BIODIVERSITAS
Volume 24, Number 10, October 2023
Pages: 5739-5746

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241056

Morpho-physiology and metabolite content of *Cosmos caudatus* Kunth. and yellow and orange *Cosmos sulphureus* Cav.

ISMAIL SALEH[1,2], SANDRA ARIFIN AZIZ[3,♥], MAYA MELATI[3], NURI ANDARWULAN[4]
[1]Program of Agronomy and Horticulture, Graduate School, Institut Pertanian Bogor. Jl. Meranti, IPB Darmaga Campus, Bogor 16680, West Java, Indonesia
[2]Program of Agrotechnology, Faculty of Agriculture, Universitas Swadaya Gunung Jati. Jl. Pemuda No. 32 Cirebon 45132, West Java, Indonesia
[3]Depatment of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, IPB Darmaga Campus, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629353, ♥email: sandraaziz@apps.ipb.ac.id.
[4]Department of Food Science and Technology, Faculty of Agricultural Engineering, Institut Pertanian Bogor. Jl. Lingkar Akademik, IPB Darmaga Campus, Bogor 16680, West Java, Indonesia

Manuscript received: 7 August 2023. Revision accepted: 30 October 2023.

Abstract. *Saleh I, Aziz SA, Melati M, Andarwulan N. 2023. Morpho-physiology and metabolite content of* Cosmos caudatus *Kunth. and yellow and orange* Cosmos sulphureus *Cav. Biodiversitas 24: 5739-5746. Cosmos* sp., known as *kenikir* in Indonesia, is an underutilized vegetable commodity. *Cosmos sulphureus* Cav. (*C. sulphureus*), with orange and yellow flowers, is commonly found as an ornamental plant. *C. sulphureus* can also be used as vegetable crops, although the *Cosmos* species widely used as a vegetable is *Cosmos caudatus* Kunth. (*C. caudatus*). This study investigated the growth, shoot production, primary and secondary metabolite content, and antioxidant activity of yellow and orange *C. sulphureus* and *C. caudatus*. This experiment was arranged using a Randomized Completely Block Design (RCBD) with one factor: *Cosmos* species (yellow and orange *C. sulphureus* and *C. caudatus*) with six replications. The result showed that plant height, leaf number, and branch number were not significantly different among the three *Cosmos* studied; however, the shoot weight per plant of orange *C. sulphureus* was significantly higher than the others. The antioxidant activity and phenol content of *C. sulphureus* were lower than that of *C. caudatus*. Meanwhile, crude fiber, flavonoid, anthocyanin, and carotenoid content were not significantly different among the three kinds of *Cosmos*. Orange *C. sulphureus* could be a functional vegetable, although its antioxidant activity is lower than *C. caudatus*.

Keywords: Antioxidant, carotenoid, flavonoid, functional vegetable, phenol

INTRODUCTION

Vegetables play an important role in the human diet. It provides some minerals, vitamins, fiber, and bioactive compounds that may act as antioxidants (Arias et al. 2022). Some studies showed that sufficient consumption of vegetables reduces disease risks such as cancer (Alzate-Yepes et al. 2023) and prevents obesity (Gariballa et al. 2023). Unfortunately, the consumption of fruit and vegetables in Indonesian society is still lower than the FAO recommendation (Khairunnisa et al. 2022). The Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommendation for fruit and vegetable consumption is 400 g per day (Růžičková and Kohout 2023). Therefore, efforts are needed to increase the consumption of vegetables, including introducing various kinds of vegetables. The introduction of several types of vegetables increases the variability of vegetable consumption in society. Some vegetables cultivated in the yard but underutilized can meet society's nutritional needs (Tanimonure et al. 2021).

Indonesia, located in a tropical climate, has an enormous biodiversity. About 20,000 Angiosperm species are found in Indonesia (Pullailah et al. 2015). *Cosmos* sp., which has a local name as *kenikir*, is an Angiosperm species usually found as wild plants in Indonesia. They are used as ornamental or vegetable crops. *Cosmos* sp. consisted of several species. The common ornamental *Cosmos* species is *Cosmos sulphureus* Cav., i.e., yellow and orange flowers. Previous research showed that *C. sulphureus* can be used as refugial plants because they host natural enemies of some pests in rice (Aldini et al. 2019), used as natural dyes, a source of pollen bees, and bioherbicides (Wroblewska et al. 2016; Aldini et al. 2019; Respatie et al. 2019). The young leaves of *C. sulphureus* are consumed as vegetables; however, *Cosmos caudatus* Kunth. is also commonly consumed as vegetable (Puttock 2022). The difference between *C. caudatus* and *C. sulphureus* is the size and color of the flower. *C. caudatus* has smaller purplish-pink flowers (Datiles 2022).

The use of *C. sulphureus* as a vegetable crop is an effort to introduce new types of vegetables to the community. Both *C. sulphureus* and *C. caudatus* could be categorized as functional vegetables because they contain several bioactive benefits for human health. Functional foods potentially positively affect health because they have ingredients that improve health or are proven to prevent certain diseases (Granato et al. 2017). Therefore, *Cosmos* sp. as vegetable crops could be used to improve human health.

Cosmos caudatus and *C. sulphureus* leaves contain several secondary metabolites such as phenols, flavonoids, anthocyanins, and vitamin C, which act as antioxidants, antibacterial, and anticancer (Andarwulan et al. 2010;

Phuse and Khan 2018). Flavonoids in *C. sulphureus* include flavonols, anthocyanins, and chalcones (Andrushchenko and Levon 2021). Anthocyanin is a flavonoid type found in leaves. Anthocyanin is an efficient antioxidant that effectively scavenges free radicals. Phenolic content acts as an antioxidant. Hence, the bioactive compound and antioxidant activity in *C. sulphureus* leaves are food supplements that increase human nutrition and benefit human health (Phuse and Khan 2018; Andrushchenko and Levon 2021).

C. sulphureus has not been widely used as a vegetable, and its health potential is unknown. There is no information regarding the differences in growth, shoot production, and metabolite content between the two types of *C. sulphureus* (yellow and orange flowers) and *C. caudatus*. This information is required to determine the potential of the two types of *C. sulphureus* to be developed as vegetable crops and ornamental plants. Therefore, this study aims to determine the differences in growth, yield (shoot production), and metabolite content of two types of *C. sulphureus* and *C. caudatus*.

MATERIALS AND METHODS

Study area

The field experiment was carried out from May to July 2022. The experiment was conducted at Mandirancan, Kuningan District, West Java, Indonesia (6°48'0.31" S, 108°28'17.5" E) at an altitude of 296 m above sea level (asl). Laboratory analysis was conducted at The Postharvest Laboratory Department of Agronomy and Horticulture, Institut Pertanian Bogor, to analyze N, P, K, total sugar, total flavonoid, and total phenolic content; and at The Laboratory of Indonesian Medicinal and Aromatic Crops Research Institute to analyze crude fiber and antioxidant activity.

Plant materials

Seeds of three *Cosmos* spp. (*C. caudatus*, *C. sulphureus* yellow flowers, and *C. sulphureus* orange flowers) were collected from Cirebon, Indonesia. Plants used for seed sources were identified at Herbarium Bogoriense, Research Center for Biosystematics and Evolution. Goat manure was used as the only fertilizer.

Procedures

This experiment used a randomized completely block design (RCBD). The treatment included three *Cosmos* species, namely *C. caudatus*, *C. sulphureus* yellow flowers (yellow *C. sulphureus*), and *C. sulphureus* orange flowers (orange *C. sulphureus*). Each treatment has six replicates; hence, there were 18 experimental units.

Each experimental unit was a plot of 2 × 3.5 m. Firstly, seeds were soaked in water for two hours and then sown for 12 days. The planting media for seedlings were soil, manure, and rice-hull charcoal (1:1:1) (v/v). After 12 days, seedlings were planted with 30 × 50 cm plant spacing. Goat manure as much as 20 t ha^{-1} was applied two weeks before planting.

Growth parameters that were observed were plant height (cm), leaf number, stem diameter (mm), shoot diameter (cm), branch number, shoot diameter, relative growth rate (g g^{-1} day^{-1}), and net assimilation rate (g cm^{-2} day^{-1}). Growth observation was conducted 2, 4, and 6 weeks after planting (WAP). Plant yield parameters included the number of harvested shoots per plant, shoot weight (g), and shoot weight per plant (g $plant^{-1}$) at the first and second harvests. Shoots were harvested at 6 WAP, then at 7 WAP. The nitrogen (N), phosphorus (P), potassium (K) content, crude fiber, sugar, and chlorophyll in leaves were measured at 6 WAP.

The analysis of bioactive compounds included phenol, flavonoid, anthocyanin, carotenoid, and antioxidant activity. Chlorophyll, anthocyanin, and carotenoid were measured using the spectrophotometric method (Sims and Gamon 2002). The gravimetric method measured crude fiber (Igile et al. 2013). Aluminum chloride colorimetric and the Folin-Ciocalteu were used to measure phenol content and total flavonoid, respectively (Vongsak et al. 2013). Next, the 2.2-diphenyl-1-picrylhydrazyl (DPPH) method was used to measure the antioxidant activity (Cheng et al. 2016) and expressed as an IC_{50} value.

Data analysis

The data were analyzed using analysis of variance (ANOVA) with a significance level (α) of 5% using SPSS software ver. 25. The differences among treatments were analyzed further with Tukey post hoc with α = 5%. The correlation analysis among the variables was analyzed by using Pearson correlation.

RESULTS AND DISCUSSION

Soil properties in the experimental site

The soil properties before the experiment were: a pH of 5.14 (acid), the content of C-organic, N-total, P-total, P-available, K-total, and cation exchange capacity was 1.11% (low), 0.17% (low), 205.83 mg P_2O_5 (100 g)$^{-1}$ (very high), 6.76 ppm P_2O_5 (low), 28.19 mg K_2O (100 g)$^{-1}$ (moderate), and 16.74 cmol kg^{-1} (low), respectively. Those conditions were unsuitable for plant growth due to the low nutrient availability for plants. Adding organic fertilizer increased C-organic in soil and the availability of N, P, and K (Lasmini et al. 2018). The rainfall during the experiment was an average of 220 mm $month^{-1}$ with an average temperature of 26°C and 80 % relative humidity.

Plant growth of *Cosmos caudatus* and *Cosmos sulphureus*

There were no significant differences in plant height, leaf number, and branch number of three types of *Cosmos* at 6 WAP. Plant descriptions of *C. caudatus* and *C. sulphureus* show a similar height of 0.3-2.0 m (Datiles 2022; Puttock 2022). In early growth, *C. caudatus* has a shorter and thinner stem diameter but a higher leaf number than *C. sulphureus*. At the end of the observation, the stem diameter of orange *C. sulphureus* was higher than that of *C. caudatus*. The lowest shoot diameter was found in yellow *C. sulphureus* (Table 1).

Variables of plant growth observed in this study were related to the shoot production of *Cosmos* sp. because the shoots were harvested for vegetable sources. Correlation analysis showed that the leaf number positively correlated ($P<0.05$) with shoot diameter (r = 0.722), branch number (r = 0.791), harvested shoot number per plant (r = 0.627), and shoot weight per plant (r = 0.626). The leaf is a plant organ that carries out photosynthesis, and the photosynthate is translocated into all plant organs. Translocated assimilate is then used to stimulate the formation of lateral shoots that will become new branches (Mason et al. 2014). Increasing the number of branches was followed by increasing shoot diameter (r = 0.714).

Relative growth rate (RGR) is defined as the growth rate relative to size per unit of time, and net assimilation rate (NAR) is an increase of plant weight per unit of time and unit of leaf area (Poorter 1989). RGR and NAR from the *Cosmos* plant were not significantly different in 2-4 WAP and 4-6 WAP (Table 2). This condition was probably caused by the small plant growth components that were not significantly different (Table 1.). The RGR value decreased from 2-4 WAP to 4-6 WAP. It means that the rapid growth of the *Cosmos* occurred in the early growth at 2-4 WAP. The plant growth curve is generally in the form of a sigmoid. The curve starts from the exponential phase (fast growth) at the beginning of growth, followed by a linear and logarithmic phase at the senescence phase (Lamont et al. 2023).

In contrast to RGR, NAR increased to 4-6 WAP. Leaf area increases as the plant ages, so the assimilate produced also increases. NAR provides information regarding CO_2 assimilation through the photosynthesis process. Photosynthate was then translocated into several organs, such as roots, stems, and young leaves (Weraduwage et al. 2015).

Table 1. Plant height, leaf number, stem diameter, branch number, and shoot diameter of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**2 WAP**	**4 WAP**	**6 WAP**
Plant height (cm)			
Yellow *C. sulphureus*	6.73 ± 0.7 a	14.72 ± 1.4 a	44.75 ± 1.6 a
Orange *C. sulphureus*	6.88 ± 0.6 a	14.10 ± 0.7 a	39.92 ± 3.2 a
C. caudatus	5.23 ± 0.6 b	12.43 ± 0.9 b	40.28 ± 6.4 a
Leaf number			
Yellow *C. sulphureus*	4.9 ± 0.6 ab	22.2 ± 7.3 a	73.6 ± 13.7 a
Orange *C. sulphureus*	4.1 ± 0.2 b	31.0 ± 3.5 a	85.9 ± 9.0 a
C. caudatus	5.2 ± 0.8 a	26.5 ± 6.0 a	86.2 ± 11.5 a
Stem diameter (mm)			
Yellow *C. sulphureus*	1.83 ± 0.2 a	3.87 ± 0.4 a	5.10 ± 0.5 ab
Orange *C. sulphureus*	1.65 ± 0.2 ab	3.94 ± 0.4 a	5.50 ± 0.4 a
C. caudatus	1.60 ± 0.2 b	3.24 ± 0.2 b	4.54 ± 0.6 b
Branch number			
Yellow *C. sulphureus*	-	7.2 ± 2.2 a	16.5 ± 1.5 a
Orange *C. sulphureus*	-	7.8 ± 0.7 a	23.0 ± 5.7 a
C. caudatus	-	7.4 ± 1.0 a	21.0 ± 4.4 a
Shoot diameter (cm)			
Yellow *C. sulphureus*	10.08 ± 1.0 b	23.43 ± 2.6 b	42.00 ± 3.9 b
Orange *C. sulphureus*	10.45 ± 1.0 b	29.87 ± 2.5 a	50.85 ± 3.7 a
C. caudatus	11.83 ± 0.6 a	29.93 ± 1.5 a	51.73 ± 3.3 a

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard. WAP: weeks after planting

Table 2. Relative growth rate (RGR) and net assimilation rate (NAR) of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**2-4 WAP**	**4-6 WAP**
RGR ($g\ g^{-1}\ day^{-1}$)		
Yellow *C. sulphureus*	0.176 ± 0.04 a	0.158 ± 0.03 a
Orange *C. sulphureus*	0.195 ± 0.03 a	0.161 ± 0.03 a
C. caudatus	0.179 ± 0.02 a	0.167 ± 0.04 a
NAR ($mg\ cm^{-2}\ day^{-1}$)		
Yellow *C. sulphureus*	1.036 ± 0.24 a	1.336 ± 0.20 a
Orange *C. sulphureus*	1.107 ± 0.19 a	1.335 ± 0.31 a
C. caudatus	1.123 ± 0.19 a	1.736 ± 0.35 a

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard. WAP: weeks after planting. RGR: relative growth rate, NAR: net assimilation rate

Shoot production of *Cosmos caudatus* and *Cosmos sulphureus*

The shoot production of *Cosmos* was determined by several yield components, i.e., the shoot number (harvested shoot) and shoot weight in both the first and second harvests. The shoot weight of three *Cosmos* sp. at the first and second harvests and the shoot number (harvested shoot) at the second harvest were significantly different; meanwhile, the internode length was not significantly different (Table 3). *C. sulphureus*, orange flowers, had a higher shoot weight per plant than the other two *Cosmos* sp. at the first and second harvests. The shoot weight of all *Cosmos* studied was lower in the second harvest than in the first harvest (Figure 1).

Harvesting shoots repeatedly causes the shoot weight per plant to decrease in the next harvest because the shoot size is smaller than the previous harvest. Cutting shoots at harvest will induce the growth of lateral branches that are relatively smaller than the main stem (Saleh et al. 2014). The third harvest in this study could not be carried out because the flower of all *Cosmos* flowers emerged at 8 WAP. The emerging flowers in *Cosmos* inhibit the formation of new leaves (Rahanita et al. 2015). Flowering shoots have smaller leaves and elongated top internode (McKim 2020).

Physiological characteristics and metabolite content of leaves

Nitrogen, phosphorus, and potassium are nutrients that plants need. Those nutrients affect some metabolism processes in plants. Although the amount of organic fertilizer added to the plants was the same, there was a difference in the uptake and content of these nutrients. *C. sulphureus* yellow flowers have the highest nitrogen content but the lowest potassium content (Table 4). Nitrogen in plants is important, especially in vegetative growth (Khan et al. 2005). From the Pearson correlation analysis, there was a correlation (P<0.05) between leaf nitrogen content with plant height (r = 0.470) and internode length (r = 0.552). Nitrogen also affects plant phenology. Increasing plant nitrogen availability causes a shorter phenological duration (Wang and Tang 2019). In this study, yellow *C. sulphureus* flower faster than the others. At 6 WAP, 50% of the plant population has emerged flower buds, while only 30% and 37% of orange *C. sulphureus* and *C. caudatus* have flower buds, respectively (Figure 2).

Phosphorus content was not significantly different among the three types of *Cosmos* sp. Phosphorus also has an essential role in the photosynthesis process. It functions in ATP formation in light reaction electron transport (Carstensen et al. 2018). In this study, Phosphorus positively correlated with harvested shoot weight in plants (P<0.05, r = 0.618). The photosynthate translocation from leaves to the sink organs was regulated by potassium (Xu et al. 2020). Potassium levels were positively correlated to the net assimilation rate in plants (P<0.05, r = 0.571). It showed that the higher the leaf potassium level, the increased net assimilation rate. The main impact of potassium deficiency is a decrease in photosynthetic capacity or CO_2 assimilation (Tränkner et al. 2018). In addition, K content in leaves also had a positive correlation with the diameter of the shoot (P<0.05, r = 0.559). The diameter of the shoot was affected by lateral branches. The growth of lateral buds into lateral branches was affected by sugar content from photosynthesis (Mason et al. 2014). Thus, photosynthesis and photosynthate translocation have an important role in plant growth.

In this study, there was no significant difference in crude fiber content among the three kinds of *Cosmos*. Meanwhile, the sugar content of *C. caudatus* leaves was higher than that of *C. sulphureus* (Table 5). Vegetable is a rich source of fiber (Wang et al. 2016). Fiber consumption reduces the risk of cancer, diabetes, and obesity (Zhang et al. 2013; Sarker and Wang et al. 2016; Sarker and Rahman 2017). However, the factors that affect consumer preference in consuming vegetables are taste and texture. Quality attribute desired by consumers when consuming *Cosmos* leaves was not too fibrous and did not taste bitter (Yurlisa et al. 2018). Therefore, it was expected that the texture of leaves between *C. caudatus* and *C. sulphureus* would be the same. *C. caudatus* leaves contain higher sugar levels and are expected to be sweeter than *C. sulphureus*. Glucose is a form of sugar highly correlated with the perception of sweet taste (Chadwick et al. 2016).

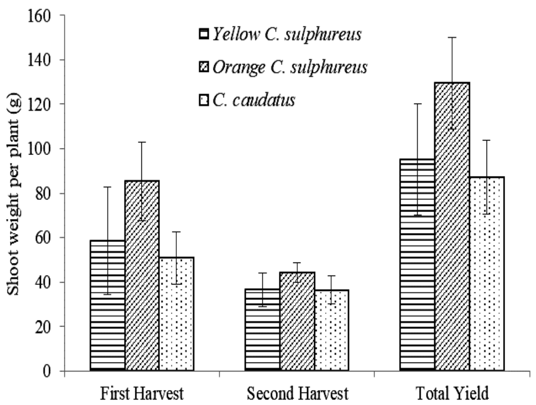

Figure 1. Shoot weight per plant at the first harvest, second harvest, and total yield of shoots of *Cosmos caudatus* and *C. sulphureus*

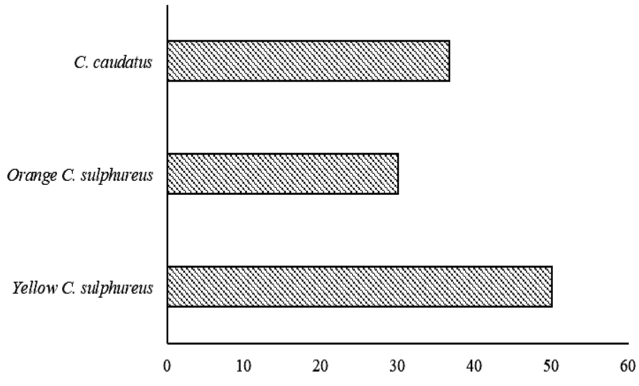

Figure 2. Percentage of flowering plants at 6 WAP of *Cosmos caudatus* and *C. sulphureus*

Table 3. Number of shoots per plant and shoot weight of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**Internode length (cm)**	**Harvested shoots number per plant**	**Shoot weight (g)**
First harvest (6 WAP)			
Yellow *C. sulphureus*	12.17 ± 1.2 a	5.0 ± 2.2 a	12.09 ± 1.5 b
Orange *C. sulphureus*	10.21 ± 1.2 a	5.9 ± 1.1 a	14.40 ± 1.0 a
C. caudatus	10.28 ± 1.9 a	4.0 ± 1.3 a	13.15 ± 1.7 ab
Second harvest (7 WAP)			
Yellow *C. sulphureus*	10.31 ± 1.9 a	5.0 ± 1.1 ab	7.44 ± 1.3 ab
Orange *C. sulphureus*	8.02 ± 1.7 a	4.7 ± 0.4 b	9.42 ± 1.1 a
C. caudatus	7.99 ± 2.1 a	6.0 ± 0.5 a	6.17 ± 1.3 b

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard. WAP: weeks after planting

Table 4. N, P, K content in leaves of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**N (%)**	**P (%)**	**K (%)**
Yellow *Cosmos sulphureus*	4.71 ± 0.3 a	0.39 ± 0.03 a	2.42 ± 0.2 b
Orange *Cosmos sulphureus*	4.28 ± 0.2 b	0.39 ± 0.03 a	2.71 ± 0.3 ab
Cosmos caudatus	4.47 ± 0.4 ab	0.41 ± 0.04 a	3.11 ± 0.2 a

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard

Table 5. Crude fiber and sugar content in leaves of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**Crude fiber (%)**	**Sugar (%)**
Yellow *Cosmos sulphureus*	17.95 ± 9.1 a	3.71 ± 0.4 b
Orange *Cosmos sulphureus*	22.17 ± 6.7 a	3.58 ± 0.7 b
Cosmos caudatus	14.34 ± 2.0 a	5.56 ± 1.0 a

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard

The content of leaf pigments, including chlorophyll a, b, and total, was not significantly different between the two *Cosmos* species (Table 6). These pigments were energy receptors from sunlight (photons), and light is the source of energy in the light reactions of photosynthesis (Taiz and Zeiger 2006). Photosynthesis produces carbohydrates for plant growth and development and is involved in secondary metabolites biosynthesis. Accessory pigments involved in photosynthesis include carotenoids (Son et al. 2021), which belong to the terpenoid group. The function of terpenoids in plants involves photosynthesis, photomorphogenesis, photoprotection, and plant growth and development. Carotenoids possess antioxidant activity and provitamin A for humans (Nisar et al. 2015; Wang et al. 2019). Carotenoid is photoreceptors of the spectral region that is not covered by chlorophyll (Collini 2019). The chlorophyll a and b contents positively correlated with carotenoids using Pearson correlation (P<0.05) with Pearson coefficients of 0.908 and 0.711, respectively. Increasing chlorophyll a, b, and carotenoid levels in leaves are thought to increase the photosynthesis rate and plant growth. The relationship between chlorophyll a and b to CO_2 assimilation also was explained by previous research in soybeans (Singh et al. 2017). From the correlation analysis, increasing carotenoid levels positively affected stem diameter (r = 0.576), harvested shoot number (r = 0.525), and shoot weight per plant (r = 0.472).

Anthocyanin and carotenoid content in the leaves of *C. sulphureus* and *C. caudatus* were not significantly different (Table 7). The average content of anthocyanin and carotenoid in *C. sulphureus* and *C. caudatus* was 14 mg 100 g^{-1} and 39 mg 100 g^{-1}, respectively. *C. caudatus* possesses high anthocyanin content (Gunasekaran et al. 2021). Anthocyanins are a group of flavonoid compounds with antioxidants and antimicrobial activities (Silva et al. 2017).

Anthocyanins in leaves protect photosynthetic apparatus from oxidative damage (Trojak and Skowron 2017); therefore, anthocyanins are related to photosynthesis. There was a correlation (P<0.05) between anthocyanin and plant growth components such as plant height (r = 0.479) and net assimilation rate (r = 0.480). It may be due to the role of anthocyanin in accelerated plant growth by delaying foliar senescence (Landi et al. 2015). In addition, anthocyanins correlated with chlorophyll b (P<0.05, r = 0.735), an antenna pigment in photosynthesis. Chlorophyll b elevated antenna size and electron transport in light photosynthesis reaction, then increased CO_2 assimilation (Voitsekhovskaja and Tyutereva 2015).

Secondary metabolites related to antioxidant activity are phenols and flavonoids (Irshad et al. 2018). The results of the study showed that the total phenol content of *C. caudatus* leaves was 27.78 mg GAE g^{-1}. This value was 32% higher than orange *C. sulphureus* (28.61 mg GAE g^{-1}) and 48% higher than yellow *C. sulphureus* (25.57 mg GAE g^{-1}). The total phenolic content of *C. caudatus* leaves in our research, similar to previous research, ranged from 36.09-37.76 mg GAE g^{-1} (Seyedreihani et al. 2017).

The antioxidant activity of *C. caudatus* was higher than yellow and orange *C. sulphureus*. IC_{50} value of *C. caudatus* is approximately 50% lower than *C. sulphureus* (Table 8). The higher the antioxidant activity of a material, the lower the IC_{50} value because the lower concentration is needed to inhibit 50% of DPPH free radicals. *C. caudatus* and *C. sulphureus* had the same flavonoid content in their leaves.

Several secondary metabolites, including phenols and flavonoids, affected the activity of antioxidants. Based on the Pearson correlation analysis, the IC_{50} negatively correlated ($P<0.05$) with flavonoids ($r = -0.497$) and phenols ($r = -0.861$) in *Cosmos* leaves. From the coefficient correlation value, in this study, phenol content had a higher influence on antioxidant activity than flavonoid content. The increased content of flavonoids and phenols increases antioxidant activity. Several vegetable commodities in previous studies showed a close correlation between total phenols and flavonoids with antioxidants (Aryal et al. 2019).

The biosynthesis of phenolic and flavonoid compounds was affected by the phenylalanine ammonia-lyase (PAL) enzyme that catalyzes phenylalanine into cinnamic acid as a precursor to the formation of the phenol group (Montoya-Garcia et al. 2018). Increasing levels of nutrients in leaves, especially N, reduce the activity of the PAL enzyme (Wang et al. 2015); therefore, the N levels in the leaves were negatively correlated with flavonoid content ($P<0.05$, $r = -0.568$). As previously mentioned, nitrogen plays a role in enhanced plant growth; there was a correlation between plant morphology and bioactive content, especially phenol and antioxidant activity. In this study, the internode length of harvested shoots negatively correlated with phenol content ($P<0.05$, $r = -0.493$). On the other hand, the IC_{50} value was positively correlated with shoot weight per plant ($P<0.05$, $r = 0.497$).

Table 6. Pigment content in leaves of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**Chl-a (mg g^{-1} fw)**	**Chl-b (mg g^{-1} fw)**	**Chl-total (mg g^{-1} fw)**
Yellow *Cosmos sulphureus*	1.45 ± 0.3 a	0.47 ± 0.09 a	1.92 ± 0.2 a
Orange *Cosmos sulphureus*	1.47 ± 0.2 a	0.46 ± 0.06 a	1.93 ± 0.2 a
Cosmos caudatus	1.35 ± 0.3 a	0.47 ± 0.09 a	1.82 ± 0.3 a

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard. Chl-a: chlorophyll a, chl-b: chlorophyll b, chl-total: Total chlorophyll

Table 7. Anthocyanin and carotenoid content in leaves of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**Anthocyanin (mg g^{-1} fw)**	**Carotenoid (mg g^{-1} fw)**
Yellow *Cosmos sulphureus*	0.14 ± 0.08 a	0.43 ± 0.08 a
Orange *Cosmos sulphureus*	0.12 ± 0.05 a	0.41 ± 0.05 a
Cosmos caudatus	0.15 ± 0.05 a	0.33 ± 0.09 a

Note: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard. fw: fresh weight

Table 8. Total phenol, total flavonoid, and antioxidant activity in leaves of *Cosmos caudatus* and *C. sulphureus*

***Cosmos* species**	**Total phenol (mg GAE g^{-1} dw)**	**Total flavonoid (mg QE g^{-1} dw)**	**Antioxidant activity (IC_{50}) (ppm)**
Yellow *Cosmos sulphureus*	25.57 ± 1.7 b	32.26 ± 2.3 a	100.54 ± 13.3 a
Orange *Cosmos sulphureus*	28.61 ± 3.4 b	34.60 ± 2.9 a	103.87 ± 18.8 a
Cosmos caudatus	37.78 ± 5.0 a	35.04 ± 3.4 a	56.24 ± 15.0 b

Notes: The numbers in a column followed by the same letter are not significantly different using the Tukey test of α = 5%. Numbers are followed by ± deviation standard. dw: dry weight, GAE: gallic acid equivalent, QE: quercetin equivalent

In conclusion, there were significant differences in plant morphology of yellow and orange *C. sulphureus* and *C. caudatus*. Orange *C. sulphureus* had a larger stem diameter than *C. caudatus*. Orange *C. sulphureus* had the highest shoot production per plant in the first and total harvests. *C. sulphureus* and *C. caudatus* had some metabolite contents that were not significantly different, i.e., crude fiber, anthocyanin, carotenoids, and flavonoids. The total phenols and antioxidant activity of *C. sulphureus* were lower than that of *C. caudatus*. The presence of remarkable bioactive compounds such as phenol, flavonoid, anthocyanin, and carotenoid in the leaves of *C. sulphureus*, so there is a possibility that *C. sulphureus* can be used as a functional vegetable.

REFERENCES

Aldini GM, Martono E, Trisyono YA. 2019. Diversity of natural enemies associated with refuge flowering plants of *Zinnea elegans*, *Cosmos sulphureus*, and *Tagetes erecta* in rice ecosystem. Jurnal Perlindungan Tanaman Indonesia 23 (2): 285-291. DOI: 10.22146/jpti.33947.

Alzate-Yepes T, Pérez-Palacio L, Martínez E, Osorio M. 2023. Mechanisms of action of fruit and vegetable phytochemicals in colorectal cancer prevention. Molecules 28 (11): 4322. DOI: 10.3390/molecules28114322.

Andarwulan N, Batari R, Sandrasari DA, Bolling B, Wijaya H. 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. Food Chem 121 (4): 1231-1235. DOI: 10.1016/j.foodchem.2010.01.033.

Andrushchenko O, Levon V. 2021. The content of flavonoids in *Cosmos sulphureus*. Plant Introd 89/90: 83-88. DOI: 10.46341/PI2021003.

Arias A, Feijoo G, Moreira MT. 2022. Exploring the potential of antioxidants from fruits and vegetables and strategies for their recovery. Innov Food Sci Emerg Technol 77: 102974. DOI: 10.1016/j.ifset.2022.102974.

Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants 8: 96. DOI: 10.3390/plants8040096.

Carstensen A, Herdean A, Schmidt SB, Sharma A, Spetea C, Pribil M, Husted S. 2018. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. Plant Physiol 177: 271-284. DOI: 10.1104/pp.17.01624.

Chadwick M, Gawthrop F, Michelmore RW, Wagstaff C, Methven L. 2016. Perception of bitterness, sweetness and liking of different genotypes of lettuce. Food Chem 197: 66-74. DOI: 10.1016/j.foodchem.2015.10.105.

Cheng SH, Khoo HE, Ismail A, Abdul-Hamid A, Barakatun-Nisak MY. 2016. Influence of extraction solvents on *Cosmos caudatus* leaf antioxidant properties. Iran J Sci Technol Trans Sci 40: 51-58. DOI: 10.1007/s40995-016-0007-x.

Collini E. 2019. Carotenoids in photosynthesis: The revenge of the "'accessory"' pigments. Chem 5: 494-504. DOI: 10.1016/j.chempr.2019.02.013.

Datiles M. 2022. *Cosmos caudatus* (Wild *Cosmos*). CABI Compend. DOI: 10.1079/cabicompendium.117946.

Gariballa S, Al-Bluwi GSM, Yasin J. 2023. Increased fruit and vegetable consumption mitigates oxidative damage and associated inflammatory response in obese subjects Independent of Body Weight Change. Nutrients 15 (7): 1638. DOI: 10.3390/nu15071638.

Granato D, Nunes DS, Barba FJ. 2017. An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. Trends Food Sci Technol 62: 13-22. DOI: 10.1016/j.tifs.2016.12.010.

Gunasekaran D, Tahir NI, Akbar MA, Basir S, Ismail I, Talip N, Ramzi AB, Baharum SN, Noor NM, Bunawan H. 2021. Discovery of anthocyanin biosynthetic pathway in *Cosmos caudatus* Kunth. using omics analysis. Agronomy 11 (4): 661. DOI: 10.3390/agronomy11040661.

Igile GO, Iwara IA, Mgbeje BIA, Uboh FE, Ebong PE. 2013. Phytochemical, proximate and nutrient composition of *Vernonia calvaona* Hook (Asteraceae): A green-leafy vegetable in Nigeria. J Food Res 2 (6): 1-11. DOI: 10.5539/jfr.v2n6p1.

Irshad M, Debnath B, Mitra S, Arafat Y, Li M, Sun Y, Qiu D. 2018. Accumulation of anthocyanin in callus cultures of red-pod okra [*Abelmoschus esculentus* (L.) Hongjiao] in response to light and nitrogen levels. Plant Cell Tissue Organ Cult 134: 29-39. DOI: 10.1007/s11240-018-1397-6.

Khairunnisa M, Nurcahyani YD, Samsudin M, Martiyana C. 2022. The influence of my plate campaign to increase local fruit and vegetable consumption. In: IOP Conf Ser Earth Environ Sci 1024 (1): 012081. DOI: 10.1088/1755-1315/1024/1/012081.

Khan A, Jan A, Bashir S, Noor M. 2005. Effect of nitrogen and seed size on maize crop. I: Stand and plant height. J Agri Soc Sci 1 (4): 380-381. DOI: 1813-2235/2005/01-4-380-381.

Lamont BB, Williams MR, He T. 2023. Relative growth rate (RGR) and other confounded variables: Mathematical problems and biological solutions. Ann Bot 20: 1-13. DOI: 10.1093/aob/mcad031.

Landi M, Tattini M, Gould KS. 2015. Multiple functional roles of anthocyanins in plant environment interactions. Environ Exp Bot 119: 4-17. DOI: 10.1016/j.envexpbot.2015.05.012.

Lasmini SA, Nasir B, Hayati N, Edy N. 2018. Improvement of soil quality using bokashi composting and NPK fertilizer to increase shallot yield on dry land. Aust J Crop Sci 12 (11): 1743-1749. DOI: 10.3316/informit.096934421301743.

Mason MG, Ross JJ, Babst BA, Wiendaw BN, Beveridge CA. 2014. Sugar demand, not auxin, is the initial regulator of apical dominance. Proc Natl Acad Sci U S A 111 (16): 6092-6097. DOI: 10.1073/pnas.1322045111.

McKim SM. 2020. Moving up-controlling internode growth. New Phytol 226 (3): 672-678. DOI: 10.1073/pnas.1322045111.

Montoya-Garcia CO, Volke-Haller VH, Trinidad-Santos A, Villanueva-Verdusco C. 2018. Change in the contents of fatty acids and antioxidant capacity of purslane in relation to fertilization. Sci Hortic (Amsterdam) 234: 152-159. DOI: 10.1016/j.scienta.2018.02.043 R.

Nisar N, Li L, Lu S, Khin NC, Pogson BJ. 2015. Carotenoid metabolism in plants. Mol Plant 8: 68-82. DOI: 10.1016/j.molp.2014.12.007.

Phuse SS, Khan ZA. 2018. Estimation of free radical scavenging activity of *Cosmos* leaves extract. Intl J Recent Sci Res 9 (8): 28355-28358. DOI: 10.24327/ijrsr.2018.0908.2445.

Poorter H. 1989. Interspecific variation in relative growth rate: on ecological causes and physiological consequences. In: Lambers H (eds). Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. The Hague: SPB Academic Publishing, Amsterdam.

Pullailah T, Bahadur B, Krishnamurty KV. 2015. Plant biodiversity. In: Bahadur B (eds). Plant Biology and Biotechnology. Springer, India.

Puttock CF. 2022. *Cosmos sulphureus* (Sulphur *Cosmos*). CABI Compend. DOI: 10.1079/cabicompendium.110395.

Rahanita P, Susila AD, Kartika JG. 2015. Pengaruh pupuk organik pada pertumbuhan dan hasil tanaman kenikir (*Cosmos caudatus*) dan Katuk (*Sauropus androgynus*). Buletin Agrohorti 3 (2): 169-176. DOI: 10.29244/agrob.v3i2.14921. [Indonesian]

Respatie DW, Yudono P, Purwantoro A, Trisyono YA. 2019. The potential of *Cosmos sulphureus* flower extract as bioherbicide for *Cyperus rotundus*. Biodiversitas 20 (12): 3568-2574. DOI: 10.13057/biodiv/d201215.

Růžičková B, Kohout P. 2023. Current nutritional guidelines in terms of the effect on gut microbiota and human health considering the WHO and FAO recommendations. Czech J Food Sci 41 (1): 1-7. DOI: 10.17221/186/2022-CJFS.

Saleh I, Aziz SA, Andarwulan N. 2014. Shoot production and metabolite content of waterleaf with organic fertilizer. Jurnal Agronomi Indonesia 42 (3): 210-214. DOI: 10.24831/jai.v42i3.9169.

Sarker M, Rahman M. 2017. Dietary fiber and obesity management-A review. Adv Obes Weight Manag Control 7 (3): 295-297. DOI: 10.15406/aowmc.2017.07.00199.

Seyedreihani SF, Tan T, Alkarkhi AFM, Easa AM. 2017. Total phenolic content and antioxidant activity of Ulam raja (*Cosmos caudatus*) and quantification of its selected marker compounds: Effect of extraction. Intl J Food Prop 20 (2): 260-270. DOI: 10.1080/10942912.2016.1155055.

Silva S, Costa EM, Calhau C, Morais RM, Pintado ME. 2017. Anthocyanin extraction from plant tissues: A review. Crit Rev Food Sci Nutr 57 (14): 3072-3083. DOI: 10.1080/10408398.2015.1087963.

Sims DA, Gamon JA. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens Environ 81: 337-354. DOI: 10.1016/S0034-4257(02)00010-X.

Singh SK, Reddy VR, Fleisher DH, Timlin DJ. 2017. Relationship between photosynthetic pigments and chlorophyll fluorescence in soybean under varying phosphorus nutrition at ambient and elevated CO_2. Photosynthetica 55 (3): 421-433. DOI: 10.1007/s11099-016-0657-0.

Son M, Hart SM, Schlau-Cohen GS. 2021. Investigating carotenoid photophysics in photosynthesis with two-dimensional electronic spectroscopy. Trends Chem 3 (9): 733-746. DOI: 10.1016/j.trechm.2021.05.008.

Taiz L, Zeiger E. 2006. Plant Physiology. Sinauer Associates Inc., Massachusetts.

Tanimonure VA, Naziri D, Codjoe SNA, Ayanwale AB. 2021. Underutilized indigenous vegetables for household dietary diversity in Southwest Nigeria. Agric 11 (11): 1064. DOI: 10.3390/agriculture11111064.

Tränkner M, Tavakol E, Jákli B. 2018. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. Physiol Plant 163: 414-431. DOI: 10.1111/ppl.12747.

Trojak M, Skowron E. 2017. Role of anthocyanins in high-light stress response. World Sci News 81 (2): 150-168.

Voitsekhovskaja O V, Tyutereva E V. 2015. Chlorophyll b in angiosperms: Functions in photosynthesis, signaling and ontogenetic regulation. J Plant Physiol 189: 51-64. DOI: 10.1016/j.jplph.2015.09.013.

Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. 2013. Maximizing total phenolics, total flavonoid contents, and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Ind Crops Prod 44: 566-571. DOI: 10.1016/j.indcrop.2012.09.021.

Wang C, Chen Y, Hou C. 2019. Antioxidant and antibacterial activity of seven predominant terpenoids. Intl J Food Prop 22 (1): 230-238. DOI: 10.1080/10942912.2019.1582541.

Wang C, Ruan RW, Yuan XH, Hu D, Yang H, Li Y, Yi L. 2015. Effects of nitrogen fertilizer and planting density on the lignin synthesis in the culm in relation to lodging resistance of buckwheat. Plant Prod Sci 18 (2): 218-227. DOI: 10.1626/pps.18.218.

Wang C, Tang Y. 2019. Responses of plant phenology to nitrogen addition: A meta-analysis. Oikos 128: 1243-1253. DOI: 10.1111/oik.06099.

Wang P, Fang J, Gao Z, Zhang C, Xie S. 2016. Higher intake of fruits, vegetables or their fiber reduces the risk of type 2 diabetes: A meta-analysis. J Diabetes Investig 7: 56-69. DOI: 10.1111/jdi.12376.

Weraduwage SM, Chen J, Anozie FC, Morales A, Weise SE, Sharkey TD. 2015. The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. Front Plant Sci 6: 167. DOI: 10.3389/fpls.2015.00167.

Wroblewska A, Stawiarz E, Masierowska M. 2016. Evaluation of selected ornamental *Asteraceae* as a pollen source for urban bees. J Apic Sci 60 (2): 179-191. DOI: 10.1515/jas-2016-0031.

Xu X, Du X, Wang F, Sha J, Chen Q, Tian G, Zhu Z, Ge S, Jiang Y. 2020. Effects of potassium levels on plant growth, accumulation and distribution of carbon, and nitrate metabolism in apple dwarf rootstock seedlings. Front Plant Sci 11: 904. DOI: 10.3389/fpls.2020.00904.

Yurlisa K, Maghfoer MD, Aini N, Yamika WSD. 2018. Preferensi konsumen terhadap atribut kualitas tiga jenis sayuran indigenous di Jawa Timur, Indonesia. Jurnal Hortikultur Indonesia 9 (3): 158-166. DOI: 10.29244/jhi.9.3.158-166.

Zhang Z, Xu G, Ma M, Yang J, Liu X. 2013. Dietary fiber intake reduces risk for gastric cancer: A meta-analysis. Gastroenterology 145 (1): 113-120. DOI: 10.1053/j.gastro.2013.04.001.