
Leaf chlorophyll content, phenolic content, flavonoid content and antioxidant activity of collard greens in response to fertilizer value of duckweed

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Abstract Biennial collard greens (*Brassica oleracea* var *acephala* cv Morris Heading) are leafy vegetables that serve as an excellent source of phenolic and flavonoid compounds. Information concerning the supplementary effects of duckweed as a sole organic source of plant nutrition to enhance the content of certain phytochemicals in this vegetable remains limited. The research assessed the extent to which duckweed pellets and dried duckweed used as fertilizer affected the chlorophyll content of collard green leaves along with the total phenolic as well as flavonoid content and antioxidant properties of the vegetable. Assessments during consecutive dry and wet seasons were carried out from January to July 2022. The treatments comprised 330 kg of organically cultivated paddy soil (control), 300 kg of organically cultivated paddy soil supplemented with the sole dried duckweed, sole duckweed pellet and various ratios of dried duckweed (duckweed pellets). The combination of dried duckweed and duckweed pellet with organically cultivated paddy soil produced the maximum leaf chlorophyll content, greater antioxidant activity, and increased overall phenolic and flavonoid content compared to that recorded for the control in each of the seasons studied. The relationship between total phenolic content and antioxidant activity was found to be strongly positive according to Pearson correlation coefficient analysis, indicating that it contributes more antioxidant activity than total flavonoid content.

Keywords: Brassica, Duckweed, Phenolic, Flavonoid, Antioxidant

Introduction

Collard greens (*Brassica oleracea* var *acephala* cv Morris Heading) are upright biennial vegetables that develop a leafy head atop a 2-3-foot stem (Stephen, 1994). Similar to other members of the *Brassicaceae* family, they provide both soluble fiber and vitamin C and are an excellent source of nutritional value.

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Meanwhile, they are also known to offer several compounds that act to prevent cancer (Vetrano *et al.*, 2013). Among these compounds are various flavonoid polyphenolic antioxidants including carotenes, lutein, zeaxanthin and cryptoxanthin (Kopsell *et al.*, 2007), which assist in the prevention of certain forms of cancer affecting the lungs and oral cavity, as well as cataracts and related chronic eye problems (Gong *et al.*, 2006). One advantage of phenolic compounds lies in their capacity to donate electrons since their hydroxyl groups can support antioxidant activity directly (Bendary *et al.*, 2013). These compounds also include a number of flavonoids, such as flavones and anthocyanidins that contribute to antioxidant activity (Saltveit, 2010; Panche *et al.*, 2016). For instance, Jerusalem artichoke tuber extracts have been shown to contain high levels of phenolic compounds. Thus, this plant demonstrates the highest levels of antioxidant capability (Amarowicz *et al.*, 2020). Furthermore, the greatest antioxidant activity according to the FRAP and DPPH assays was reported for *Labisia pumila* Benth fertilized with 90 kg N·ha⁻¹ (through both organic and inorganic fertilization). However, there was a clear decline in secondary metabolite content as well as antioxidant capabilities when the nitrogen dosage was increased to 270 kg·ha⁻¹ (Ibrahim *et al.*, 2013). Similarly, the DPPH assay and total phenolic content showed a close positive correlation in the case of *Helianthus tuberosus* L., confirming the role of phenolic compounds in promoting antioxidant capabilities (Amarowicz *et al.*, 2020). Several researchers also demonstrated similar outcomes for *Satureja hortensis* (Alizadeh *et al.*, 2010), *Brassica rapa* L. chinensis (Zhao *et al.*, 2009), and selected Brassicaceae species (Ferreira *et al.*, 2015). The phenolic compounds found in flavonoids are an essential part of the secondary metabolites in plants (Dhaniaputri *et al.*, 2021), which are produced through the shikimic and malonic acid pathways (Saltveit, 2010). According to Dhaniaputri *et al.* (2021), these pathways are interconnected to the primary metabolism that provides glucose, which plays a key role in metabolizing the secondary metabolite compounds.

One important index for photosynthetic activity is the level of chlorophyll content (Larcher, 1995). It is considered a primary metabolite (Bhuvana *et al.*, 2018) that is directly involved in the primary metabolism process as photosynthesis to produce glucose, which in turn plays a key role in the metabolism of secondary metabolites (Dhaniaputri *et al.*, 2021). Duckweed belongs to the family *Lemnaceae* (Bog *et al.*, 2020), which contains the smallest flowering plants on Earth (Journey *et al.*, 1993) and is among the most rapidly growing (Ziegler *et al.*, 2015). *Lemna minor* is also a member of this particular family. Generally, duckweed contains 6.8 to 45% crude protein (Landolt and Kandeler, 1987) or 1.08 to 7.20% N. According to Jilimane (2019), the macronutrient and micronutrient compositions of *Lemna minor* vary in ranges of 3.14-4.47% N, 0.40-1.70% P, 2.35-8.79% K, 1.20-2.40% Ca, 0.40-0.60% Mg, 41.00-122.00 mg/kg Zn, 6.10-32.60 mg/kg Cu, 4153-15276

mg/kg Mn, 1465-3117 mg/kg Fe and 140.00-1207 mg/kg Al. The availability of nutrients in *Lemna minor* increases its potential as a reference organic material and its fertilizer value. However, information concerning the tangible effect of using dried duckweed and duckweed pellets as an organic fertilizer in *Brassica oleracea* var *acephala* cv Morris Heading remains limited. Thus, the current research sought to examine their influence on TCC (total chlorophyll content), TFC (Total flavonoid content), TPC (total phenolic content), and AA (antioxidant activity).

Materials and methods

All experiments in this research study were performed at facilities located at the Faculty of Agriculture and Agricultural Industry, Surindra Rajabhat University, Thailand (latitude 16.8191844: longitude 100.2569015) during the dry season from January 2022 to May 2022, and the wet season from May 2022 to July 2022. Commercial collard seeds were sown in a plastic seed tray under greenhouse conditions on December 10, 2021. Twenty-five-day-old seedlings were hand transplanted into an 8 x 13 cm white HDPE bag. The leaf chlorophyll content (LCC) on the harvest day of the dry and wet seasons was assessed by taking measurements from the top of the plant using the fourth fully expanded topmost leaves. A chlorophyll meter (Minolta SPAD502, Japan) was used for this purpose. The evaluations were performed via CRD (completely randomized design), and ten replications were carried out with each replication making use of three different plants. In all, there were six treatments comprised of 330 kg of OCPS as a Control (T₁), 300 kg of OCPS + 30 kg of DD (T₂), 300 kg of OCPS + 30 kg of DP (T₃), 300 kg of OCPS + 15 kg of DD + 15 kg of DP (T₄), 300 kg of OCPS + 20 kg of DD + 10 kg of DP (T₅) and 300 kg of OCPS + 10 kg of DD + 20 kg of DP (T₆). The DP component consisted of 1 kg of DD, 1 kg of cane molasses, 1 kg of 150-mesh dolomite (MgO not less than 20% and CaO₃ not less than 30%) and 1L of plain water. The diameter and length of the pellets were 0.5 x 2.0 cm.

Chemical properties analysis of the OCPS

Paddy soil that had been cultivated organically for three years was used as the main component for growing media in the treatments. A 1: 2 ratio of soil and deionized water was used to establish the soil pH, while the EC (electrical conductivity) was assessed at a ratio of 1: 5. The soil pH and EC were examined using a pH meter and EC meter, respectively (Land Development Department, 2010). The Walkley and Black method was used to measure the organic matter (Walkley and Black, 1947). The cation exchange capacity (CEC) was measured

by using the ammonium saturation method (Land Development Department, 2010). Extractable ammonium (NH_4^+) and nitrate (NO_3^-) were obtained using the KCl extraction method (Siwasin, 1984). The available phosphorus (P) was extracted using Bray II measured by the ammonium molybdate method and determined using a spectrophotometer at a wavelength of 882 nm (Bray and Kurtz, 1945). Exchangeable potassium (K) and sodium (Na) were extracted using the ammonium acetate method and measured by using an atomic emission spectrophotometer (AES). Exchangeable calcium (Ca) and magnesium (Mg) were extracted using the method as well as K and Na, which were measured by using an atomic absorption spectrophotometer (AAS) (Land Development Department, 2010). Extractable sulphur (S) and boron (B) were obtained using the barium chloride method and calcium chloride-manital extraction, respectively (Theerachindakajorn, 2011). The available iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) were extracted using the diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell, 1978).

Chemical properties analysis of the DD

The evaluation of total N was performed using a Leco FP 828 nitrogen analyzer (LECO Corporation, 2018), while total P was evaluated through the vanadomolybdate method (Land Development Department, 2010). AES was used to evaluate total K and Na, while AAS was employed to evaluate Ca, Cu, Fe, Mg, Mn, and Zn (Hanlon, 1998). Finally, a colorimetric approach was employed to evaluate total B (Suwanawong, 2001).

Chemical properties analysis of the DP

An EC meter and pH meter were used to evaluate DP electrical conductivity and pH, respectively (Land Development Department, 2010). The KCl extraction technique (Siwasin, 1984) was used to obtain the extractable ammonium (NH_4^+) and nitrate (NO_3^-), whereupon the total P could be evaluated through the vanadomolybdate method (Land Development Department, 2010). AAS was then used to complete the measurement of Ca, Cu, Fe, K, Mg, Mn, and Zn (Hanlon, 1998), while the barium chloride technique enabled the evaluation of S (Theerachindakajorn, 2011).

Plant sample extraction

Leaves with petiole at the harvested stage of collard were harvested on May 13, 2022 and July 13, 2022 for the dry season and wet season, respectively. The leaves with petiole were allowed to dry at a temperature of 70°C. Once dried, the

samples were ground to a powder and stored in a sealed plastic bag until used. Subsequently, 20 ml of 99.9% methanol (v/v) was added to 1.0 g of the dried ground sample and extracted by 60 Hz of ultrasound wave for one hour at 50°C. The resulting extract then underwent filtration to separate only the solution used to determine TPC, TFC and AA.

Total phenolic content (TPC) assay

TPC determination was performed via the Folin-Ciocalteu method, as described by Yingngam *et al.* (2014). The gallic acid solution was employed at different concentrations (0, 25, 50, 75, 100, 125, 150, 175, and 200 mgL⁻¹) as the standard phenolic compound. One-fourth of 1 ml of each concentration of gallic acid solution was mixed thoroughly in distilled water (2.5 ml), to which 0.5 ml of 10% (v/v) Folin-Ciocalteu reagent was added along with 0.5 ml of 7.5% (w/v) sodium carbonate (Na₂CO₃). This mixture was incubated at room temperature under conditions of darkness for 40 minutes, whereupon absorbance measurements were taken at 765 nm (UV-Vis spectrophotometer, Perkin Elmer, Lampda 365). The standard curve was obtained through varying concentrations of gallic acid (0-200 mgL⁻¹) measured against absorbance.

The standard curve ($R^2 = 0.993$) with a slope of 0.006 and a y-intercept of 0.0523 ($y = 0.006x - 0.0523$) was used to calculate the TPC from the sample extract and expressed as mgGAE/gDW using the following formula:

$$\text{mgGAE/gDW} = \left[\frac{A_{765} + 0.0523}{\text{slope}} \right] \frac{\text{dilution} \times \text{volume}}{1000 \times W}$$

where A represents the absorbance value (765 nm) of the sample solution with the addition of Folin-Ciocalteu reagent 10% v/v along with sodium carbonate 7.5% w/v, slope represents the slope of the standard curve for a gallic acid solution (0.006), and volume represents the quantity of sample extract (20 ml). Dilution represents the value of sample extract dilution (2) prior to analysis, and W represents the sample dry weight (1g).

Total flavonoid content (TFC) assay

To establish the TFC, the aluminum trichloride (AlCl₃) technique adapted from Kaewnarin *et al.* (2008) was employed. Different concentrations of quercetin solution were used as the standard flavonoid compound. Twenty-five mg of 99% quercetin (Research Laboratories, SRL, assay) was mixed with 25 ml of 99% methanol (v/v) and measured to 25 ml of volume. The standard quercetin solution was diluted to 0, 50, 100, 150, 200, 250 and 300 mgL⁻¹, respectively. One-half ml of the standard quercetin solution was added to distilled water (2

mL) to create a mixture to which 0.15 ml of 5% (w/v) sodium nitrite (NaNO_2) was also added. Following the reaction for 5 minutes, the process required the addition of 0.15 ml of 10% (w/v) AlCl_3 before 15 additional minutes of reaction, whereupon the absorbance was measured at 415 nm (UV-Vis spectrophotometer, Perkin Elmer, Lampda 365). A standard curve was obtained using various quercetin concentrations (0-300 mgL^{-1}) against absorbance. The standard curve ($R^2 = 0.996$) with a slope of 0.0032 and a y-intercept of 0.0214 ($y = 0.0032x - 0.0214$) was used to calculate TFC from the sample extract, with results presented in the form of mg QE/g DW using the following formula:

$$\text{mgQE/gDW} = \left[\frac{A_{415} + 0.0214}{\text{slope}} \right] \frac{\text{dilution} \times \text{volume}}{1000 \times W}$$

where the sample solution (NaNO_2 5% (w/v) and AlCl_3 10% (w/v)) absorbance value at 415 nm is indicated as A, slope represents the slope of standard curve of quercetin solution (0.0032), volume represents the quantity of sample extract (20 ml), dilution represents the value of sample extract dilution (5) prior to analysis, and W represents the sample dry weight (1g).

Determination of antioxidant activity

The DPPH assay was employed to evaluate the collard extract antioxidant activity in line with the approach recommended by Yingngam *et al.* (2014), in which ascorbic acid solution at various concentrations (0, 5, 10, 20, 30, 40 mgL^{-1}) was used to create the reference standards. One point five mL of 0.1 mM DPPH in methanol was thoroughly mixed with each concentration of the reference standard (0.3 ml) before incubation at room temperature under conditions of darkness. Finally, absorbance measurements were taken at 517 nm (UV-Vis spectrophotometer, Perkin Elmer, Lampda 365) and compared with the DPPH solution (1.5 ml) with methanol (0.3 ml). The equation below was used to calculate the plant extract inhibition:

$$\text{Inhibition (\%)} = \left[\frac{A_0 - (A_1 - A_2)}{A_0} \right] \times 100$$

where A_0 , A_1 , and A_2 represent the respective absorbance values for the DPPH solution (1.5 ml) combined with methanol (0.3 ml), the DPPH solution (1.5 ml) combined with sample solution (0.3 ml), and methanol (1.5 ml) combined with sample solution (0.3 ml).

A standard curve was obtained using a freshly made ascorbic acid solution with different concentrations (0-40 mgL^{-1}) against inhibition. The resulting standard curve ($R^2 = 0.992$) with a slope of 2.3871 and a y-intercept of 5.2143 (y

= 2.3871×-5.2143) was used to calculate the antioxidant activity and expressed in terms of mgVitaminC/gDW according to the following formula:

$$\text{mgVitaminC/gDW} = \left[\frac{\% \text{ inhibition} + 5.2143}{\text{slope}} \right] \frac{\text{dilution} \times \text{volume}}{1000 \times W}$$

where W represents the sample dry weight (1g), slope represents the slope of the standard curve of ascorbic acid (2.3871), volume represents the quantity of sample extract (20 ml), and dilution represents the value of sample extract dilution (3) prior to analysis.

Data for primary and secondary metabolites were analyzed using analysis of variance (ANOVA) as well as Pearson correlation between variables, which was carried out using specialized statistical software. The means were then compared using Duncan's multiple range test (DMRT) with a significance level of $p \leq 0.05$.

Results

Analysis of the chemical properties of the OCPS, DD, and DP

The OCPS chemical properties evaluated in the soil included soil pH, EC, CEC, organic matter, extractable NH_4^+ , NO_3^- , B, S, available P; Fe, Mn, Cu, Zn, exchangeable K; Ca and Mg, as tabulated in Table 1. The quantities of dried duckweed chemical properties for total N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B were tabulated as well (Table 1). The quantities of duckweed pellet chemical properties for pH, EC, extractable NH_4^+ and NO_3^- , P, K, Ca, Mg, Fe, Mn, Zn, Cu and S were also tabulated (Table 1).

Leaf chlorophyll content, total phenolic content, total flavonoid content and antioxidant activity observed during the dry season

The dry season LCC, exhibited a maximal value of 47.82 SPAD Units for T6, which significantly exceeded the alternatives, demonstrating that LCC could be enhanced by the use of 10 kgDD + 20 kgDP (Table 2). The total N content attributable to DD stood at around 3.08% according to Table 1, while DP was determined to contain 416.47 mgkg NH_4^+ and 49.41 mgkg NO_3^- , which served to maximize the accumulation of chlorophyll. N plays a key role in the structure of both proteins and chlorophyll. The P content in DD (0.48%) and DP (0.37%) could also be considered relatively high, according to Table 1, since these P quantities exceeded the OCPS and thus supported chlorophyll synthesis. Table 2 reveals that the TPC recorded during the dry season had a maximum of 6.26

mgGAE/gDW under T5, which was highly significant over all other treatments. The content of 20 kgDD+ 10 kgDP was observed to increase the TPC compared to T₁. In contrast, further variation in the dose to 10 kgDD + 20 kgDP in T₆ revealed a significant decrease in the TPC. The N content in DD and DP as the main nutrient supplied to the tested plant may have affected the synthesis of TPC. The TFC in the dry season, as shown in Table 2, was found to be the highest (25.69 mgQE/gDW) under T₅, which was highly significant over all other treatments. The results indicated that application 20 kgDD + 10 kgDP increased the TFC compared to T₁. The AA of the dry season, as shown in Table 2. It was found to be at maximum (2.10 mgVit.C/gDW) under T₄. It was observed that the equal amounts of DD and DP used in this treatment significantly affected the maximum value of the AA. However, the AA content showed a different variation pattern.

Table 1. Chemical properties analysis of the organically cultivated paddy soil, dried duckweed and duckweed pellets

Organically cultivated paddy soil		Dried duckweed		Duckweed pellet	
pH	6.16	Total N (%)	3.08	pH	5.99
EC (mS/cm)	0.63	P (%)	0.48	EC	7.03
CEC	6.25	K (%)	1.99	NH ₄ ⁺ (mgkg ⁻¹)	416.47
(meq/100g)	0.59	Ca (%)	4.73	NO ₃ ⁻ (mgkg ⁻¹)	49.41
OM (%)	21.18	Mg (%)	0.17	P (%)	0.37
NH ₄ ⁺ (mgkg ⁻¹)	17.65	Fe (%)	0.47	K (%)	1.50
NO ₃ ⁻ (mgkg ⁻¹)	1.94	Mn (mgkg ⁻¹)	3,122.50	Ca (%)	22.10
P (mgkg ⁻¹)	124.25	Zn (mgkg ⁻¹)	33.93	Mg (%)	2.25
K (mgkg ⁻¹)	900.00	Cu (mgkg ⁻¹)	<1.00	Fe (mgkg ⁻¹)	946.60
Ca (mgkg ⁻¹)	64.93	B (mgkg ⁻¹)	165.83	Mn (mgkg ⁻¹)	924.86
Mg (mgkg ⁻¹)	87.19			Zn (mgkg ⁻¹)	188.66
Fe (mgkg ⁻¹)	59.07			Cu (mgkg ⁻¹)	<1.00
Mn (mgkg ⁻¹)	<1.00			S (%)	0.30
Cu (mgkg ⁻¹)	43.16				
Zn (mgkg ⁻¹)	0.02				
B (mgkg ⁻¹)	417.54				
S (mgkg ⁻¹)					

Leaf chlorophyll content, total flavonoid and phenolic content, and antioxidant activity of the wet season

The maximum value of the LCC (52.72 SPAD Unit) was found under T₆, which was highly significant over all other treatments (Table 2). N and Mg content in the DD (Table 1) may promote the synthesis of chlorophyll. The TPC of the wet season shown in Table 2 was found to be at maximum (4.76 mgGAE/gDW) under T₃, significantly exceeding the alternative treatments. The TFC of the wet season was found to be at maximum (15.92 mgQE/gDW) under T₄, which was

significantly higher than other treatments (Table 2). The AA of the wet season shown in Table 2 was found at maximum (1.77 mgVit.C/gDW) under T₄, which significantly exceeded the alternative treatments. However, the AA content showed a different variation pattern.

Table 2. Leaf chlorophyll content, total phenolic content, total flavonoid content and antioxidant activity as affected by various doses and forms of duckweed in the dry and wet seasons

Treatments	Leaf chlorophyll content (SPAD Unit)		Total phenolic content (mgGAE/gDW)		Total flavonoid content (mgQE/gDW)		Antioxidant activity (mgVit.C/gDW)	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
T ₁ : Control	36.56d	45.25e	4.69c	4.08c	16.71d	11.64b	1.62d	1.65b
T ₂ : 300kgOCPS+ 30kgDD	42.13c	45.51e	4.77c	3.12f	20.60c	8.47d	1.75c	1.31e
T ₃ : 300kgOCPS+ 30kgDP	42.01c	48.14d	5.51b	4.76a	23.42b	7.40e	1.91b	1.58c
T ₄ : 300kgOCPS+ 15kgDD+15kgDP	41.26c	45.89c	5.99ab	4.41b	21.89c	15.92a	2.10a	1.77a
T ₅ : 300kgOCPS+ 20kgDD+10kgDP	46.25b	50.97b	6.26a	3.40e	25.69a	9.91c	1.99b	1.23f
T ₆ : 300kgOCPS+ 10kgDD+20kgDP	47.82a	52.72a	5.65b	3.76d	17.62d	9.60c	1.94b	1.51d
F-test	**	**	**	**	**	**	**	**
C.V. (%)	4.02	2.19	10.33	7.78	7.48	5.01	5.03	5.14

** = Significantly different at $p \leq 0.01$.

The Pearson correlation coefficients (PCC) for the dry season/wet season linking TPC, TFC, AA, and LCC. For TPC and TFC in the dry season, the PCC = 0.478 ($p < 0.01$) confirms a moderately positive relationship. For TPC and LCC, TFC and AA, and AA and LCC, the respective PCC values were 0.434, 0.477, and 0.457 ($p < 0.01$), again confirming a moderately positive relationship (Table 3). For TPC and AA, however, the PCC = 0.700 ($p < 0.01$), indicative of a strongly positive relationship, suggesting that the major contribution to AA came from TPC. The wet season relations between TPC and TFC, TPC and LCC, and TFC and LCC had respective PCC values of 0.228, 0.065, and 0.113 ($p > 0.05$), confirming an insignificant level of correlation. In the case of TPC and AA, however, PCC was equal to 0.708 ($p < 0.01$) representing a strong positive correlation, suggesting that the main contribution to AA came from TPC. Meanwhile, a moderately positive relationship was observed for TFC and AA with PCC = 0.587 ($p < 0.01$).

Table 3. Pearson correlation coefficient of total phenolic content, total flavonoid content, antioxidant activity and leave chlorophyll content in the dry season and wet season (n = 60)

	Dry season				Wet season			
	TPC	TFC	AA	LCC	TPC	TFC	AA	LCC
TPC	1.0	0.478**	0.700**	0.434**	1.0	0.228	0.708**	0.065
TFC		1.0	0.477**	0.273*		1.0	0.587**	0.113
AA			1.0	0.457**			1.0	-0.075

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Discussion

Earlier work from Bojovic and Stojanovic (2005) produced similar outcomes revealing that chlorophyll formation and stability in plants are affected by the presence of P. Likewise, Skwarylo-Bednarz and Krzepilko (2009) replicated these findings in amaranth, Purbajanti *et al.* (2019) in peanuts, and Uysal (2018) in apples. Table 1 also confirms that chlorophyll synthesis is affected by the presence of K and Mg in both DD and DP. It has widely been reported that chlorophyll concentrations fall under K-deficient conditions (Zhao *et al.*, 2001; Lu *et al.*, 2016). In addition, K and Mg contribute to the organizational structure of grana and stroma lamellae, adding to the integrity of chloroplasts. Notably, much of the magnesium content within the leaves becomes bound as the central atom in the tetrapyrrole ring of chlorophyll a and b molecules (Tränkner, 2018).

It is understood that phenolic compound synthesis can be influenced by fertilization with nitrogen (Treutter, 2010). For instance, it has been reported that increasing the level of N fertilization resulted in the total phenolic content in *Solanum lycopersicum* L. increasing (Vinha *et al.*, 2014). Similar results were reported by Amarowicz *et al.* (2020) in Jerusalem artichoke and Barroso *et al.* (2018) in *Stevia rebaudiana* Bertoni. However, the results of other investigations are inconclusive and have a particular dose of N supply. When the dosage of nitrogen fertilizer in red cabbage was increased from 150 kg·ha⁻¹ to 250 kg, the phenolic compound content was reduced (Biesiada, 2010). Furthermore, increasing nitrogen levels also caused a significant decline in phenolic compound levels in mustard leaves (Li *et al.*, 2008).

It was shown that this rate of application promoted the maximum value of TFC. Alhasan and Al-Ameri (2021) reported that secondary metabolite production in medicinal plants could be significantly influenced by the application levels of both macro- and micronutrients. Furthermore, the application of nutrients at an optimal rate can in turn maximize the secondary

metabolite rate (Nagaveni *et al.*, 2018). DD and DP contain both micro- and macronutrients, as presented in Table 1, which can significantly influence TFC generation. According to Cristina and Constantin (2011), copper can promote flavonoid production in *Digitalis lantana* Ehrh. Plants, while zinc is also notable in its ability to boost overall flavonol content (Hanif *et al.*, 2017). However, T₆ resulted in the lowest TFC value (17.62 mgQE/gDW) as well as T₁ (16.71 mgQE/gDW).

The micronutrient content found in DD and DP may enhance the synthesis of AA. Panhwar *et al.* (2015) reported that applying Zn, Cu and Mo along with the advised NPK fertilizer rates significantly increased the antioxidant activities in dehusked rice compared to those that applied only N, P and K. In addition, Powell (2000) observed that micronutrients served as cellular antioxidants, while zinc was able to improve antioxidant activity by inducing metallothioneins (Di Silvestro and Cousind, 1984). Ebrahimian *et al.* (2021) also reported that the antioxidant properties of milk thistle in dryland conditions could be enhanced when micronutrients including B, Fe, Mn, and Zn were sprayed. Hanif *et al.* (2017) reported similar outcomes in the case of *Ocimum basilicum* L.

Nitrogen is a key element in the structure of both proteins and chlorophyll, thus potentially influencing chloroplast formation and chlorophyll accumulation (Daughtry, 2000). Furthermore, Mg and N are also vital components of chlorophyll molecules, and the chlorophyll concentration could be increased when N and Mg occur in greater quantities (Pandey *et al.*, 2015). Furthermore, Mg plays a vital role in supporting the growth of plants and comprises 2.7% of the total molecular weight of chlorophyll (Kasinath *et al.*, 2014). Mg also acts as the central atom in chlorophyll pigment and is essential for biosynthesis to take place (Tränkner *et al.*, 2018). It has also been found that the level of nitrogen supply influences 5-aminolevulinic acid biosynthetic rates as well as porphobilinogen synthase activity while simultaneously governing the content of chlorophyll and carotenoids (Chu *et al.*, 2006). Reports from Uysal (2018) maintain that nitrogen and chlorophyll content levels tend to increase in parallel. In terms of the effects of Mg on chlorophyll content, it was explained by Farhat *et al.* (2016) that chlorophyll levels in plants would decline when insufficient Mg was present. This finding matched the work of Wang *et al.* (2004) who revealed that Mg in suitable quantities would lead to an increase in chlorophyll levels in soybeans. Moreover, when adequate levels of P were present, sugarcane exhibited increased chlorophyll content as the level of Mg rose (Zheng *et al.*, 2019).

The DP supplied to the tested plants may be attributed to enhanced TPC content due to its macronutrients and micronutrient content. Treutter (2010) also reported that the use of N as fertilizer has an effect on phenolic compound

synthesis, in concurrence with the work of Vinha *et al.* (2014) in *Solanum lycopersicum* L., Amarowicz *et al.* (2020) in Jerusalem artichoke, and Barroso *et al.* (2018) in *Stevia rebaudiana* Bertoni. The previous studies also showed how the application of different quantities of micronutrients would affect a variety of plants. Meanwhile, Mudau *et al.* (2007) showed that adding NPK nutrients could increase the total polyphenol levels in cultivated plants to levels that exceeded the highest levels observed in wild plants. Sharafzadeh (2011) also reported that the highest TPC was recorded on 50 mg N, P₂O₅, K₂O/kg soil of pot (equal to 100 kg/ha). In terms of some single macronutrients, the use of P for fertilization resulted in enhanced phenolic compound accumulation in the context of *Mentha spicata* (Chrysargyris *et al.*, 2019), while Joel *et al.* (2017) claimed that the application of 40 kgP/ha affected the highest levels of phenolic and antioxidant properties in African nightshade. According to Hussein *et al.* (2012), the foliar application of 100 or 200 ppmK increased total phenols concentration in the leaves of pepper plants by 2.1 to 2.6-fold compared to the plants used as the control. This study, therefore, demonstrated that the incorporation of DP to OCPS provided the optimal and balanced level of plant nutrients to increase the TPC of the tested plants in the wet season.

Incorporating 15 kgDD+15kgDP to 30 kgOCPS was the optimum amount to boost the TFC to the highest level in tested plants during the wet season. Nagaveni *et al.* (2018) reported that secondary metabolites reached their highest rate when optimal levels of plant nutrition were employed. Hence, N content in DD and DP (Table 1) may influence the synthesis of TFC. Zhao *et al.* (2021) demonstrated that using N at the rate of 130 kg/ha affected the highest flavonoid content in *Allium fistulosum*. Secondary metabolite production in medicinal plants is dependent upon the appropriate application of both macro- and micronutrients (Alhasan and Al-Ameri, 2021). Critina and Constantin (2011) found that flavonoid content in *Digitalis lantana* Ehrh plants was associated with Cu application. Khathutshelo *et al.* (2016) reported that when zinc and copper were applied to the leaves at 100 mL, L-1 could maximize the total flavonoid content found in bush tea. Meanwhile, Hanif *et al.* (2017) added that zinc served as a crucial micronutrient with an important contribution to increases in TFC.

In this study, incorporating equal amounts of DD and DP into OCPS was the most suitable for increasing the AA of the tested plants. The variation between the different treatments was found to be significant (Table 2). The combination of macronutrients and micronutrients found in the DD and DP may cause the maximum AA. Joel *et al.* (2017) revealed that the application of 40 kgP/ha resulted in the highest levels of phenolics and antioxidants. Panhwar *et al.* (2015) reported that applying Zn, Cu and Mo alongside the advised NPK fertilizer rates brought about a significant rise in the antioxidant properties of the rice. In

addition, zinc also served to improve the antioxidant properties by inducing metallothioneins (Di Silvestro and Cousind, 1984). Meanwhile, milk thistle cultivated under dryland conditions showed enhanced antioxidant capabilities due to the spraying of Fe, Zn, B and Mn (Ebrahimian *et al.*, 2021).

The results of work by Muflihah *et al.* (2021) revealed that the contribution to AA is greater from TPC than TFC. Moreover, other studies have confirmed the lesser role of TFC in contributing to AA (Saeed *et al.*, 2012; Li *et al.*, 2018). Positive correlations for antioxidant activity with TPC, TFC, and flavonoid compounds in common buckwheat and fenugreek seeds were reported by Salehi *et al.* (2018). In contrast, the relationship between TFC and LCC was only slightly positive, with $PCC = 0.273$ ($p < 0.05$), suggesting that the contribution to TFC from LCC was minimal. These findings were in concurrence with those of Li *et al.* (2018), who found that both TPC and TFC contribute significantly to AA and are frequently found in plants.

The findings of the dry season study demonstrated that the use of 300 kgOCPS + 10 kgDD+20kgDP significantly maximized LCC. The greatest levels of TPC and TFC were recorded for 300kgOCPS+20kgDD+10kgDP. The use of 300 kgOCPS+15kgDD+15kgDP significantly increased the highest AA. A strong positive linear association was observed to link TPC and AA according to correlation analysis. The highest LCC of the wet season was measured with the use of 300 kgOCPS + 10 kgDD + 20kgDP. TPC and TFC were maximized when using 300 kgOCPS + 20kgDD + 10kgDP. The use of 300kgOCPS + 15kgDD + 15kgDP significantly promoted the highest AA. This study proved that the use of DD and DP incorporated into OCPS at the optimal level was able to increase LCC, TPC, TFC, and AA when compared to control in both the dry season and wet season. It was confirmed that TPC is the major antioxidant compound. The AA of the dry season tended to perform higher than the wet season. The incorporation of dried duckweed and duckweed pellets into organically cultivated paddy soil boosted the significant phytochemicals of collard green cv. Morris Heading.

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Conflicts of interest

The authors declare no conflicts of interest.

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