Jurnal Agronomi Indonesia
(Indonesian Journal of Agronomy)

ISSN 2085-2916 | e-ISSN 2337-3652
December 2024, *52*(3), 340-348
DOI: https://dx.doi.org/10.24831/jai.v52i3.60164

Research Article

Plant growth, fruit production, and total terpenoid production in bitter melon (*Momordica charantia* L.) with guano fertilizer application

Anna Moslihat Jamil [1], Maya Melati [2, 3,*], and Sandra Arifin Aziz [2,3]

[1] Agronomy and Horticulture Study Program, Graduate School of IPB University (Bogor Agriculture University), Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, INDONESIA

[2] Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University (Bogor Agriculture University), Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, INDONESIA

[3] Tropical Biopharmaca Research Center, IPB University (Bogor Agricultural University), Bogor 16680, INDONESIA

* Corresponding author (✉ maya_melati@apps.ipb.ac.id)

Edited by:
Ahmad Rifqi Fauzi
Trilogy University

Received:
4 November 2024
Accepted:
18 December 2024

Citation:
Jamil, A. M., Melati, M., & Aziz. S. A. (2024). Plant growth, fruit production, and total terpenoid production in bitter melon (*Momordica charantia* L.) with guano fertilizer application. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, *52*(3), 340-348

ABSTRACT

Bitter melon (Momordica charantia L.) is valued for its anti-diabetic properties due to terpenoid compounds like charantin, but production struggles to meet market demands. This study aimed to analyze the response of plant growth characteristics, fruit production, and terpenoid compound production in bitter melon fruit by improving phosphorus availability through guano fertilizer application as additional organic fertilizer in organic cultivation. The experiment was conducted from May to September 2024 at the IPB experimental station in Cikarawang, Bogor, Indonesia. The experiment used a randomized complete block design with varying guano fertilizer doses (0, 152, 304, and 456 g per plant). Observations were conducted on 10 sample plants per experimental unit. The observed characteristics included vegetative and reproductive growth, fruit production, and total terpenoid production. Terpenoid analysis was performed using a colorimetric method with an ELISA reader. The results showed that guano fertilizer significantly affected several plant growth and production variables. Higher doses increased several values of vegetative growth parameters, flower numbers, and fruit production. The 304 g guano per plant yielded the highest concentration and production of terpenoids. This study demonstrates the potential of guano fertilizer to enhance bitter melon growth, yield, and terpenoid content, contributing to improved production to meet market demands.

Keywords: net assimilation rate; organic; phosphorus; relative growth rate

INTRODUCTION

Bitter melon (*Momordica charantia* L.) is a functional vegetable rich in secondary metabolites (Rahmasari & Wahyuni, 2019), such as terpenoids, phenolics, flavonoids, and alkaloids (Chaudhury, 2023). Terpenoids are the main group of natural compounds that have proven anti-diabetic properties. Terpenoids, particularly charantin, are known for their ability to normalize blood sugar levels by countering vascular dysfunction associated with diabetes (Putta et al., 2016). Despite its high economic potential, cultivation at the farmer level is still conducted conventionally and suboptimally, failing to meet the demand for both vegetables and traditional medicine in terms of quality and quantity (Novi, 2015).

Plant growth must be optimized through proper fertilization to enhance terpenoid compound production in bitter melon fruit (Suwandi et al., 2015). Organic cultivation is highly recommended as a functional vegetable with potential as a source of traditional medicine (Melati et al., 2021). The organic approach seeks to reduce chemical use, enhance soil fertility, and promote environmentally friendly, sustainable agriculture (Suwandi et al., 2015). Although secondary metabolite production generally increases under stress, research on Orange Jessamine plants has shown that appropriate use of organic fertilizers significantly increases secondary metabolite production. This increase positively correlates with better plant growth and increased biomass, indicating a direct relationship between organic nutrition, plant development, and biosynthesis of bioactive compounds (Taufika et al., 2016).

Chicken manure is one of the organic fertilizers that is commonly used for crop production. Several benefits of chicken manure are due to its easy decomposition and high phosphorus content (Melati et al., 2021). This fertilizer contains C-organic of 17.48%, total nitrogen of 1.43%, P_2O_5 of 5.26%, and K_2O of 3.70% (Jabary et al., 2023). This composition indicates that chicken manure has a higher nutritional value compared to other manures (Ritonga et al., 2022). Previous research has shown the positive impact of chicken manure application on the growth and yield of okra plants (Fhonna et al., 2023). Other research results also showed a significant increase in productivity with the application of chicken manure at 30 tons ha^{-1}, which increased productivity up to 9.09 tons ha^{-1} of bitter melon (Jabary et al., 2023). In an effort to improve the production of terpenoids, which are secondary metabolites, additional sources of phosphorus may be needed in addition to chicken manure. Guano is an organic fertilizer that is commonly used as a source of phosphorus. Studies demonstrate guano's potential to improve soil nutrient profiles by enhancing N, P, and K levels in gold mining tailings (Syofiani & Oktabriana, 2017), increasing secondary metabolite content in *Physalis angulata* (Suhartono et al., 2023), and optimizing phosphorus availability and soil pH in podsolic soil (Puspitasari et al., 2023).

One factor expected to influence the production of secondary metabolite compounds in bitter melon fruit is phosphorus availability. Phosphorus availability in soil plays a crucial role in supporting plant growth and secondary metabolite compound production. Phosphorus plays a crucial role in the terpenoid biosynthesis pathway by functioning as an essential component in organic molecules such as nucleic acids, phospholipids, ATP, and NADPH. Additionally, phosphorus plays a vital role in a series of phosphorylation reactions that help activate terpenoid precursors (Bustamante et al., 2020). Guano fertilizer is an organic fertilizer that serves as a phosphorus source with a composition of 9.47% P_2O_5, which has a higher phosphorus content compared to agricultural waste and manure. Research has shown that guano fertilizer, with its high phosphorus content, has the potential to increase phosphorus availability in soil and enhance plant growth and yield (Lukman, 2022). Previous research has demonstrated that using phosphorus fertilizer can significantly impact the biosynthesis of secondary metabolite compounds in *Vitex negundo* Linn plants (Peng & Ng, 2022). However, to date, there have been no reports regarding using guano fertilizer to increase terpenoid compound production in bitter melon fruit.

This study aimed to analyze the response of plant growth characteristics, fruit production, and terpenoid compound production in bitter melon fruit by applying guano fertilizer. The research problem to be investigated was determining the optimal dose of guano fertilizer to increase phosphorus availability in soil and total terpenoid compound production in bitter melon fruit. The research provides critical benefits by offering recommendations for optimal guano fertilizer dosage to enhance phosphorus availability in soil, optimize terpenoid compound production, and develop organic fertilization strategies that improve bitter melon fruit quality and quantity.

MATERIALS AND METHODS

The experiment was conducted at the IPB Experimental Station in Cikarawang A, Dramaga, at coordinates 6°33'30" S, 106°43'43" E, at an elevation of 160 m above sea level in Bogor, West Java, Indonesia, from May to September 2024. The experiment used a randomized complete block design (RCBD) with one factor: variations in guano fertilizer doses. The recommended dose from PT. East West Seed Indonesia was 76 g guano per plant (100%). The treatments consisted of four different total doses across three applications: 0 g per plant (0% of recommended dose), 152 g per plant (50% of recommended dose), 304 g per plant (100% of recommended dose), and 456 g per plant (150% of recommended dose). Each treatment was replicated 4 times, resulting in 16 experimental units, with each unit comprising 24 plants.

Land preparation began with soil cultivation using a cultivator. Subsequently, beds were formed with dimensions of 9 m long, 1 m wide, and 0.3 m high, with 0.5 m spacing between beds and 1 m spacing between replications. Each bed was made with 24 planting holes at a spacing of 0.5 m x 0.75 m. Two weeks before transplanting, each planting hole received 1.1 kg of chicken manure (30 tons ha^{-1}), 75 g of agricultural lime/dolomite (2 tons ha^{-1}), and guano fertilizer according to treatment doses (0; 38; 76; and 114 g per planting hole). Mulching was then applied, and planting holes were prepared according to the specified planting distance.

Guano fertilizer was applied in three stages. The first stage was conducted two weeks before planting with doses of 0, 38, 76, and 114 g of guano per plant. The second stage was carried out at the beginning of the generative phase with the same doses. The third stage was at the first harvest, with doses doubled to 0, 76, 152, and 228 g of guano per plant. This dose increase was implemented to enhance harvest yields (Nisaa et al., 2017).

Vegetative and reproductive characteristics were observed on 10 sample plants per experimental unit. Reproductive variables included leaf number and stem length (cm), measured at 2, 3, and 4 weeks after planting (WAP), relative growth rate (RGR), and net assimilation rate (NAR).

- RGR (g.week^{-1}) = $\frac{\text{InW2} - \text{InW1}}{\text{t2} - \text{t1}}$
- NAR (g.cm^{-2}. week^{-1}) = $\frac{\text{W2} - \text{W1}}{\text{A2} - \text{A1}}$ X $\frac{\text{InW2} - \text{InW1}}{\text{t2} - \text{t1}}$

Description:

W1 and W2 = Plant dry weight at the 1st and 2nd observations, respectively
t1 and t2 = Plant age at the 1st and 2nd observations
A1 and A2 = Leaf area of plants in the 1st and 2nd observations

Reproductive traits encompass flowering time and flower number. Fruit production was assessed by fruit number per plant, weight per fruit, fruit weight per plant, fruit moisture content, and dry fruit weight. Leaf nutrient analysis (N, P, K) was conducted at 4 WAP (just before the reproductive phase began), while fruit P analysis was performed on the fourth harvest (at peak harvest). Leaf N analysis was conducted using the Kjeldahl method. P and K were extracted using the wet ash method using HNO_3 and $HClO_4$. P levels were determined using a spectrophotometer, while K was determined using an atomic absorption spectrophotometer (AAS).

Terpenoid analysis followed the method of Lukowski et al. (2022), using a colorimetric approach with an ELISA reader to determine total terpenoid content. Samples were collected from the fourth harvested fruit, sliced, and then dried using two methods: an oven and a dehydrator. One gram of dried sample (simplicia) was weighed and mixed with 14 mL of cold 95% methanol. The sample was then incubated for 48 hours at room temperature. Following incubation, the sample was centrifuged at 4,000 rpm for 15 minutes at 25 °C. The resulting supernatant was transferred to a test tube, and 1.5 mL of chloroform was added and homogenized using a vortex. Subsequently, 100 µl of 10% H_2SO_4 was added to the sample and incubated for 1.5 hours at room temperature. After this, 1.5 mL of 95% methanol was added, and the sample was again homogenized with a vortex until the precipitation was completely dissolved. The sample was then analyzed using an ELISA reader at a wavelength of 520 nm. Terpenoid content was determined using a nerol standard curve at various concentrations (0.150-0.050 mg NE/µl methanol). Terpenoid production (mg plant^{-1}) was calculated by multiplying the dry weight (simplicia weight per plant) by the terpenoid content (mg g^{-1} simplicia).

Data were analyzed using an F-test at a 5% significance level, followed by Duncan's multiple range test (DMRT). Statistical analysis was performed using Microsoft Excel and R Studio software.

RESULTS AND DISCUSSION

Vegetative growth phase

The application of 152 g guano fertilizer per plant resulted in a 37% increase in plant height compared to unfertilized plants ($P<0.05$) (Table 1) 3 weeks after planting (WAP). This increase in plant height was consistent with the number of leaves at 4 WAP, which also showed a 63% higher result compared to unfertilized plants at the same dose. This occurred because guano fertilizer improved nitrogen availability, which plays a crucial role in cell division and enlargement that directly contributes to increased plant height and leaf number (Gu et al., 2018). Guano fertilizer also increases soil organic matter content, which plays an important role in improving soil structure and increasing water-holding capacity, thus supporting optimal root growth and nutrient uptake (Gerke, 2022). At 4 WAP, although plant height did not show significant differences, leaf number differed significantly between the 152 g treatment and the control. However, increasing guano fertilizer doses above 152 g per plant showed no further increase in plant height and leaf number parameters, indicating that this dose was enough for the vegetative growth of the plant.

Table 1. Stem length and leaf number at 2, 3, and 4 WAP with variations in guano fertilizer doses

Treatment (g guano per plant)	Stem length (cm)			Leaf number		
	Plant age (weeks after planting)					
	2	3	4	2	3	4
0	34.4±3.9	74.9±4.9ab	134.9±8.5	10.2±0.5	22.4±1.0b	50.5±3.8b
152	41.1±5.2	102.7±9.3a	170.0±14.8	11.4±0.8	27.9±1.6a	82.3±12.1a
304	28.3±2.1	69.8±7.9b	133.1±2.7	10.1±0.3	23.8±1.5ab	50.5±1.1b
456	40.4±4.6	97.8±11.8ab	157.8±14.3	11.5±0.9	28.0±1.7a	81.7±12.4a

Note: Numbers followed by different letters in the same column were significantly different according to DMRT at α = 5% level, and mean values are followed by the standard error (s.e. n = 4).

Leaf N, P, and K content

Analysis of leaf N, P, and K levels showed no significant differences with guano fertilizer application (Table 2). Nitrogen and phosphorus content in bitter melon leaves were within the sufficient reference range. However, potassium in bitter melon plants showed lower levels compared to the reference range for cucumber plants. The reference range for nutrient sufficiency levels in cucumber plants is N = 4.5-6.0%, P = 0.3-1.3%, and K = 3.9-5.0% (Silva & Uchida, 2000).

Table 2. Concentrations of leaf N, P, and K with various guano fertilizer doses.

Treatment (g guano per plant)	Nitrogen (%)	Phosphorus (%)	Potassium (%)
0	5.16±0.03	0.47±0.02	2.94±0.17
152	5.19±0.03	0.52±0.04	3.06±0.07
304	5.21±0.07	0.49±0.03	2.77±0.25
456	5.14±0.14	0.50±0.03	3.21±0.23

Note: Mean values were followed by the standard error (s.e. n = 4).

Various guano fertilization treatments did not significantly affect the relative growth rate (RGR). During plant development, interesting patterns emerged in both RGR and net assimilation rate (NAR). At 2-4 weeks after planting (WAP), plants showed higher RGR values but relatively low NAR, with the application of 304 g guano per plant resulting in the lowest NAR, significantly different from the control. This pattern shifted at 4-6 WAP, where the application of 152 g guano fertilizer per plant yielded the highest NAR, increasing by 186% compared to unfertilized plants (Table 3) ($P<0.05$). It is hypothesized that bitter melon plants prioritize root system development for nutrient absorption in the early stage (2-4 WAP), explaining the higher RGR but lower NAR values. As plants enter the next stage (4-6 WAP), resource allocation shifts towards canopy growth and enhanced photosynthetic efficiency, reflected in increased NAR values. This increase in NAR demonstrates that guano fertilizer application enhances the plant's ability to produce greater biomass per unit leaf area, likely due to improved photosynthetic activity resulting from better nutrient availability in the soil (Hidayat et al., 2020).

Table 3. Relative growth rate and net assimilation rate due to various guano fertilization doses.

Treatment (g guano per plant)	Relative growth rate (g g^{-1} day^{-1})		Net assimilation rate (g cm^{-2} day^{-1})	
	Plant age (weeks after planting)			
	2-4	4-6	2-4	4-6
0	0.29±0.03	0.32±0.02	0.015±0.001a	0.07±0.01b
152	0.39±0.02	0.25±0.01	0.012±0.001ab	0.20±0.03a
304	0.32±0.05	0.24±0.01	0.007±0.001b	0.06±0.02b
456	0.35±0.03	0.26±0.03	0.012±0.003ab	0.11±0.05ab

Note: Numbers followed by different letters in the same column were significantly different according to DMRT at α = 5% level, and mean values were followed by the standard error (s.e. n = 4).

Reproductive growth phase

The application of 152, 304, and 456 g of guano fertilizer per plant resulted in the highest number of male flowers. In comparison, 456 g guano fertilizer per plant resulted in the highest number of female flowers, increasing by 142% compared to unfertilized plants, even though it did not significantly differ with 152 g treatment (Table 4) at 6 weeks after planting (WAP). This indicated that guano fertilizer can enhance flower formation in bitter melon plants. This increase may be due to better nutrient availability, especially phosphorus, which plays a crucial role in developing plant reproductive organs (Malhotra et al., 2018). Although guano fertilizer treatments did not show a statistically significant difference in the days to reach 50% of the plant population that had flowered within 1 plot, the 152 g dose significantly accelerated flowering compared to the control.

Table 4. Number of male and female flowers per plant at 4 and 6 WAP, and days to 50% due to various guano fertilization doses.

Treatment (g guano per plant)	Number of male flowers		Number of female flowers		Days to 50% flowering
	Plant age (weeks after planting)				
	4	6	4	6	
0	17.4±1.8	40.7±0.6b	2.6±0.3	5.7±0.6c	27.8±0.3
152	30.6±5.7	51.8±3.8a	5.0±1.2	10.1±1.5ab	26.3±0.5
304	19.5±2.4	51.4±0.6a	2.2±0.4	9.0±1.2bc	27.0±0.4
456	29.5±7.7	57.7±3.0a	4.8±1.0	13.8±1.6a	26.5±0.3

Note: Numbers followed by different letters in the same column were significantly different according to DMRT at α = 5% level, and mean values were followed by the standard error (s.e. n = 4).

Yield

The application of 456 g guano fertilizer per plant showed a significant increase in the number of fruits per plant, which increased by 46% compared to unfertilized plants, even though it did not significantly differ with 152 g guano fertilizer treatment ($P<0.05$) (Table 5). However, no significant differences were observed in other characteristics, such as fresh weight per fruit, fruit moisture content, or fruit weight per plant, although the 456 g dose tended to produce higher values for these parameters. These results suggested that higher doses of guano fertilizer increased fruit number. This increase can be attributed to improved soil conditions and more balanced nutrient availability due to the addition of guano fertilizer. This aligns with the opinion of Assefa and Tadesse (2019) that organic fertilizers can enhance soil fertility and plant productivity by providing and ensuring that plants have access to balanced and appropriate nutrients. Lukman (2022) highlighted the important role of phosphorus and nitrogen from guano fertilizer on sweet corn fruit weight and emphasized that the availability of phosphorus and potassium greatly influences that yield component.

While the number of fruit per plant was higher with the 456 g guano, the application of 152 g guano fertilizer per plant increased the dry weight of fruit by 48% compared to unfertilized plants ($P<0.01$) (Table 5). The dry weight of fruit with 152 g of guano was not significantly different from 304 g of guano, but they were higher than the control and 456 g guano fertilizer treatments. The 456 g guano resulted in fruit with higher water content (Tabel 5).

Table 5. Number of fruits per plant, fresh weight per fruit, fruit moisture content, dry weight of fruit, and fruit weight per plant due to various guano fertilization doses.

Treatment (g guano per plant)	Number of fruits per plant	Fresh weight per fruit (g)	Fruit moisture content (%)	Dry weight of fruit (g)	Fruit weight per plant (g)
0	8.1±0.5b	210.4±3.0	91.67±0.02	14.7±0.3b	1444.3±148.5
152	10.4±0.4ab	222.7±5.9	88.26±0.03	21.7±0.3a	1626.1±176.3
304	8.8±1.0b	216.5±1.2	88.21±0.03	21.2±03a	1441.5±156.3
456	11.8±0.9a	223.1±6.5	91.43±0.01	15.6±0.3b	1842.8±236.9

Note: Numbers followed by different letters in the same column were significantly different according to DMRT at α = 5% level, and mean values are followed by the standard error (s.e. n = 4).

Fruit: Phosphorus levels, terpenoid concentration, and terpenoid production

The application of guano fertilizer showed no significant effect on phosphorus levels in bitter melon fruit (Table 6). This result can be related to the sufficient P in the leaf, as shown in Table 2.

Terpenoid content in bitter melon fruit varied depending on the dose of guano fertilizer and the drying method used. A comparison between oven and dehydrator-dried samples was conducted to evaluate the difference in terpenoid analysis between both drying methods. From both drying methods, the application of guano fertilizer did not show a significant effect.

Based on the study, terpenoid production using the oven drying method ranged from 22.1-42.5 mg NE plant^{-1}, while the dehydrator method produced a range of 18.4-33.2 mg NE per plant (Table 6). The application of 304 g guano fertilizer per plant resulted in the highest total terpenoid production, increasing by 92% compared to unfertilized plants (Table 6). The total terpenoid production with 304 g guano was not significantly different from 152 g guano. Low terpenoid production in unfertilized plants was caused by nutrient limitations, not only phosphorus but also nitrogen and potassium, which led to suboptimal vegetative (Table 1) and reproductive growth (Table 4). Phosphorus plays a crucial role in terpenoid biosynthesis as it is required in the formation of terpenoid precursors that need high-energy phosphate bonds, such as IPP (isopentenyl diphosphate), DMAPP (dimethylallyl pyrophosphate), GDP (geranyl diphosphate), and FDP (farnesyl diphosphate). According to Bustamante et al. (2020), phosphorus availability is more limited to terpenoid production than carbon availability due to phosphorus's essential role in two main terpenoid biosynthetic pathways: the MVA (mevalonate) and MEP (methylerythritol phosphate) pathways. These findings align with Honorato et al. (2024), who demonstrated that optimizing organic fertilization can enhance secondary metabolite production in medicinal plants through increased plant biomass and metabolic efficiency. However, the highest guano dose of 456 g per plant resulted in lower terpenoid production that was not statistically different from the control and significantly different from doses of 152 and 304 g per plant, which was caused by lower terpenoid concentration and lower dry weight (Table 5).

Table 6. Phosphorus concentration in fruit, fruit terpenoid concentration, and terpenoid production due to various guano fertilization doses.

Treatment (g guano per plant)	Phosphorus (%)	Fruit terpenoid concentration (mg NE g^{-1} simplicia)		Terpenoid production (mg NE per plant)	
		Dehydrator	Oven	Dehydrator	Oven
0	0.45±0.02	1.3±0.4	1.5±0.2	19.5±5.5	22.1±3.4b
152	0.47±0.05	1.4±0.3	1.8±0.1	30.3±5.9	38.7±3.2a
304	0.47±0.02	1.6±0.2	2.0±0.2	33.2±5.7	42.5±4.2a
456	0.51±0.01	1.2±0.3	1.5±0.3	18.4±4.7	23.5±4.9b

Note: Numbers followed by different letters in the same column were significantly different according to DMRT at α = 5% level, and mean values are followed by the standard error (s.e. n = 4).

Based on the morphological and reproductive characteristics of terpenoids in bitter melon, the study showed that guano fertilizer had a significant positive impact on stem length, leaf number, net assimilation rate, number of flowers, number of fruits per plant, fruit dry weight, and terpenoid production through several mechanisms. Firstly, guano increased the availability of essential macronutrients for plants (Table 7). This nutrient enhancement supports vegetative growth, flower and fruit formation, and the production of secondary metabolites such as terpenoids (Alhasan & Al-Ameri, 2021).

Table 7. Soil analysis due to various guano fertilization doses.

Treatment (g guano per plant)	pH H_2O	C-organic (%)	N total (%)	P potential (mg 100 g^{-1})	K potential (mg 100 g^{-1})
0	6.99	3.63	0.50	491.48	50.73
152	7.60	8.11	0.89	1,985.46	698.94
304	7.51	7.09	1.03	1,268.51	338.14
456	7.56	6.97	0.91	1,795.85	474.07

Secondly, guano improved soil's properties by increasing organic matter content (Table 7). This improvement contributes to enhanced soil structure, increased porosity, and improved water retention capacity, creating a better root environment for plant growth (Hasibuan, 2015). The high organic matter content also boosts soil microbial activity, which plays a crucial role in decomposing organic matter and gradually releasing nutrients. It also contributes to nutrient cycling, ensuring a continuous supply of essential nutrients to plants (Hussain et al., 2023).

The third significant influence of guano was because of its ability to increase soil pH (Table 7), which is crucial for optimizing nutrient uptake and overall plant health. Most plant nutrients are most readily available in slightly acidic to neutral soil conditions (Khaled & Sayed, 2023). Guano's pH-regulating effect creates an environment where essential nutrients like nitrogen (N), phosphorus (P), potassium (K), and various micronutrients become more bioavailable to plants.

CONCLUSIONS

The application of guano fertilizer significantly increased stem length, leaf number, net assimilation rate, number of flowers, number of fruits per plant, fruit dry weight, and total terpenoid compounds in bitter melon (*M. charantia* L.). The doses of 152 g and 456 g guano per plant increased vegetative growth parameters, namely plant height and leaf number. The application of 456 g per plant enhanced reproductive growth in terms of male and female flower numbers. Production parameters showed that a dose of 456 g per plant increased fruit number, while fruit dry weight improved at doses of 152 g and 304 g per plant. The 304 g guano fertilizer per plant was the best dose for total terpenoid production, achieving a 92% increase compared to unfertilized plants. Guano fertilization enhanced terpenoid synthesis by improving nutrient availability for the MVA and MEP pathways.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Ministry of Education, Culture, Research, and Technology (Kemdikbudristek) for funding this research through the Student Thesis Research (PTM) BIMA Program in the 2024 academic year under contract number 027/E5/PG.02.00.PL/2024 and subcontract number 22274/IT3.D10/PT.01.03/P/B-/2024. This study, titled "Production of total terpenoid compounds in bitter melon (*M. charantia* L.) fruit with guano fertilizer application," was made possible through their support.

REFERENCES

Alhasan, A. S., & Al-Ameri, D. T. (2021). Effects of macronutrient fertilization on plant growth, essential oil content, and chemical composition in medicinal and Aromatic plants grown under different environmental conditions: a review. *Natural Volatiles & Essential Oils Journal, 8*(6), 2588-2601.

Assefa, S., & Tadesse, S. (2019). The principal role of organic fertilizer on soil properties and agricultural productivity - a review. *Agricultural Research & Technology, 22*(2), 46-50. https://doi.org/10.19080/ARTOAJ.2019.22.556192

Bustamante, M. A., Michelozzi, M., Caracciolo, A. B., Grenni, P., Verbokkem, J., Geerdink, P., Safi, C., & Nogues, I. (2020). Effects of soil fertilization on terpenoids and other carbon-based secondary metabolites in *Rosmarinus officinalis* plants: a comparative study. *Plants, 9*(7), 830. https://doi.org/10.3390/plants9070830

Chaudhury, P. (2023). Role of secondary metabolites of *Momordica charantia* in combating copper-induced stress. *American Journal of Applied Bio-Technology Research, 4*, 5-29.

Fhonna, T. N., Melati, M., & Aziz, S. A. (2023). Twice applications of laying hen manure produced the highest yield of organic okra pods (*Abelmoschus esculentus* L. Moench.). *Journal of Tropical Crop Science, 10*(03), 176-185. https://doi.org/10.29244/jtcs.10.03.176-185

Gerke, J. (2022). The central role of soil organic matter in soil fertility & carbon storage. *Soil Systems, 6*(2), 33. https://doi.org/10.3390/soilsystems6020033

Gu, J., Li, Z., Mao, Y., Struik, P. C., Zhang, H., Liu, L., Wang, Z., & Yang, J. (2018). Roles of nitrogen and cytokinin signals in root & shoot communications in maximizing plant productivity and their agronomic applications*. Plant Science, 274*, 320-331. https://doi.org/10.1016/j.plantsci.2018.06.010

Hasibuan, A. S. Z. (2015). Utilization of organic materials in improving several properties of sandy soil on the south coast of Kulon Progo. (In Indonesian.). *Planta Tropika (Journal of Agro Science), 3*(1), 31-40. https://dx.doi.org/10.18196/pt.2015.037.31-40

Hidayat, Y. V., Apriyanto, E., & Sudjatmiko, S. (2020). Public perception of the new rice field printing program in Air Kering Village, Padang Guci Hilir district, Kaur Regency, and its impact on the environment. (In Indonesian.). *NATURALIS, 9*(1), 41-54.

Honorato, A. da C., de Assis, R. M. A., Maciel, J. F. A., Nohara, G. A., de Carvalho, A. A., Pinto, J. E. B. P., & Bertolucci, S. K. V. (2024). Fertilization with different manure sources & doses provides quantitative-qualitative gains in the production of *Thymus vulgaris* L. *South African Journal of Botany, 164*, 345-355. https://doi.org/10.1016/j.sajb.2023.11.052

Hussain, A., Bashir, H., Zafar, S. A., Rehman, R. S., Khalid, M. N., Awais, M., Sadiq, M. T., & Amjad, I. (2023). The importance of soil organic matter (som) on soil productivity and plant growth. *Biological and Agricultural Sciences Research Journal, 2*(11), 1-9.

Jabary, M., Melati, M., & Aziz, S. A. (2023). Determination of the dosage of chicken manure for organic paria production. (In Indonesian.). In N. Kristina et al. (Eds.), *Prosiding Nasional Seminar Perhorti* (pp. 37-46).

Khaled, F., & Sayed, A. (2023). Soil pH and its influence on nutrient availability and plant health. *International Journal of Advanced Chemistry Research, 5*(2), 61-63. https://doi.org/10.33545/26646781.2023.v5.i2a.204

Lukman, L. (2022). Utilization of guano fertilizer in sustainable agricultural systems and its impact on sweet corn (*Zea mays* saccharata L) growth and yield. (In Indonesian.). *Jurnal Ilmu Pertanian Indonesia, 27*(4), 590-595. https://doi.org/10.18343/jipi.27.4.590

Lukowski, A., Jagiello, R., Robakowski, P., Adamczyk, D., & Karolewski, P. (2022). Adaptation of a simple method to determine the total terpenoid content in needles of coniferous trees. *Plant Science, 314*, 111090. https://doi.org/10.1016/j.plantsci.2021.111090

Malhotra, H., Vandana, S., & Pandey, R. (2018). Phosphorus nutrition: Plant growth in response to deficiency and excess. In M. Hasanuzzaman, et al. (Eds.), *Plant nutrients and abiotic stress tolerance* (p. 171-190). Springer. https://doi.org/10.1007/978-981-10-9044-8_7

Melati, M., Ari, A. N. H. G., & Aziz, S. A. (2021). Plant growth and morphological characters of *Sonchus arvensis* L. from different chicken manure rates and harvest intervals with ratooning practices. *Journal of Tropical Crop Science, 8*(2), 60-70. https://doi.org/10.29244/jtcs.8.02.60-70

Nisaa, B., Sudiarso, & Aini, N. (2017). Application of NPK compound and filter cake compost to increase growth and yield of tomato (*Solanum lycopersicum*) between cabbage (*Brassica oleraceae*). (In Indonesian.). *Jurnal Produksi Tanaman, 5*(6), 925-931.

Novi, R. (2015). Vegetative growth of bitter melon plants (*Momordica charantia* L.) given rice washing water at various concentrations. (In Indonesian.). *Bio Concetta, 1*(2), 67-73.

Peng, L. C., & Ng, L. T. (2022). Impacts of nitrogen and phosphorus fertilization on biomass, polyphenol contents, and essential oil yield and composition of *Vitex Negundo* Linn. *Agriculture, 12*(6), 859. https://doi.org/10.3390/agriculture12060859

Puspitasari, I. A., Septiana, M., & Razie, F. (2023). The effect of guano fertilizer and dolomite on phosphorus nutrient availability in Podzolic soil. (In Indonesian.). *Acta Solum, 2*(1), 1-6. https://doi.org/10.20527/actasolum.v2i1.2273

Putta, S., Yarla, N. S., Kilari, E. K., Surekha, C., Aliev, G., Divakara, M. B., Santosh, M. S., Ramu, R., Zameer, F., Prasad M., Chintala, R., Rao, P. V., Shiralgi, Y., & Dhananjaya, B. L. (2016). Therapeutic potentials of triterpenes in diabetes and its associated complications. *Current Topics in Medicinal Chemistry, 16*(23), 2532-42. https://doi.org/10.2174/1568026616666160414123343

Rahmasari, I., & Wahyuni, E. S. (2019). The effectiveness of *Momordica charantia* (bitter melon) in reducing blood glucose levels. (In Indonesian.). *Infokes, 9*(1), 57-64.

Ritonga, M. N., Aisyah, S., Rambe, M. J., Rambe, S., & Wahyuni, S. (2022). Processing chicken manure into environmentally friendly organic fertilizer. (In Indonesian.). *Jurnal Adam IPTS, 1*(2), 137-141.

Silva, A., & Uchida, S. (2000). *Plant nutrient management in Hawaii's soils: Approaches for tropical and subtropical agriculture*. University of Hawaii.

Suhartono, S., Djunaedy, A., Maknun, L., Suryono, E., & Khoiri, S. (2023). The effect of various guano fertilizer doses on *Physalis angulata* growth, flavonoid content, and saponin content. (In Indonesian.). *Journal of Science in Agrotechnology, 1*(2), 53-60.

Suwandi, S., Sopha, S., & Yufdy, M. P. (2015). The effectiveness of organic fertilizer, NPK, and biofertilizer management on growth and yield of shallots. (In Indonesian.). *Jurnal Hortikultura, 25*(3), 208-221.

Syofiani, R., & Oktabriana, G. (2017). Application of guano fertilizer in improving N, P, K nutrients and soybean plant growth on gold mine tailings planting media. In N. Fitriah et al. (Eds.), *Prosiding Seminar Nasional Fakultas Pertanian UMJ* (pp. 98-103). UMJ

Taufika, R., Aziz, S. A., & Melati, M. (2016). Flavonoid production of orange jessamine leaf (*Murraya paniculata* L. Jack) with various organic fertilizer and harvest intervals. (In Indonesian.). *Buletin Penelitian Tanaman Rempah dan Obat, 27*(1), 27-36. http://dx.doi.org/10.21082/bullittro.v27n1.2016.27-35