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Shoot Production and Metabolite Content of *Cosmos sulphureus* Cav. Leaves with Different Rates of Goat Manure

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ABSTRACT

Cosmos sulphureus Cav. is a plant species commonly cultivated for ornamental purposes, with its young leaves being consumed as vegetables. Therefore, this study aimed to investigate the effect of goat manure rates and different harvest criteria on shoot production as well as the metabolite of *C. sulphureus*. The experiment was conducted in Kuningan Regency, West Java, Indonesia, from October to November 2022, using a factorial randomized complete block design. Goat manure rates examined were 0, 100, 200, and 300 kg N/ha, equivalent to 0, 6, 12, and 18 tons of goat manure/ha. Shoot harvest criteria were two and four top nodes, with each treatment replicated three times. The result showed that applying goat manure significantly increased the shoot production of *C. sulphureus*. The harvest criteria affected the shoot weight per plant, where the four-node harvest was 98.3% higher than the two-node. The metabolite content, such as crude fiber, phenolic, and antioxidant activity, was not significantly different between the upper and lower leaves. However, the lower leaves had a total flavonoid and sugar content of 38.9 and 12.6%, higher than the upper leaves.

Keywords: Antioxidant, crude fiber, flavonoid, leaves age, phenol

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INTRODUCTION

Cosmos sulphureus Cav. is a plant species from Mexico (Vargas-Amado et al., 2020) and a member of the Asteraceae family. Furthermore, it is characterized by orange or yellow flowers and is often cultivated for ornamental purposes (Puttock, 2022). The various uses of *C. sulphureus* include as a bioherbicide (Respatie et al., 2019), natural dyes from its flower (Rahayuningsih et al., 2016), refugial flowering plants (Aldini et al., 2019), and the young shoots and leaves are eaten as vegetables (Lim, 2013).

The consumption of vegetables in Indonesia, currently at approximately 122 g, is still lower than the recommended daily intake of 300–400 g (Vermeulen et al., 2019). To address this issue and increase the consumption of various diversities, introducing underutilized vegetables to the community is crucial to overcoming malnutrition problems (Jena et al., 2018). Consequently, optimizing the use of underutilized vegetables is crucial, such as *C. sulphureus*, including *Cosmos caudatus* in Indonesia (Santosa et al., 2015).

Vegetables are essential for human diets, providing vitamins, minerals, fiber, and bioactive compounds (Ramya & Patel, 2019). Several types of vegetables have high bioactive content and antioxidant properties, such as polyphenols (Lima et al., 2014). A previous study showed that *C. sulphureus* has a high phenolic, flavonoid, and tannin content, serving as an antioxidant (Ortega-Medrano et al., 2023). The potential use of *C. sulphureus* as vegetables and some of the bioactive compounds in its leaves requires the proper cultivation techniques to maximize the yield of shoot production and metabolite content.

Fertilization is an essential aspect of plants, which is carried out by applying inorganic or organic fertilizers to provide nutrients to plants. The advantage of organic fertilizers also includes improving the physical and biological properties of the soil to prevent acidification and increase organic matter content (H. Wang et al., 2019). Furthermore, using organic fertilizers such as goat manure potentially produces high yields and quality leafy vegetables (Steiner et al., 2019). The addition of goat manure to the soil is capable of increasing pH, organic matter, total nitrogen, and available P (Uwah & Eyo, 2014), which are included in the metabolic process in plants (Tripathi et al., 2014).

Nitrogen is an essential nutrient plants need (Krapp, 2015) and is usually applied to enhance vegetative growth (Aminifard et al., 2012). Sufficient nitrogen supply must be considered to increase the growth and shoot production of *C. sulphureus*. Rates of nitrogen also affect metabolite content in leaves, while increasing organic fertilizer in plants decreases the antioxidant activity (Hassan et al., 2012). It shows that higher nutrient uptake in plants reduces the accumulation of phenolic compounds (Olarewaju et al., 2018). Therefore, it is necessary to study the appropriate nitrogen rates to optimize the growth and metabolite levels of *C. sulphureus* leaves.

The shoot production of *C. sulphureus* is also affected by harvest techniques, requiring further investigation to obtain optimal yields because *C. sulphureus* shoot is obtained by cutting some of the top nodes, where their differences affect the leaves harvested. The younger leaves have higher nitrogen and lower fiber content (de los Santos et al., 2016), while the age of the leaves influences the metabolite content. Total phenolics and flavonoid content increased with *Moringa oleifera* leaves' maturity (Nobossé et al., 2018).

The number of harvested nodes also affects future shoot production. This phenomenon occurs because harvesting more nodes leads to increased lateral shoots, originating from leaves axils, reducing the number of shoots available for the next harvest. Therefore, this study aims to analyze the interaction effect of organic fertilizer rates and harvesting criteria on the repeated production and metabolite content of *C. sulphureus*.

MATERIALS AND METHODS

Study Area

The experiment was conducted from September to November 2022 at Mandirancan, Kuningan District, West Java, Indonesia, with coordinates 6°48'0.31" S, 108°28'17.5" E, and an altitude of 296 m above sea level. Laboratory analysis was carried out at the Postharvest Laboratory Department of Agronomy and Horticulture IPB University and Food and Biochemistry Laboratory, Agriculture Faculty, Sebelas Maret University, Indonesia.

Materials

The materials used in this experiment included seeds of *C. sulphureus* with orange flowers. The seeds were obtained from cultivated *C. sulphureus,* and the organic fertilizer was composted goat manure.

Procedures

The experiment was carried out using a factorial randomized complete block design. The first factor was rates of goat manure consisting of four levels, namely 0, 100, 200, and 300 kg N/ha. The proportion of C-organic, N total, phosphorus pentoxide (P_2O_5), and potassium oxide (K_2O) concentrations were 34.20, 2.45, 1.70, and 2.01%, respectively. The amount of goat manure applied to the soil was based on the concentration of nitrogen (2.45%) and water content at 33%, yielding rates of 0, 6, 12, and 18 ton/ha. The second factor was harvesting criteria comprising the harvesting of two and four top nodes. Each treatment was repeated thrice, resulting in 24 experimental units, represented by a plot of 2 m x 3.5 m, individually.

Cosmos sulphureus seeds were collected from the plants and soaked with water for two hours. Subsequently, the seeds were sown for 12 days using soil, manure, and rice-hull charcoal media. The seedlings were planted in a plot with a plant spacing of 30 cm x 50 cm, and goat manure was applied two weeks before planting.

Shoot harvesting was carried out twice in one week, with the first harvesting executed six weeks after planting (WAP). The yield observation included internode length and shoot weight per plant in the first and second harvests, harvested shoot number, and shoot weight in the second harvest. The criteria for the second harvest were the same as those for the first harvest.

The metabolite content of *C. sulphureus* leaves was analyzed in the first harvest. The leaves were categorized based on their position in the first and second nodes (upper leaves) and the third and fourth nodes (lower leaves). The observations included leaf N, P, and K concentration, total sugar, crude fiber, phenol, flavonoid, and antioxidant activity.

Sample Preparation

The leaves dried using the drying oven at 50°C for 24 hr were ground with mortar, and the powder form obtained was stored in a refrigerator for analysis.

Determination of Nitrogen Concentration

Approximately 0.250 g of leaf powder was weighed and put into a digest tube, followed by adding 1 g selenium mixture (Merck, Germany) and 3 ml concentrated sulfuric acid (H_2SO_4, Merck, Germany). The solution was digested to 350°C for 3–4 hr, complete when white steam came out and obtained a clear extract. Subsequently, the extract was diluted with deionized water to 50 ml and shaken until homogenous, and the solution was left overnight for the particle to settle. A total of 10 ml of extract was piped into a vaporizer tube and added with 10 ml of 40% sodium hydroxide (NaOH, Merck, Germany). Erlenmeyer containing 10 ml of 1% boric acid (Merck, Germany) and three drops of Conway indicator (Merck, Germany) were prepared as a reservoir for the released ammonia (NH_3). Distillation was carried out until the container volume reached 50-75 ml. The distillate was titrated with 0.05 N H_2SO_4 (Merck, Germany) to achieve a pink color, and the titration volume was recorded. The formula for calculating N levels was as follows:

$$\text{N (\%)} = \frac{(\text{V}_{\text{sample}} - \text{V}_{\text{blank}}) \text{ x } 14.08 \text{ x Dilution factor x Correction factor x Normality}}{\text{Sample weight (mg)}} \text{ x } 100\%$$

where, N = nitrogen content (%), V = titration volume (ml).

Determination of Phosphorus and Potassium Concentration

The weight of 0.5 g of leaf powder was put into the digest tube, 5 ml of nitric acid (HNO3, Merck, Germany) and 0.5 ml of concentrated perchloric acid ($HClO_4$, Merck, Germany). The solution was left to stand for one night and heated at 100°C for 1.5 hr after incubation. Subsequently, the temperature was raised to 150°C for 2.5 hr until the yellow steam was exhausted and further increased to 165°C for 1 hr to achieve white steam formation. For P and K measurements, the extract was diluted with deionized water to a volume of 50 ml, shaken until homogenous, and left overnight. A total of 1 ml of each sample extract was piped and standard series into a test tube for P measurement, added with 9 ml of deionized water and shaken. The 1 ml of diluted extract was pipetted, and the standard series was introduced into the test tube and added with 9 ml of color reagent of P. The solution obtained was incubated for 30 min, and the phosphorus concentration was measured using a spectrophotometer (Shimadzu UV 1800, Japan) at a wavelength of 889 nm. For K measurement, 1 ml of each sample extract was pipetted, and the standard series was introduced into a test tube. It was followed by adding 0.25% lanthanum chloride ($LaCl_3$, Merck, Germany) and shaking until homogenous, while K was measured using atomic absorption spectrophotometry (AAS, PG Instrument PG-990, China).

Determination of Leaf Sugar Content

Approximately 100 mg of leaf powder sample was placed into a centrifuge tube, and 10 ml of 80% ethanol (Merck, Germany) was added. A glass ball was placed on top of the tube and kept in a water bath at 80–85°C for 30 min. The solution was centrifuged, decanted into a 50 ml beaker glass, and repeated three times. Subsequently, the alcohol extract was evaporated in a water bath at 80–85°C to remove most alcohol. The distilled water was added to 25 ml, while 5 ml of extract was transferred to a 100 ml volumetric flask of distilled water. The 5 ml of diluted sugar extract (Merck, Germany) was put into a Pyrex test tube and an ice bath. To each tube, 10 ml of the anthrone reagent (HiMedia, India) was slowly added, allowing the reagent to run down the side of the test tube and stir with a glass rod. The tubes were put into a boiling water bath for 7.5 min and immediately cooled in ice, and the absorbance was measured at 630 nm.

Determination of Crude Fiber Content

The crude fiber was measured using the gravimetric method (Igile et al., 2013). During this process, filter paper was dried in an oven at 105°C and weighed. One gram of leaves powder was weighed, placed in Erlenmeyer glass, added with 200 ml of H_2SO_4 (Supelco, Germany), and boiled for 30 min. The solution was filtered with a Buchner funnel, and the residue was washed using hot distilled water until the washing water was no longer acidic. The residue obtained was transferred from filter paper to Erlenmeyer, and 200 ml of NaOH (Supelco, Germany) was added. The solution was boiled for 30 min, filtered with a Buchner funnel, and washed with hot distilled water to a non-alkaline state, followed by washing with 15 ml of 10% K_2SO_4 (Merck, Germany). The residue was washed with hot distilled water and added to 15 ml of 95% alcohol (PT. Brataco, Indonesia). Subsequently, the remnant was left on filter paper, dried in an oven at 105°C to achieve a constant weight equivalent to crude fiber, and cooled in a desiccator.

Determination of Total Phenol and Total Flavonoid Content

Total phenol was measured using Folin-Ciocalteau, while total flavonoid was determined with aluminum chloride colorimetric (Vongsak et al., 2013). Initially, 0.01 g of leaf powder was added with 4 ml of ethanol and macerated for 72 hr at room temperature and in a dark room. The solution was added to 10 ml with 70% ethanol (Supelco, Germany), shaken, and centrifuged at 4,427 x *g* for 7 min. Total phenol levels were measured by pipetting 100 µl, added with 2.5 ml of distilled water and 100 µl of Folin-Ciocalteau reagent (Merck, Germany). The solution was vortexed and incubated at 45°C for 15 min, followed by absorbance measurement using a spectrophotometer at a wavelength of 765 nm with the gallic acid standard. Flavonoid levels were measured by pipetting 2 ml of supernatant, adding 2 ml of 2% aluminum chloride ($AlCl_3$, Merck, Germany), and homogenizing. The solution was incubated for 10 min at room temperature and in a dark room. After incubation, the absorbance was measured using a spectrophotometer (Shimadzu UV-Vis Type 1280, Japan) at a wavelength of 415 nm with quercetin standard.

Determination of Antioxidant Activity

Antioxidant activity was measured using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) method (Cheng et al., 2016) and expressed by percentage of inhibition. The sample solution was made by dissolving the leaves powder with methanol in a ratio of 1:10. Subsequently, 0.1 ml of sample solution was pipetted to the test tube and added with 1 ml of 0.35 mM DPPH (Sigma Aldrich, Germany), and 4 ml ethanol (Supelco, Germany) was added, followed by vortexing, and incubation in a dark room as well as at room temperature for 30 min. The absence was measured using a spectrophotometer (Shimadzu 1240 UV-VIS, Japan) at a wavelength of 517 nm. The percentage of inhibition was calculated in the formula as follows.

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{x } 10$$

Data Analysis

The data were tested for normality using Shapiro-Wilk at $\alpha = 5\%$. Meanwhile, the normal distribution data were analyzed using analysis of variance (ANOVA) at $\alpha = 5\%$. When treatment had a significant effect, the analysis continued with Duncan's multiple range test at $\alpha = 5\%$. Shoot weight per plant at the first, second, and total harvest were analyzed using regression analysis.

RESULTS AND DISCUSSION

Soil Properties and Climate Conditions at Experimental Site

The soil properties showed a pH of 5.6 (slightly acid), with low C-organic and N-total at 1.77 and 0.18%, respectively. The content of total P_2O_5 was 203.51 mg/100 g (very high), total K_2O was 23.61 mg/100 g (medium), and the soil texture was silty clay loam. Consequently, *C. sulphureus* benefited from increased nitrogen input through fertilizer to enhance its vegetative growth.

The application of goat manure as organic fertilizer increased plant nutrient availability and the C-organic, including the N content of soil (Ekwealor et al., 2020). Furthermore, organic fertilizer increases soil pH, which correlates with plant nutrient availability and other biogeochemical processes such as soil enzyme activities, rhizosphere, and mineralization of organic matter (Neina, 2019).

In this study, an increase in soil pH was observed after planting. Nutrients absorbed by plant roots reduce the nutrient content in the soil. The addition of goat manure minimized the reduction of N, with P_2O_5 total increasing at 200 and 300 kg N/ha rates, while K_2O content increased at 200 and 300 kg N/ha, as shown in Table 1. Goat manure contains other macro and micronutrients (Uwah & Eyo, 2014). The P_2O_5 and K_2O content in goat manure was 1.7 and 2.01%, respectively. During the experiment, the average rainfall was estimated at 341.5 mm/month, with a temperature of 25.2°C and relative humidity of 68%.

Table 1
Soil properties before planting and after planting with the addition of different rates of goat manure

Parameters	Before planting	After planting with different rates of goat manure (kg N/ha)			
		0	100	200	300
pH H_2O	5.6 (SA)	5.9 (SA)	5.9 (SA)	6.3 (SA)	6.3 (SA)
C-organic (%)	1.77 (L)	1.32 (L)	1.39 (L)	1.66 (L)	1.61 (L)
N-total (%)	0.18 (L)	0.17 (L)	0.17 (L)	0.18 (L)	0.18 (L)
P_2O_5 (mg/100 g)	203.51 (VH)	195.6 (VH)	198.01 (VH)	217.99 (VH)	216.05 (VH)
K_2O (mg/100 g)	23.61 (M)	19.92 (L)	27.72 (M)	61.41 (VH)	41.67 (H)

Note. H_2O = Water; P_2O_5 = Phosphorus pentoxide; K_2O = Potassium oxide; SA = Slightly acid; L = Low; M = Medium; H = High; VH = Very high

Shoot Production of *C. sulphureus*

The yield component of *C. sulphureus* shoot production was evaluated based on weight and shoot number. The internode length of *C. sulphureus* was affected by rates of organic fertilizer in the first and second harvests. Based on the results presented in Table 2, an increase in the rates of goat manure significantly led to elevated internode length. It was attributed to the influence of nitrogen, enhancing stem development, including cell division and elongation (Souza & Tavares, 2021). An increase in shoot weight per plant in both the two and four-node harvesting criteria was also observed. The optimum rate of goat manure at the four-node was 266 kg N/ha, while the curve in the two-node harvesting

Table 2
Effect of goat manure rates and harvest criteria on internode length of shoot

Rates of goat manure (kg N/ha)	Internode length (cm)	
	First harvest	Second harvest
0	6.02±0.50b	6.45±0.21c
100	7.80±1.48ab	8.60±2.03b
200	9.64±2.95a	9.23±1.57b
300	9.91±1.01a	11.28±2.24a

Note. The number followed by the same letter in a column is not significantly different by the Duncan's multiple range test at the level of α = 5%. Numbers were followed by ± standard deviation

criteria was linear, as shown in Figure 1. The increasing rates of shoot weight in four nodes due to the addition of nitrogen rates were higher than in two-node harvesting.

The second harvest was carried out one week after the first harvest by cutting all the lateral branches according to harvesting criteria. An interaction was observed between the effect on shoot weight in the second harvest. As shown in Table 3, an increasing shoot weight of two-node harvesting was obtained from 300 kg N/ha compared to the control treatment, while the four-node shoot weight increased to 100 kg N/ha. Shoot numbers in the second harvest were affected by harvesting criteria and rates of goat manure, where the two nodes yielded higher results, as shown in Table 4. This result variation was caused by fewer shoots that met the four-node harvesting criteria. More shoots were carried out at the first four-node harvesting from the bud at the leaves axillar or the nodes.

The increasing availability of nutrients by adding organic fertilizer also enhanced branch growth in the second harvest. Table 4 shows that the application of goat manure plays a significant role in vegetative growth, increasing the harvested shoot number in the second harvest. This result is in line with

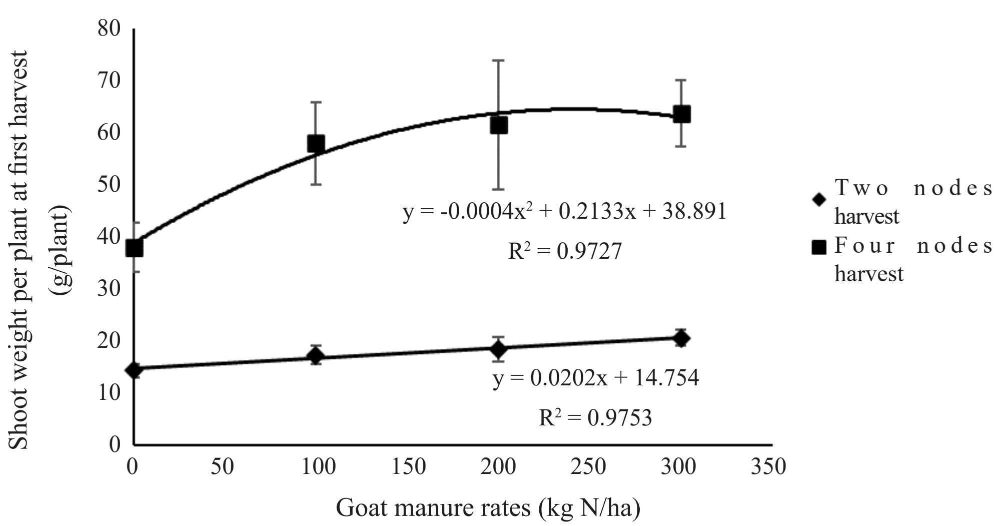

Figure 1. The increasing shoot weight per plant at several rates of goat manure at the first harvest

Table 3
Interaction effect of goat manure rates and harvest criteria on shoot weight (g) at second harvest

Treatment (kg N/ha)	Two-node	Four-node
0	4.81±1.71d	9.29±0.70c
100	5.42±1.85d	13.39±0.42b
200	5.37±1.51d	14.78±0.60b
300	6.16±0.92cd	17.91±0.46a

Notes. *Note*. The number followed by the same letter is not significantly different by the Duncan's multiple range test at the level of α = 5%. Numbers were followed by ± standard deviation.

Table 4
Effect of goat manure rates and harvest criteria on shoot number in the second harvest

Treatment	Shoot number
Harvest criteria	
Two-node	9.2±1.79a
Four-node	5.6±3.81b
Rates of goat manure (kg N/ha)	
0	6.2±1.79b
100	7.1±2.34ab
200	8.3±2.29a
300	8.1

Note. The number followed by the same letter in a column is not significantly different by the Duncan's multiple range test at the level of α = 5%. Numbers were followed by ± standard deviation

a previous study, where the application of organic fertilizer increased the significance of branches on chili (Khandaker et al., 2017). Similarly, there was an increase in shoot weight and shoot weight per plant in the second harvest at an optimum rate of 300 kg N/ha. This condition showed that goat manure was needed for *C. sulphureus* shoot production with repeated harvesting. The optimum rates of goat manure to obtain maximum shoot weight per plant in the second harvest for two and four were 509.5 and 608.5 kg N/ha, respectively, as presented in Figure 2. The difference in the optimum points during the first and second harvests showed that adding goat manure repeatedly or increasing the goat manure rates to the soil was still necessary to obtain the maximum yields in the second harvest.

The total harvest was the accumulated shoot weight of the first and second harvests. Based on the results, harvesting four nodes of *C. sulphureus* resulted in a significant 98.3% increase in the average total shoot weight per plant. The 324.0 and 739.5 kg N/ha were the optimum rates to obtain the maximum yield of *C. sulphureus* with four and two-node harvest criteria, respectively (Figure 3).

Goat manure contains macro and micronutrients essential for plant growth, contributing significantly to soil fertility. Apart from nitrogen addition into the soil, goat manure was also added at rates of 6-, 12-, and 18-ton/ha to 69.4, 138.8, and 208.2 kg of P_2O_5, as well as 82, 164.1, and 246.1 kg of K_2O, respectively. Furthermore, goat manure enhances soil organic content, improving proper soil structure conducive to root growth and increasing plants' uptake of water and nutrients. Although N is essential in plant vegetative growth, it is also affected by macronutrients such as P and K. Phosphorus availability to plants is beneficial to root growth (D. Liu, 2021), causing an increase in nutrient

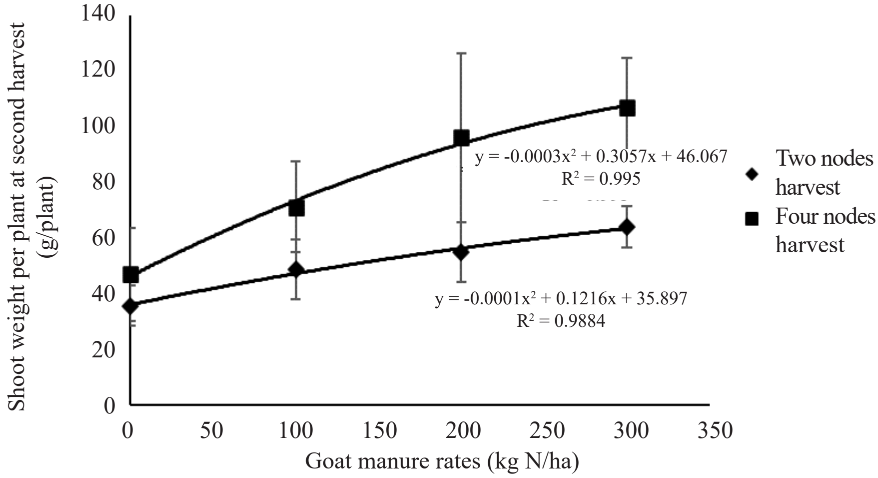

Figure 2. The increasing shoot weight per plant at several rates of goat manure at the second harvest

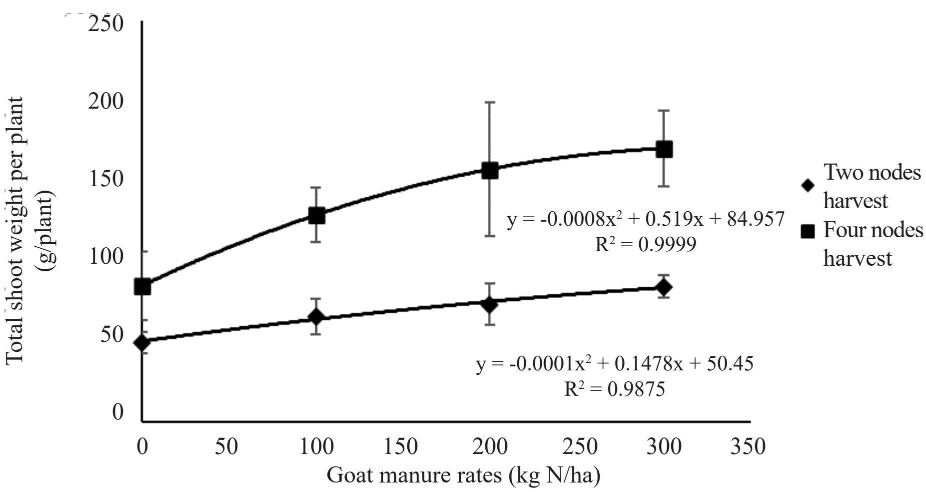

Figure 3. The increasing total shoot weight per plant at several rates of goat manure

uptake. Pearson correlation analysis showed that P concentration was positively correlated (P<0.05) with N (r = 0.87) and K concentration (r = 0.46) in leaves (Figure 4).

Phosphorus plays a significant role in adenosine triphosphate (ATP) formation and is included in some plant metabolism, such as photosynthesis, specifically in electron transport in light reactions (Carstensen et al., 2018). Potassium is also included in the essential macronutrients for plants, functioning in carbon dioxide (CO_2) assimilation in photosynthesis and photosynthate translocation from source to sink (Mengel, 2016). Photosynthesis is the primary plant metabolism that directly affects plant growth and development. All the roles of the nutrient nutrients in organic fertilizer are included in photosynthesis, which affects plant growth.

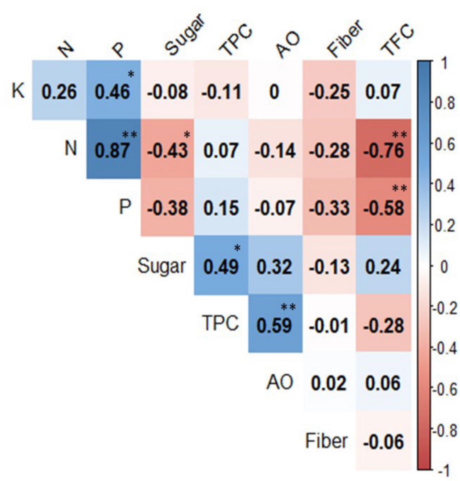

Figure 4. Pearson correlation analysis of metabolite content in leaves of *Cosmos sulphureus*

Note. TPC = Total phenolic content; TFC = Total flavonoid content; AO = Antioxidant activity; ** = Correlation significant at the 0.01 level; * = Correlation significant at the 0.05 level

Metabolite Content and Antioxidant Activity of Different Leaves Position

In this study, four-node harvest criteria showed a higher yield of *C. sulphureus*, showing the need to explore the physiological characteristics of leaves from different positions. Harvesting four included leaves in the third and fourth nodes below the first and second nodes. The results also showed that the lower leaves were older, showing an age difference between the two types. It suggested that the physiological characteristics of leaves, namely fiber and secondary metabolites, were also different (de los Santos et al., 2016; Nobossé et al., 2018). The nutrient content of leaves, including nitrogen, phosphorus, and potassium, was significantly different between the positions of leaves, as shown in Table 5. The upper leaves have higher N, P, and K content, which are the mobile nutrients in plant tissue. These elements were translocated from older leaves into young ones, resulting in decreased nitrogen content as the age increased (Onyango et al., 2012).

Despite goat manures supplying more plant nutrients, N, P, and K levels did not significantly increase in leaves. It was attributed to the characteristic slow release of nutrients from goat manure as organic fertilizer, resulting in an insignificant increase in nutrient levels. The nitrogen content in leaves also did not increase with manure application in amaranth, but a significant elevation was observed when mineral fertilizer was supplied (Onyango et al., 2012). However, nutrient uptake of *C. sulphureus* increased due to enhanced biomass (yield) and the addition of organic fertilizer. It is consistent with C.-W. Liu et al. (2014), who stated that applying organic fertilizer increased the dry weight of lettuce.

In contrast with N, P, and K concentrations, the sugar content was higher in the upper leaves (Table 6). As old leaves tend to meet senescence, nitrogen is translocated into young leaves, increasing the hexose/sucrose ratio at the start of senescence and accumulating soluble sugar in leaves (Agüera & De la Haba, 2018). However, the low content of minerals led

Table 5
Effect of goat manure rates and leaf position on N, P, and K concentration in leaves

Treatment	N (%)	P (%)	K (%)
Leaf position			
Upper leaves	5.01±0.17a	0.47±0.02a	2.35±0.63a
Lower leaves	4.25±0.20b	0.31±0.03b	1.91±0.54b
Rates of goat manure (kg N/ha)			
0	4.64±0.46a	0.38±0.10a	2.15±0.50a
100	4.55±0.50a	0.40±0.10a	2.20±0.80a
200	4.60±0.41a	0.39±0.08a	2.19±0.65a
300	4.73±0.44a	0.40±0.09a	1.98±0.62a

Note. The number followed by the same letter in a column is not significantly different by the Duncan's multiple range test at the level of $\alpha = 5\%$. Numbers were followed by ± standard deviation

Table 6
Effect of goat manure rates and leaf position on total sugar and crude fiber in leaves

Treatment	Total sugar (%)	Crude fiber (%)
Leaf position		
Upper leaves	2.30±0.39b	11.17±1.59a
Lower leaves	2.59±0.26a	12.31±2.13a
Rates of goat manure (kg N/ha)		
0	2.59±0.34a	11.01±1.40b
100	2.52±0.46a	11.13±1.18b
200	2.43±0.23a	11.41±1.22b
300	2.24±0.35a	13.42±2.77a

Note. The number followed by the same letter in a column is not significantly different by the Duncan's multiple range test at the level of $\alpha = 5\%$. Numbers were followed by ± standard deviation

to an increase in soluble carbohydrates (Sung et al., 2015), which occurred in the organic fertilizer rates treatments without affecting the sugar content of leaves. A negative correlation was also observed between nitrogen and leaf sugar content in *C. sulphureus* leaves (P<0.05, r = -0.43) (Figure 4). This condition occurred in stevia, where the increased nitrogen content was followed by a decrease in sugar levels (Sun et al., 2019).

The consumption of vegetables is essential for supplying dietary fiber, comprising residues from edible plant cells, including cellulose, hemicellulose, pectin, and noncarbohydrate residues such as lignin that resist hydrolysis by human digestive enzymes (Dai & Chau, 2017; DeVries et al., 1999). The fiber content in leafy vegetables must be considered due to its influence on consumer preference. Furthermore, texture and flavor are important characteristics of consumer acceptance of leafy vegetables

(Gil et al., 2012). This study observed no significant difference in crude fiber content between upper and lower leaves (Table 6). The fiber content of *C. sulphureus* leaves was affected by rates of organic fertilizer. The 300 kg N/ha rates significantly increased the crude fiber compared to lower nitrogen application. The fertilizer application of NPK and manure increased crude fiber content in amaranth compared to the control treatment (Oyedeji et al., 2014). In contrast with the previous study in radishes, high nitrogen input decreased crude fiber in leaves (Yousaf et al., 2021).

Phenols and flavonoids are secondary metabolites in plants, playing a significant role as antioxidants (Zeb, 2020). In this study, the total phenolic content (TPC) and total flavonoid content (TFC) of *C. sulphureus* leaves ranged from 19.05 to 20.66 mg GAE/g dry weight and 14.11 to 19.60 mg QE/g dry weight, respectively. These values were lower compared to a previous study, where the TPC of *C. sulphureus* leaves was 40.74 mg GAE/g dry extract (Ortega-Medrano et al., 2023). Although organic fertilizer rates and leaves position did not affect the phenol content, there was a difference in total flavonoid content between the upper and lower leaves. As presented in Table 7, the lower leaves had a 38.9% higher total flavonoid than the upper ones. In line with studies in *Moringa oleifera* leaves, TPC was increased with maturity (Nobossé et al., 2018), while total flavonoids decreased (B. Wang et al., 2018).

A negative correlation was found between nitrogen and total flavonoid content in leaves ($P<0.05$, $r = -0.76$) (Figure 4). Meanwhile, nitrogen affected phenylalanine ammonia-lyase (PAL) enzyme activity that catalyzed flavonoid biosynthesis (Deng et al., 2019). The phenol and flavonoids were also synthesized through shikimate

Table 7
Effect of goat manure rates and leaf position on total phenolic contents, total flavonoid contents, and antioxidant activity in leaves

Treatment	TPC (mg GAE/g dw)	TFC (mg QE/g dw)	Antioxidant activity (% inhibition)
Leaf position			
Upper leaves	20.52±3.87a	14.11±2.77b	76.82±15.72a
Lower leaves	19.55±3.12a	19.60±3.63a	81.31±14.93a
Rates of goat manure (kg N/ha)			
0	20.66±3.01a	16.99±4.53a	74.07±15.91a
100	21.11±4.60a	18.86±3.86a	76.84±19.27a
200	19.05±3.06a	16.06±4.74a	76.94±16.78a
300	19.31±3.48a	15.50±4.05a	88.42±2.41a

Note. The number followed by the same letter in the column is not significantly different by the Duncan's multiple range test at the level of α = 5%. Numbers were followed by ± standard deviation; TPC = Total phenolic contents; TFC = Total flavonoid contents; GAE = Gallic acid equivalent; QE = Quercetin equivalent; dw = Dry weight pathways, which catalyzed carbohydrates to produce aromatic amino acids. A positive correlation was observed between starch and C content with flavonoid content (Deng et al., 2019). Furthermore, total phenol content was positively correlated with sugar content ($P<0.05$, $r = 0.49$) (Figure 4). It showed that the high sugar content of *C. sulphureus* leaves also enhanced the phenol content.

The position of leaves and organic fertilizer rates did not affect antioxidant activity but were influenced by metabolite content. In this study, antioxidant activity was positively correlated with total phenol content ($P<0.05$, $r = 0.59$) (Figure 4). Some studies with different species also showed a correlation between TPC and antioxidant activities (Aryal et al., 2019). Furthermore, phenolic compounds have shown the ability to stabilize free radicals by donating H-atom to free radical substrate (Chen et al., 2020; Zeb, 2020).

CONCLUSION

This study showed that applying organic fertilizer increased the shoot production of *Cosmos sulphureus* Cav. The harvest criteria affected the shoot weight per plant, where the four-node harvest was 98.3% higher than the two-node. The characteristics of leaves and metabolite content consumers consider when consuming vegetables, such as crude fiber, phenolic content, and antioxidant activity, were not significantly different between upper and lower leaves. Furthermore, the lower leaves had a higher flavonoid and total sugar content at 38.9 and 12.6% than the upper leaves. Based on these results, a four-node harvest is recommended as the criteria for the shoot harvest of *C. sulphureus*.

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