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Plant Growth, Fruit Production and Total Terpenoid Compounds in Bitter Gourd (*Momordica charantia* L.) at Various Levels of Phosphorus Fertilization

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Abstract

Bitter gourd (*Momordica charantia* L.) fruits have been reported to have pharmacological functions such as anti-bacterial, lowering blood sugar, and preventing cardiovascular disease. Terpenoids are the bioactive compounds that play a role in those functions. The phosphorus is essential in the biosynthesis of terpenoids. This research aimed to determine plant growth characteristics, fruit production, and terpenoid production in bitter gourds fertilized with various phosphorus. The experiment was conducted at the IPB experimental station in Cikarawang, 6°32'58.3" S south latitude and 106°43'54.8" E east longitude, Bogor, Indonesia, from July to October 2023. The experiment used a completely randomized block design with a single factor: fertilizer treatments and three replications. The treatments were without fertilizer, chicken manure only, and varying dosages of SP-36 (0, 20, 40, 60 g per plant*)*. Plants treated with 40 g SP-36 per plant significantly had longer stems at four weeks after planting (WAP) (153.5 cm), substantially more female flowers at 5 WAP (6.4 flowers), heavier fresh weight per fruit (243.98 g), and higher fruit carotene level (86 $\mu g.g^{-1}$). Plants treated with 60 g SP-36 per plant had the heaviest fresh fruit weight per plant (2,820.9 g). On the other hand, chicken manure fertilizer resulted in an elevated number of female flowers at 7 WAP (6.8 flowers), leaf potassium content (3.41%), chlorophyll a (250 $\mu g.g^{-1}$), chlorophyll b (114 $\mu g.g^{-1}$), and total chlorophyll concentration (363 $\mu g.g^{-1}$). There were no significant differences in IC50, terpenoid content, and terpenoid production; however, plants treated with 60 g SP-36 per plant tended to have lower IC50 (1,347.67 ppm) and terpenoid content (95,227 µmol NE.g^{-1} dry fruit). In contrast, plants treated with 40 g SP-36 per plant tended to have higher terpenoid production (15,995 mmol NE per plant) than other treatments.

Keywords: antioxidant, chicken manure, chlorophyll, precursors

Introduction

Bitter gourd (*Momordica charantia* L.) is an annual plant widely grown in tropical and subtropical regions including East Africa, Asia, the Caribbean, and South America (Paul et al., 2009). Bitter gourd is commonly utilized as a vegetable and a medicinal potential plant (Yaldiz et al. 2015). Bitter gourd contains various secondary metabolite compounds (Slamet., 2020), one of the secondary metabolite compounds is a group of terpenoids. Bitter gourd-derived terpenoid compounds include cucurbitanes (Huang et al., 2020), charantin (Weng et al., 2013), and momordicin (Zhao et al., 2005). These compounds have been shown to possess pharmacological benefits, such as anti-cancer and anti-bacterial properties (Cuong et al., 2017), suppressing cardiovascular disease disorders (Tuan et al. 2017), also as anti-diabetic, at a level of 300 $mg.kg^{-1}$ bitter gourd can reduce blood sugar levels by 31.64% and increase insulin levels by 27.35% (Mahwish et al., 2021).

Due to its numerous properties and benefits, bitter gourd has attained significant economic value as a commercial commodity in the market (Lee et al., 2021). However, the current requirements of bitter gourd, both in terms of quantity and quality, are not yet optimal. Therefore, efforts are necessary to enhance bitter gourd production, particularly by increasing the yield of secondary metabolites such as terpenoid compounds, which can be achieved through fertilization techniques. Fertilization has been demonstrated to enhance plant production

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(Purba et al., 2021) and can significantly influence the production of secondary metabolite compounds (Yang et al., 2018).

Among the fertilizers, phosphorus is a crucial factor influencing secondary metabolite compounds. Phosphorus plays a pivotal role in the production of terpenoid compounds within the plant, as terpenoid precursors, including IPP (Isopentenyl diphosphate), DMAPP (Dimethylallyl pyrophosphate), GDP (Geranyl diphosphate), and FDP (Farnesyl diphosphate), contain high-energy phosphorus bonds. Additionally, phosphorus constitutes an essential component of ATP and NADPH molecules, which are vital for synthesizing terpenoids through both the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways. Thus, phosphorus serves as a fundamental element in the production of terpenoid compounds (Bustamante et al., 2020).

Several research results reveal that applying P fertilizer has a significant role in the biosynthesis of secondary metabolite compounds (Nell et al., 2009). Increasing the amount of P fertilizer can increase terpenoid biosynthesis (Peng and Teik, 2022). Based on the research results of Naorem et al., (2019), the application of P fertilizer (90 kg.ha^{-1}) gave the highest results in the fruit number per plant, fruit weight, fruit length, fruit diameter, fruit weight per plant, and fruit weight per hectare on bitter gourd plants. This is because the absorption of macro and micronutrients increases with the addition of P and this is also related to improved root growth due to the addition of P (Fageria et al., 2016). P utilization efficiency can be increased when applied along with other nutrients, primarily N and K (Duan et al., 2004).

Phosphorus can be sourced from both inorganic and organic fertilizers. Inorganic fertilizers like SP-36 contain phosphorus, while organic fertilizers such as chicken manure also provide a high phosphorus level. Considering the role of phosphorus in increasing the yield and production of secondary metabolite compounds, especially terpenoid compounds, research is necessary to determine the best phosphorus fertilizer treatment that can increase growth, fruit production, and total terpenoid production in bitter gourd.

Materials and Methods

The experiment was conducted from July to October 2023 at the Cikarawang experimental station, IPB, Bogor, West Java, Indonesia. The materials used are bitter gourd seeds of the Opal F1 variety, laying chicken manure, urea fertilizer, SP-36 fertilizer, KCl fertilizer, and silver-black plastic mulch. The tools used include a UV-vis spectrophotometer, centrifuge, sonication, and micropipette.

The experiment used a randomized complete block design with one factor consisting of six fertilizer treatments: without fertilization, chicken manure, and four SP-36 fertilizer doses consisting of 0, 20, 40, and 60 g SP-36 per plant (equivalent to 0, 50, 100, and 150% of the recommended dose from PT. East-West). Each treatment was replicated thrice, so there were 18 experimental units, each consisting of 32 plants.

Experimental Procedures

Bitter gourd seeds were sown in trays for 14 days, then transplanted to the experimental field with a plot size of 1 m x 11.75 m, planting distance of 0.5 m x 0.75 m, distance between plots of 0.5 m, and distance between replicates of 1 m. The seedlings with the criteria of 2-4 leaves fully opened were transplanted. Chicken manure as a treatment (0.73 kg per plant) was applied two weeks before transplanting. The SP-36 fertilizer ($Ca(H_2PO_4)$) was applied one week after transplanting. SP-36 fertilizer with doses of 20, 40, and 60 g SP-36 per plant (equivalent to 35.5 kg.ha^{-1}; 71 kg.ha^{-1}; and 106.5 kg.ha^{-1}) was given in 6 times-applications. The first application of each treatment was 10, 20, and 30 g SP-36 per plant, and the rest was applied five times with doses of 2, 4, and 6 g SP-36 per plant every two weeks.

For the four SP-36 treatments, N and K fertilizers were also given with a dose of 45 g urea per plant and 30 g KCl per plant, respectively. The urea and KCl were also applied six times with the first application being 20 g urea per plant and 10 g KCl per plant. The next application of N and K fertilizer was 5 g urea per plant urea and 4 g KCl per plant every two weeks. The first fruit harvest was done 43 days after transplanting. The fruit harvest can be done 10 times with specific criteria, including shiny dark green color, fruit length of approximately 26 cm, and fruit weight of approximately 305 g.

Plant Growth and Development

Stem height and leaf number were measured from 2 weeks after planting (WAP) to 5 WAP at 1-week intervals. The number of stems was counted at 4 and 5 WAP.

The number of male and female flowers was counted on each plant sample. Days to 50% flowering are recorded based on the age at which flowers are first initiated.

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Relative growth rate (RGR) was calculated based on plant dry weight per unit time ($g.g^{-1}$ per day), whereas net assimilation rate (NAR) is calculated based on plant weight per unit leaf area within a specific time (g.cm-2 per day). RGR and NAR are calculated at 3-5 and 5-7 WAP, respectively.

Leaf N, P, and K Content

The mature leaves' N, P, and K contents were measured at 4 WAP. The leaves are air-dried and then dried in the oven at 60oC until reaching a constant weight. Leaf N analysis was conducted using the Kjeldahl method. P and K were extracted using the wet ash method using HNO3 and HClO4. P levels were calculated using a spectrophotometer, while K was calculated using an atomic absorption Spectropotometer (AAS) (Balitttanah, 2005).

Yield

Fruit number, fresh weight per fruit (g), fresh weight of fruit per plant (g), and dry weight of fruit per plant (g) were measured as yield components. The fresh weight per fruit was calculated by weighing each unit of bitter gourd fruit sample that had been harvested. The fresh fruit weight per plant was calculated by adding up all the weights of fruit per plant that had been harvested. Fruit dry weight per plant was calculated using the following formula:

Dry weight of fruit per plant = fresh weight of fruit per plant x (100% - fruit water %)

Fruit Pigment Concentration

The fruit pigment was determined from the fourth-harvested fresh fruit using the Sims and Gamon (2002) method. The analysis procedure was initiated by grinding the fruit samples and then adding 2 mL acetone Tris. The mixture was then centrifuged at 14000 rpm for 10 minutes. Next, 1 mL of supernatant was added with 3 mL of acetone Tris and homogenized. The absorbance of the mixture was measured using a Shimadzu UV-1201 UV-VIS spectrophotometer at wavelengths of 663, 647, 537, and 470 nm.

Inhibiting Concentration (IC50)

The IC50 in bitter gourd fruit was determined by using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method (Salazar et al., 2011) The procedure for this analysis begins by preparing 100 mg of concentrated bitter gourd fruit extract, dissolving it with 1 ml of DMSO, and then diluting it to various concentrations (125 - 8000 $ug.ml^{-1}$). 100uL of sample extract at different concentrations was added with 100uL of DPPH 125 Um into a 96-well microplate. Next, the extract solution was incubated in the dark for 30 minutes. The absorbance was measured at a wavelength of 517 nm. Ascorbic acid was used as a standard at a concentration of (0.3125 – 10 ug ascorbic acid per ml of methanol). The capacity value is expressed in percent IC50 (effective concentration of the sample capable of inhibiting 50% of DPPH radicals). The IC50 value is calculated based on the line equation obtained by entering the value 50 as the y variable and then determining the x variable value as the concentration of the sample.

Total Terpenoid Production

The principle of determining total terpenoids using spectrophotometry is based on the colorimetric method (Łukowski et al., 2022). A total of 0.2 g of dry fruit was weighed, then cold 95% methanol was added. The mixture was incubated for 48 hours at room temperature. The samples were then centrifuged at 4000 g for 15 minutes at room temperature. The resulting supernatant was transferred to a test tube then 1.5 mL of chloroform was added and homogenized using a vortex. Once homogeneous, 100 µL of H_2SO_4 was added to the sample and incubated at room temperature for 1.5–2 hours. After incubation, 1.5 mL of 95% methanol was added to the sample and homogenized using a vortex until the precipitate dissolved again. The samples were then analyzed with a UV-Vis spectrophotometer at 520 nm. Total terpenoids were determined based on the Nerol standard curve. Terpenoid contents were quantified in Nerol equivalents (NE). Determination of terpenoid productions (mmol NE per plant) was obtained by multiplying the dry weight (weight of dry fruit per plant) by the terpenoid content (µmol NE. g^{-1} of dry fruit).

Data Analysis

Data were analyzed using ANOVA at α=0.05 and continued with the Duncan multiple range test (DMRT) if the means between treatments were significant. Statistical analysis was performed using R Studio software version 4.3.1.

Result and Discussion

Plant Growth and Development

The application of 40 g SP-36 per plant led to a significant 17.09% increase in stem length at four weeks after planting (WAP) ($P<0.05$) compared to without fertilizer (Table 1), but leaf numbers were not different among all fertilizer treatments.

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Leaf N, P and K Content

Various phosphorus fertilizations resulted in significant differences in the leaf potassium content (P<0.05) (Table 2). The highest potassium content was found in chicken manure, with an increase of 39.75% compared to 0 g SP-36 per plant. On the other hand, various phosphorus fertilizations did not affect leaf nitrogen and phosphorus content.

The differences in the number of female flowers due to various fertilizer treatments occurred at 5 and 7 WAP (Table 3). At 5 WAP, the highest flower number was obtained from the 40 g SP-36 per plant with a 45.45% increase compared to 0 g SP-36 per plant and 68.42% increase compared to without fertilizer (P<0.05). On the other hand, 60 g SP-36 per plant led to a 44.44% increase in 7 WAP (P<0.05) compared to without fertilizer. The number of male flowers at 5, 7 WAP, and the days to 50% flowering were not significantly different among fertilizer treatments.

Fertilization treatment did not significantly affect the relative growth and net assimilation rates at 3-5 and 5-7 WAP (Table 4).

Yield

The application of 40 g SP-36 per plant, resulted in a significantly 10.84% higher fresh weight per fruit compared to 20 g SP-36 per plant and 29.38% higher than without fertilizer (P<0.01, Table 5). The application of 60 g SP-36 per plant produced 29.43% higher fresh weight of fruit per plant compared to without fertilizer (P<0.05, Table 5). Various levels of phosphorus fertilization did not affect the fruit number and dry weight of fruit per plant.

Fruit Pigment Concentration

Applying 40 g of SP-36 caused a higher concentration of chlorophyll a, chlorophyll b, carotene, and total chlorophyll (P<0.05) by 200%, 166.66%, 207.14%, and 185.83%, respectively, compared to those without fertilizer (Table 6.).

Table 1. Stem length and leaf number at 3, 4, and 5 WAP with various levels of phosphorus fertilization

Treatment	3 WAP		4 WAP		5 WAP	
	Stem length (cm)	Leaf number	Stem length (cm)	Leaf number	Stem length (cm)	Leaf number
Without fertilizer	68.6	17.3	131.1b	51.6	172.2	105.2
Chicken manure	73.7	19.2	133.3b	55.7	174.7	105.8
0 g SP-36 per plant	79.3	21.9	151.6a	69.2	176.6	111.4
20 g SP-36 per plant	75.0	23.2	144.5ab	62.4	178.2	106.9
40 g SP-36 per plant	81.2	24.1	153.5a	66.1	177.3	114.4
60 g SP-36 per plant	71.5	22.8	143.9ab	55.9	177.8	122.9
P-Value	0.305	0.366	0.034	0.415	0.965	0.666
Sig.	ns	ns	*	ns	ns	ns

Note: values followed by different letters in the same column are significantly different in the Duncan test; ns=non-significant;
* = significant at α=0.05

Table 2. Leaf N, P, and K content at different levels of phosphorus fertilization

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Without fertilizer	4.54	0.28	2.73abc
Chicken manure	4.89	0.31	3.41a
0 g SP-36 per plant	4.35	0.22	2.44bc
20 g SP-36 per plant	4.66	0.27	2.96abc
40 g SP-36 per plant	4.39	0.23	2.08c
60 g SP-36 per plant	4.59	0.28	3.27ab
P-Value	0.202	0.199	0.040
Sig.	ns	ns	*

Note: values followed by different letters in the same column are significantly different in the Duncan test; ns=non-significant;
* = significant at α=0.05

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Table 3. Number of male and female flowers per plant at 5 and 7 WAP, and days to 50% at different levels of phosphorus fertilizations.

Treatment	Number of males flowers		Number of female flowers		Days to 50% flowering
	5 WAP	7 WAP	5 WAP	7 WAP	
Without fertilizer	3.6	5.6	3.8b	4.5b	28.0
Chicken manure	3.2	6.6	5.2ab	6.8a	27.7
0 g SP-36 per plant	4.5	5.8	4.4b	5.6ab	27.3
20 g SP-36 per plant	3.7	6.5	4.8b	6.2a	27.3
40 g SP-36 per plant	3.7	6.3	6.4a	6.4a	27.0
60 g SP-36 per plant	3.7	7.1	4.2b	6.5a	27.3
P-Value	0.380	0.171	0.017	0.017	0.775
Sig.	ns	ns	*	*	ns

Note: values followed by different letters in the same column are significantly different according to DMRT; ns=non-significant; * = significant at α=0.05

Table 4. Plant relative growth rate (RGR) and net assimilation rate (NAR) at 3-5 WAP and 5-7 WAP with various phosphorus fertilization

Treatment	Relative growth rate ($g.g^{-1}$ per day)		Net assimilation rate ($g.cm^{-2}$ per day)	
	3-5 WAP	5-7 WAP	3-5 WAP[1)]	5-7 WAP[1)]
Without fertilizer	3.46	4.12	0.08	0.13
Chicken manure	3.66	4.22	0.10	0.10
0 g SP-36 per plant	3.53	4.46	0.07	0.18
20 g SP-36 per plant	3.64	4.35	0.09	0.14
40 g SP-36 per plant	3.78	4.47	0.10	0.14
60 g SP-36 per plant	3.84	4.44	0.11	0.13
P-Value	0.486	0.569	0.855	0.877
Sig.	ns	ns	ns	ns

Note: ns=non-significant; [1)] Data was transformed using the formula $\sqrt{x + 0{,}5}$

Table 5. Fruit number, fresh weight per fruit (g), fresh weight of fruit per plant (g), and dry weight of fruit per plant with various phosphorus fertilization

Treatment	Harvest			
	Fruit number per plant	Fresh weight per fruit (g)	Fresh weight of fruit per plant (g)	Dry weight of fruit per plant (g)
Without fertilizer	11.7	188.54d	2,179.5b	90.74
Chicken manure	12.1	237.32ab	2,828.4a	118.79
0 g SP-36 per plant	12.2	225.12bc	2,714.9a	114.63
20 g SP-36 per plant	12.5	220.07c	2,725.8a	134.85
40 g SP-36 per plant	11.5	243.93a	2,820.4a	163.75
60 g SP-36 per plant	11.8	240.05ab	2,820.9a	163.90
P-Value	0.737	0.000182	0.00194	0.314
Sig.	ns	**	**	ns

Note: values followed by different letters in the same column are significantly different according to the DMRT; ns= non-significant; *= significant at α=0.05; ** = significant at α=0.01.

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Table 6. Fruit pigment concentration with various levels of phosphorus fertilization.

Treatment	Chlorophyll a ($\mu g.g^{-1}$)	Chlorophyll b ($\mu g.g^{-1}$)	Carotene ($\mu g.g^{-1}$)	Chlorophyll total ($\mu g.g^{-1}$)
Without fertilizer	80b	39b	28b	120b
Chicken manure	250a	114a	83a	363a
0 g SP-36 per plant	240a	106a	84a	344a
20 g SP-36 per plant	190a	85a	67a	279a
40 g SP-36 per plant	240a	104a	86a	343a
60 g SP-36 per plant	220a	101a	75a	320a
P-Value	0.018	0.019	0.023	0.017
Sig.	*	*	*	*

Note: values followed by different letters in the same column are significantly different according to the DMRT; ns=non-significant; * = significant at α=0.05.

Table 7. Antioxidant activity (IC50), fruit terpenoid content, and terpenoid production with various levels of phosphorus fertilization.

Treatment	IC50 (ppm)	Fruit terpenoid content (μmol NE.g^{-1} of dry fruit)[1)]	Terpenoid production (mmol NE per plant)[1)]
Without fertilizer	2,214.5	80,830	6,791
Chicken manure	1,448.6	79,821	8,162
0 g SP-36 per plant	1,602.8	85,266	11,630
20 g SP-36 per plant	1,658.6	94,292	12,888
40 g SP-36 per plant	1,669.0	92,950	15,995
60 g SP-36 per plant	1,347.7	95,227	14,494
P-Value	0.326	0.985	0.624
Sig.	ns	ns	ns

Note: values followed by different letters in the same column are significantly different in the Duncan test; ns=non-significant; [1)] Data was transformed using the formula $\sqrt{x + 0,5}$; NE = Nerol equivalent

IC50, Fruit Terpenoid Content, and Plant Terpenoid Production

Various levels of phosphorus fertilization did not significantly affect IC50, but plants that received 60 g SP-36 tended to have the lowest IC50 (Table 7). Terpenoid content tended to be higher in plants treated with 60 g SP-36 compared to control (Table 7).

Discussion

Bitter gourd applied with 40 g SP-36 per plant at 4 WAP showed significantly a longer stem than without fertilizer. This positive effect of fertilizer shows that fertilization is irreplaceable in increasing growth, yields and the quality of plant growth and development (Li and Shang., 2021). Fertilization is one of the main inputs for providing nutrients. Bitter gourd treated with 60 g SP-36 per plant exhibited a higher leaf number at 5 weeks after planting (WAP) compared to 0 g SP-36 per plant, although this difference was not statistically significant. Consistent with prior studies, phosphorus has been observed to enhance plant vegetative growth, increasing leaf number (Martin et al., 2018) and stem length (Razaq et al., 2017). Phosphorus, as a macronutrient, plays a crucial role in the growth and development of plants (Wang et al., 2021), particularly in flowering (Dey et al., 2021). Higher phosphorus doses have been linked to increased flower production in cucumber (*Cucumis sativus* L.) (Vaudo et al., 2022), attributed to its ability to enhance the assimilation, translocation, and partitioning of floral components (Sahu et al., 2021). In this study, the application of various phosphorus fertilizers had a significant impact on female flowers at 5 and 7 WAP, in line with the same family, the use of phosphorus significantly can increase the ratio of female flowers to watermelon (Maluki et al., 2016). This is because phosphorus is crucial for enzymatic activity and hormone synthesis that support female flower development (Choudhary et al., 2013).

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Applying 40 g SP-36 per bitter gourd plant can increase fruit weight compared to plants treated with 0 g SP-36 per plant. This is due to phosphorus, with a significant portion being translocated to the fruit area. This translocation is driven by the high energy demand during seed and fruit production (Johan et al., 2021). Furthermore, phosphorus is an essential element in cell energy transfer because it is part of adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), uridine triphosphate (UTP), phosphoenolpyruvate, and other phosphorylated intermediates (Malhotra et al., 2018). Increasing the dose of phosphorus fertilizer to 100 kg ha^{-1} can increase the weight of bitter gourd fruit, significantly different from doses of 80, 60, 40, 20, and 0 ha^{-1} (Ashraf et al. 2019). Increasing phosphorus concentration in the same Cucurbitaceae family can produce larger cucumber fruit (Lee et al., 2024). The application of phosphorus can also increase Citrus yields compared to without phosphorus (Li et al., 2020). Citrus yields increased with phosphorus doses from 0.1 to 0.3 kg per plant (Li et al., 2019). In line with this research, increasing the dose of phosphorus fertilizer can increase fruit weight but was not significantly different from the chicken manure treatment. Chicken manure can improve soil's physical, chemical, and biological properties (Lima et al., 2021). In addition, chicken manure contains two to four times more phosphorus than other manures, ranging from 13.6 to 25.4 g P_2O_5 kg^{-1}dm (Kacprzak et al., 2022). Chicken manure had the best effect on the weight of bitter gourd fruit per plot, weighing 464 grams (Wardana et al., 2020). Moreover, chicken manure can increase the weight of bitter gourd up to 54.68% per plant (Miguel et al., 2023).

Bitter gourd without fertilizer significantly resulted in a lower concentration of chlorophyll a, chlorophyll b, carotene, and total chlorophyll compared to those treated with fertilizer (chicken manure only and various SP-36 dosages treatments), this is because those treatments have macronutrients such nitrogen, phosphorus, and potassium which have an essential role to increase photosynthetic capacity by increasing chlorophyll content (Deng et al., 2020). Nitrogen is used in chloroplasts with thylakoids and photosynthetic enzymes (Mu et al., 2016). Phosphorus is involved in cellular processes, including energy conservation, metabolic regulation, and signal transduction (Carstensen et al., 2018). Phosphorus deficiency can reduce stomata opening. If stomata opening is reduced, the lower CO_2 can be captured, reducing triose phosphate, significantly reducing the recycling of ATP and NADPH, inhibiting photosynthetic capacity (Neocleous and Savvas., 2018). Potassium can increase photosynthetic assimilation and improve nutrient absorption (Sustr et al., 2019). Potassium deficiency can significantly inhibit the biosynthesis of chlorophyll a and b and total chlorophyll (Thornburg et al., 2020). The maximum value of total chlorophyll content was obtained at a dose (N:P:K: 300:120:100 kg^{-1}ha), significantly different from the treatment without fertilization (Ashraf et al., 2019).

Based on the results of this research, bitter gourd treated with 60 g SP-36 tend to have a lower IC50 value; namely, 1,347.67 ppm sample concentration is needed to inhibit 50% of free radicals. On the other hand, plants treated with 0 g SP-36 tend to have higher IC50 values, namely 1,602.8 ppm sample concentration is needed to inhibit 50% of free radicals, although it was not significantly different. It can be inferred that bitter gourds treated with 0 g SP-36 per plant had lower antioxidant activity because the higher the IC50 value, the lower the antioxidant activity (Cruz et al., 2020). This value is calculated based on the antioxidant concentration required to reduce the DPPH concentration by 50% (Moreno et al., 1999). However, antioxidant enzyme activity increases when phosphate fertilizer is applied (Hekmati et al., 2023). Conversely, low phosphorus availability will reduce antioxidant enzyme activity (Kayoumu et al., 2023).
Increasing the dose of phosphorus to 60 g SP-36 per plant tends to increase the total terpenoid content compared to 0 g SP-36 per plant, although it did not have a significant effect. Terpenoid content and dry fruit weight per plant are the main factors determining terpenoid production per plant. Therefore, in this study, various SP-36 treatments tended to have the potential to increase terpenoid production because they had higher total terpenoid and heavier fruit dry weight than 0 g SP-36 treatment. However, it did not have a significant effect. This is because phosphorus plays a vital role in secondary metabolites because it is a constituent of nucleic acids and phospholipids and plays an important role in cell metabolism (Marschner 2002). One group of secondary metabolites that require phosphorus is terpenoids, terpenoid precursors including IPP (Isopentenyl diphosphate), DMAPP (Dimethylallyl pyrophosphate), GDP (Geranyl diphosphate), and FDP (Farnesyl diphosphate) contain high-energy phosphate bonds so that phosphate becomes an element for producing terpenoid compounds and expect to influence terpenoid production (Li et al., 2023). Terpenoid production in *Rosmarinus officinalis* is influenced by phosphorus availability (Bustamante et al., 2020). There is a positive influence between phosphorus fertilization on the terpenoid content in *Pinus halepensis* under drought conditions, although it is not significantly different (Branch et al., 2009). In addition, in the same species, there is a positive and significant effect on the terpenoid content in phosphorus fertilization (Ormeno et al., 2008).

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Phosphorus is also involved in the synthesis of ATP and NADPH where later these molecules are needed to synthesize terpenoids via the mevalonic acid (MVA) pathway and the methylerythritol phosphate (MEP) pathway (Stirbet et al., 2020). Therefore, phosphorus is an essential component in producing secondary metabolites, especially terpenoid compounds.

Conclusion

The application of 40 g SP-36 per plant, in addition to the basic fertilizer N and K, increased stem height at 4 WAP, number of female flowers, fresh weight per fruit, fresh weight of fruit production per plant, and leaf chlorophyll a, chlorophyll b, carotene, and total chlorophyll. Bitter gourd plants without fertilizer, with chicken manure, and fertilized with 20, 40, and 60 g SP-36 per plant produced similar amounts of terpenoid, i.e., 6,791, 8,162, 11,630, 12,888, 15,995, and 14,494 mmol NE per plant, respectively.

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