursery at the age of 4-10 WAP. Differences in the planting media composition caused significant differences in plant height, stem diameter, and number of Moringa leaves at 4, 6, and 10 WAP. Planting media with soil:sand:manure composition (1:1:2) gave higher plant growth compared to soil:sand:manure composition (1:2:1). The difference in plant height was due to the planting media with a composition of 2 parts fertilizer, resulting in a more loose and fertile soil structure so that microbial activity in the soil was better.

Improved fertility will provide suitable soil conditions for plant growth. Soil moisture and temperature become stable, making it easier for plants to absorb nutrients. This condition will make nutrient availability in the soil more accessible, so plant roots absorb more. Adding organic matter such as manure can improve soil structure to become more crumbly and increase water capacity so that drainage is not excessive [10]. Utilization of manure will enrich microorganisms [11] such as rhizobacteria around plant roots. Rhizobacteria are capable of

Table 9. Moringa stem diameter resulted in the interaction of PGR, Planting media, and Nitrogen fertilizer at 6 WAP

Treatment	Nitrogen (N)				Average M×G
Planting media × PGR	N_0	N_1	N_2	N_3	
M_1G_1	8.00^{abc}	9.83^{ab}	8.67^{abc}	8.50^{abc}	8.75^{a}
M_1G_2	9.50^{ab}	8.83^{abc}	8.83^{abc}	9.00^{abc}	9.04^{a}
M_1G_3	7.50^{bc}	9.00^{abc}	8.50^{abc}	8.33^{abc}	8.33^{a}
M_2G_1	8.50^{abc}	7.67^{abc}	9.00^{abc}	8.17^{abc}	8.33^{a}
M_2G_2	8.00^{abc}	10.33^{a}	8.33^{abc}	7.67^{abc}	8.58^{a}
M_2G_3	7.83^{abc}	8.33^{abc}	6.33^{c}	8.33^{abc}	7.71^{a}
Average M	8.22^{a}	9.00^{a}	8.28^{a}	8.33^{a}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Table 10. Fresh weight of Moringa plants treated with Nitrogen fertilizer and Planting media at 10 WAP

Treatment	Planting media (soil:sand:manure)		Average N
Nitrogen (kg/ha)	M_1 (1:1:2)	M_2 (1:2:1)	
N_0 (0)	251.67^{a}	85.67^{a}	168.67^{a}
N_1 (50)	208.17^{a}	143.17^{a}	175.67^{a}
N_2 (100)	246.17^{a}	91.67^{a}	168.92^{a}
N_3 (150)	235.17^{a}	175.67^{a}	205.42^{a}
Average M	235.29^{a}	124.04^{a}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Table 11. Protein content (%) of Moringa leaves treated by PGR and Planting media at 10 WAP

	Planting media (soil:sand:manure)		Average G
Plant Growth Regulator	M_1 (1:1:2)	M_2 (1:2:1)	
G_1 (Fresh water)	3.048^{abc}	4.870^{a}	3.956^{a}
G_2 (Coconut water)	2.201^{bc}	0.987^{c}	1.596^{b}
G_3 (Gibberellins/GA_3)	1.770^{bc}	3.723^{ab}	2.746^{ab}
Average M	2.341^{a}	3.192^{b}	

The numbers followed by the same letter show no significant difference at 5% of DMRT.

Table 12. Calcium content (%) of Moringa leaves treated by Planting media and Nitrogen (M×N) at 10 WAP.

Treatment	Planting media (soil:sand:manure)		Average N
Nitrogen (kg/ha)	M_1 (1:1:2)	M_2 (1:2:1)	
N_0 (0)	1.95^{a}	2.72^{a}	2.34^{a}
N_1 (50)	2.21^{a}	1.72^{a}	1.95^{a}
N_2 (100)	1.87^{a}	2.61^{a}	2.24^{a}
N_3 (150)	2.15^{a}	1.83^{a}	1.99^{a}
Average M	2.04^{a}	2.22^{a}	2,13

The numbers followed by the same letter show no significant difference at 5% of DMRT.

producing IAA hormones which will increase seedling growth.

Stem diameter was affected by the planting media and significantly different between the composition of M_1 compared to M_2 at 4 and 8 WAP. The number of leaves was significantly different between groups of Moringa grown on M_1 and M_2. In general, the increase in stem diameter between Moringa grown on M_1 media was greater than that of Moringa grown on M_2 because, based on the composition of the media, M_1 contained more manure than M_2. It is necessary to fertilize or add organic matter to increase soil fertility and crop production [12]. Application 3.6 kg/plot of cow manure [13] produced the heaviest fruit weight of cucumber at 0.74 kg/plot. Plants need nutrients in growth processes, such as physiological processes and the formation of plant structures [14].

The improvement of available groundwater pores is most likely a factor that can provide better yields for production, especially those grown on marginal lands [15]. Soil fertility is a determining medium for plant growth to available nutrients in the soil. Soil fertility is specific both in terms of location and plants. It means that fertile soil for one crop is not indeed fertile for another. A broader concept is soil productivity which is related to the ability of the soil to sustain plant growth [16].

Growth Regulators significantly affected plant height at 4, 6, and 10 WAP, the number of leaves at 4 WAP, and root length at 10 WAP. Meanwhile, stem diameter and root volume were not significantly different due to PGR.

Growth Regulator play vital roles in a plant's life from dormancy to senescence so it's an important field in agriculture. PGR under optimal conditions allows certain hormones to work actively of stretch in the cell wall [17]. Cell-stretching hormones stimulate cells to elongate and cell walls to grow thicker. This elongated and thickened cell wall occurs due to the accumulation of additional cellulose made of sugar. They are triggering agents or substances because they initiate a biochemical process that ultimately leads to growth. It will accelerate the growth of stems, leaves, and root systems. The application of the hormone IAA significantly affected the number of leaves and plantlet height of the *Chrysanthemum* in vitro [18].

The concentration of PGR was not significantly different for all plant vegetative parameters [19]. It was due to the positive response of plants to the application of growth regulators. It is influenced by several factors, including the type of plant, the phase of plant growth, the type of growth regulator, concentration, and the method of application of growth regulators [20].

Coconut water contains auxin, cytokinins and gibberellins, which stimulate root and shoot growth [21; 22]. Cytokinins found in coconut water support cell division, and together with other chemical components promote plant growth [23].

The optimal giving of GA_3 can accelerate the process of plant stem elongation [24] stated the optimal giving of GA_3 can accelerate the process of plant stem elongation. Besides giving GA_3 encourages gibberellins also interact with endogenous auxins. The content of endogenous auxin causes cell elongation reactions and can increase plant height.

Nitrogen fertilization caused significant differences in plant height at 4, 6 and 10 WAP, volume and root length at harvest (10 WAP). The number of leaves and stem diameter were not significantly different due to nitrogen treatment. The combination of Planting media, PGR and Nitrogen treatments caused significant differences in plant height at 4, 6 and 10 WAP and the number of leaves at 6 WAP. Meanwhile, stem diameter, length and volume of roots were not significantly different due to the effect of the combination of MGN treatment.

The Plant height will increase along with the addition of N nutrients over time [25]. Nitrogen is a component of amino acids, nucleic acids, and chlorophyll. Although the mechanism is unclear, it is possible that at lower temperatures, it will quickly convert ammonia nitrogen into nitrate available to plants [26]. Nitrogen (N) is an abundant element on earth and plays an essential role in modern agriculture [27]. N shapes organ construction, material metabolism, yield, and fruit quality [28].

Sufficient N application will increase the photosynthetic efficiency of leaves, flowers, and fruit [29;30]. However, N application should not be excessive because it will increase production costs, and reduce yield and fruit quality [31]. Excess N application also caused negative ecological effects, including increased N deposition, intensification of the greenhouse effect, soil acidification, and eutrophication of water bodies [32;33]. Element N is an important ingredient in amino acids and an essential for cell division, cell enlargement and plant growth. N is needed in large quantities in every plant growth, especially at the vegetative growth stage, such as increasing the number of leaves. The application of biofertilizers can increase the total dry weight of plants, nutrient absorption, and secondary metabolite content. The content of metabolic compounds is influenced by fruit weight and plant nitrogen uptake [34].

CONCLUSION

The composition of the planting media caused significant differences in plant height at 4, 6 and 10 WAP, the number of leaves at 4 WAP, and root length at 10 WAP. Plant Growth Regulators (PGR) significantly affect plant height at 4, 6 and 10 WAP, the number of leaves at 4 WAP and root length at harvest. Nitrogen fertilization caused significant differences in plant height at 4, 6 and 10 WAP, volume and root length at harvest (10 WAP). The combination of Planting media, PGR and Nitrogen treatments caused significant differences in plant height at 4, 6 and 10 WAP and the number of leaves at 6 WAP. The research, furthermore, could be done by giving a higher dose of nitrogen fertilizer, and the composition of the planting media is more than two combinations. The best composition for growth and leaf production in this study was M_1 (soil:sand:manure = 1:1:2)

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