Effects of organic compost, gypsum, and topdress sulfur rates on bioactive compounds and physicochemical characteristics of collard greens

Compostos bioativos e caracterização físico-química da couve-de-folha sob composto orgânico, gessagem e doses de enxofre em cobertura

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This study evaluates the use of sulfur, gypsum, and organic compost as to their effects on bioactive compounds and physicochemical characteristics of collard greens. Twelve treatments were assessed in randomized blocks in a split-plot scheme, where the main plots received three types of soil amendments (pre-planting incorporation of organic compost; gypsum; and organic compost + gypsum), and the subplots received four rates of topdress-applied sulfur (0; 80; 160; and 240 kg ha^{-1} of S). Flavonoid bioactive compounds, total antioxidant activity, and total phenolic compounds were evaluated. The physicochemical characteristics assessed were: reducing sugars, total sugars, sucrose, soluble solids, pH, titratable acidity, fiber, protein, pigments (chlorophyll a, b, anthocyanins, and total carotenoids), and the relative chlorophyll index (SPAD index). The treatment whose soil was prepared with organic compost and a topdressing of sulfur from ammonium sulfate increased the flavonoid content in the collard plants. Gypsum application to soil increased the antioxidant activity in the collard leaves but, when combined with organic compost, it decreased the levels of phenolic compounds thereof. The rates of topdress-applied sulfur and the use of organic compost or gypsum in the soil, or the combination of both methods, did not affect the physicochemical characteristics and the pigments of commercial collard leaves. The use of organic compost combined with gypsum during soil preparation decreased the SPAD index in commercial collard leaves. The rates of topdress-applied sulfur showed no influence on the SPAD index in commercial collard leaves.
Keywords: *Brassica oleracea* L. var. *acephala,* organic fertilization, gypsum.

Estudou-se a utilização do enxofre, gesso e composto orgânico sobre compostos bioativos e características físico-químicas de couve-de-folha. Foram avaliados doze tratamentos, delineados em blocos casualizados em esquema de parcelas subdivididas, sendo três tipos de preparo de solo as parcelas (incorporação pré-plantio de composto orgânico; gesso agrícola e composto orgânico + gesso agrícola), e quatro doses de enxofre em cobertura as subparcelas (0; 80; 160 e 240 kg ha^{-1} de S). Foram avaliados flavonoides, atividade antioxidante total e compostos fenólicos totais, características físico-químicas: açúcares redutores, açúcares totais, sacarose, sólidos solúveis, pH, acidez titulável, fibras, proteína, pigmentos (clorofila a, b, antocianinas e carotenoides totais) e o índice relativo de clorofila (índice SPAD). O preparo de solo com composto orgânico associado ao enxofre via sulfato de amônio em cobertura aumenta os teores de flavonoides na couve-de-folha. O gesso aplicado no preparo de solo aumenta a atividade antioxidante da couve-de-folha. Mas quando associado ao composto orgânico diminui os teores de compostos fenólicos contidos nessa hortaliça. A aplicação de doses de enxofre em cobertura, o preparo do solo com adubação orgânica, gessagem e a combinação das duas técnicas, não afetam as características físico-químicas e pigmentos presentes nas folhas comerciais de couve-de-folha. A utilização do composto orgânico mais gessagem no preparo de solo diminui o índice SPAD em folhas comerciais de couve-de-folha. O uso de doses de enxofre em cobertura não interfere no índice SPAD das folhas comerciais de couve-de-folha.
Palavras-chave: *Brassica oleracea* L. var. *acephala*, adubação orgânica, gesso.

1. INTRODUCTION

Kale or collards (*Brassica oleracea* var. *acephala*) are a widely grown and consumed vegetable in the world. China is the world's main producer of kale, followed by India, Russia, South Korea, Ukraine, and Japan [1]. Kale and collards form a group of plants of the same species, i.e. *Brassica oleracea,* which includes cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botritys*), broccoli (*B. oleracea* var. i*talica*), and others that differ from each other according to their botanical varieties [2].

Collard greens are a good source of calcium, potassium, magnesium, fiber, vitamin A, and vitamin C, and have a low calorie value [3, 4, 5]. Carotenoids (lutein and beta-carotene), anthocyanins and chlorophylls present in their leaves reduce free radicals in the human body and help prevent some degenerative chronic diseases [6, 7].

Additionally, they are rich in nutrients and bioactive compounds [8]. In kale or collard cultivars, the content and amount of secondary metabolites varies significantly according to endogenous developmental factors, such as gene expression and protein modification, along with environmental conditions [9].

Sulfur is a component of the amino acids cysteine and methionine in plants. Deficiency of this nutrient reduces protein synthesis [10]. It plays an important role in the growth of *Brassica* plants, as this species requires from 70 to 80 kg ha^{-1}thereof, while for most crops the range is from 15 to 50 kg ha^{-1} [11, 12]. Previous studies on cauliflower identified sulfur as the fourth most accumulated nutrient in the plant, and the second in the seeds [13]. The same was found in broccoli seeds [14], thus showing the importance of this nutrient for the *Brassica* family.

The absorption of sulfur in the plant takes place in the form of sulfate-sulfur (SO_4^{2-}), and its primary source in the soil derives from the decomposition of organic matter. However, gypsum application also contributes to the supply of sulfur and calcium in the form of calcium sulfate. In addition, gypsum can reduce aluminum toxicity in the soil and favor deep root growth, thus leading to greater water absorption and increased drought tolerance [15, 16].

Despite the importance of kale/collard greens in the human diet, there has been little research regarding this crop. In view of that, this study aims to evaluate the effects of sulfur, gypsum, and organic compost application on the contents of bioactive compounds and physicochemical characteristics of collard plants.

2. MATERIAL AND METHODS

Collard seedlings were grown in an area of typical dystrophic Red Latosol with sandy texture in the São Manuel Experimental Farm of the Faculty of Agronomic Sciences (FCA), Sao Paulo State University (UNESP), Botucatu Campus, located in the municipality of São Manuel (22°46'S; 48°34'W), State of São Paulo, at 750 m altitude. The physicochemical analyses were carried out at the Laboratory of Fruit and Vegetable Post-Harvest of the FCA's Department of Horticulture.

The predominant climate of the municipality of São Manuel is Cfa, according to the Köppen classification, a temperate/mesothermal, humid subtropical climate with hot summers, with the warmest month averaging above 22ºC [17].

The results of the soil chemical analysis at a 0-20 cm depth were: pH ($CaCl_2$) = 6.3;OM = 13 g dm^{-3}; P_{resin}= 82 mg dm^{-3}; H + Al = 12 mmol$_c$ dm^{-3}; K = 1.2 mmol$_c$ dm^{-3}; Ca = 28 mmol$_c$ dm^{-3}; Mg = 8 mmol$_c$ dm^{-3}; S = 3 mg dm^{-3}, SB = 37 mmol$_c$ dm^{-3}; CEC = 49 mmol$_c$ dm^{-3}; and V% = 76.

Twelve treatments were evaluated in a split plot design, with three types of soil amendment applied to the main plots, and four topdress sulfur rates applied to the subplots. The experiment was arranged in a randomized block design with four replications, with 18 plants per plot distributed in three planting rows, the net plot area being the four central plants, from which leaves were collected.

Three methods of soil amendment were implemented: pre-planting incorporation of Visafértil® organic compost (30 t ha^{-1}), containing 120 kg ha^{-1} of S; gypsum (1.2 t ha^{-1}), containing 156 kg ha^{-1} of S; and organic compost (30 t ha^{-1}) + gypsum (1.2 t ha^{-1}), containing 276 kg ha^{-1} of S. All plots received 2 t ha^{-1} of the 04-14-08 formulated fertilizer for the soil pre-planting treatment. The rates were based on the low sulfur content found in the soil chemical analysis. The treatments and fertilizers were incorporated into the soil with a bed shaper rotary tiller.

The chemical analysis of Visafértil®, carried out at the FCA's Laboratory of Soils and Environmental Resources, yielded values expressed in percentage, in the natural state, and indicated the following contents: 0.7 N; 1.0 P_2O_5; 0.7 K_2O; 6.8 Ca; 0.4 Mg; 0.4 S; 24 OM-Total; and 13 C-Total; and in mg kg^{-1}: 2558 Na; 67 Cu; 17316 Fe; 577 Mn; 97 Zn; 19.1 of C/N ratio; and pH = 6.0.

Based on Trani and Raij's (1996) [18] recommendations for topdressing fertilizers for 'butter collard', the total sulfur rates applied were 0; 80; 160; and 240 kg ha^{-1} of S, corresponding to 0; 400; 800; and 1200 kg ha^{-1} of ammonium sulfate fertilizer. To standardize the nitrogen (N) topdressing at 240 kg ha^{-1} N, the amounts of 540, 356, 179, and 0 kg ha^{-1} of urea were used. The total rates of topdressing fertilizers were divided into six applications, with an interval of 14 days between them.

Topdressing fertilizers were applied onto the soil surface around each plant. The first topdressing, as planned for the treatments, was applied at 15 days after transplanting (DAT) the seedlings to the field, then repeated every 14 days. Potassium chloride (20 kg ha^{-1} K_2O in each application) was applied from the third topdressing onwards. Sprinkler irrigation was performed with 3 mm day^{-1}, and crop management followed recommendations by Filgueira (2008) [2] and Trani et al. (2015) [19].

Hi-Crop® hybrid collard seeds were sown on 162-cell black polypropylene trays, one seed per cell, filled with Carolina Soil® substrate, on July 29, 2016. At 27 days after sowing, the seedlings were transplanted to permanent beds spaced at 0.50 m between row sand 1.60 m between plants, following the quincunx system and with three rows per vegetable patch, which resulted in 12,500 plants per hectare.

The first harvest of collard leaves took place on 09/30/2016, then repeated every 10 days. At the sixth harvest (88 DAT), three samples of commercial leaves were collected at an intermediate height in the collard plants from each subplot. The leaves were taken to the laboratory, washed with tap water, and left to dry naturally for later assessment of their flavonoid bioactive compounds [20, 21] expressed in mg quercetin 100 g^{-1}; total antioxidant activity [22] expressed in percentage; and total phenolic compounds [23] in mg gallic acid 100 g^{-1}. Furthermore, the following physicochemical characteristics were evaluated: reducing sugars; total sugars; sucrose [24, 25] expressed in percentage; soluble solids [26] in ° Brix; pH [27], titratable acidity [27] in g citric acid 100g^{-1}; fiber [26] in percentage; protein [27] in percentage; chlorophyll *a*, chlorophyll *b*, anthocyanins, and carotenoids [28] expressed in mg 100 g^{-1}; and relative chlorophyll index (SPAD index).

The obtained data were analyzed by analysis of variance, and significance was verified by the F test. The means of the soil amendment treatments were compared by the Tukey's test at a 0.05 significance level. The sulfur rates were examined by regression analysis. The software application used to analyze the data was Sisvar [29].

3. RESULTS AND DISCUSSION

The analysis of bioactive compounds showed a significant influence of the soil amendment factor on total antioxidant activity and total phenolic compound contents, but the S rates had no influence on the variables under study (Table 1). However, the results showed a significant interaction between the factors studied for flavonoid contents.

The highest antioxidant activity was found for the soil amendments organic compost +gypsum (54.56%) and gypsum alone (50.10%), whereas the lowest was observed for organic compost alone (38.29%). The addition of sulfur and calcium via gypsum, coupled with the content already present in the organic compost, may have directly influenced the increase in the antioxidant activity. Armesto et al. (2019) [30] reported high antioxidant activity in Galega collard (*Brassica oleracea* var. *acephala* cv. Galega), with 84.79%. Rigueira et al. (2016) [31] observed high antioxidant activity in leaf and stem samples of 'butter collard' (*Brassica oleracea* L. var. *acephala*) grown in an organic system in comparison with plants grown in conventional systems.

Table 1. Flavonoid contents (mg quercetin 100g^{-1}), total antioxidant activity (%), and total phenolic compounds (mg gallic acid100g^{-1}) in commercial collards grown in different soil amendments and four rates of topdress-applied sulfur. São Manuel-SP, 2016.

Soil amendment	Flavonoids (mg quercetin 100g^{-1})	Total antioxidant activity (%)	Total phenolic compounds(mg gallic acid100 g^{-1})
Organic compost (O.C.)	24.90	38.29 b	326.0 a
Gypsum (G)	28.94	50.10 a	322.2 a
O.C. + G	24.21	54.56 a	288.1 b
S rates (kg ha^{-1})	**Flavonoids (mg quercetin 100g^{-1})**	**Total antioxidant activity (%)**	**Total phenolic compounds (mg gallic acid100 g^{-1})**
0	25.92	41.11	308.5
80	26.85	45.11	320.9
160	23.56	51.08	298.8
240	27.77	53.30	320.2
Overall mean	26.03	47.65	312.1
C.V.1 (%)	18.8	16.0	6.9
C.V.2 (%)	22.7	23.6	13.3
F(soil.a)	0.07^{ns}	0.002**	0.005**
F(S)	0.36^{ns}	0.052^{ns}	0.523^{ns}
F(soil.a x S)	0.01*	0.119^{ns}	0.674^{ns}

ns not significant, * significant by the F test at the 0.05 significance level, and ** significant by the F test at the 0.01 significance level.

[1]Means followed by the same letter in the column do not differ statistically by the Tukey's test at the 0.05 significance level.

The highest contents of phenolic compounds were found for the soil amendments with organic compost alone (326 mg of gallic acid 100g^{-1}) and gypsum alone (322.2 mg of gallic acid 100g^{-1}). However, the soil prepared with organic compost + gypsum resulted in the lowest content of phenolic compounds (288.1 mg of gallic acid 100g^{-1}). Rigueira et al. (2016) [31] found no significant difference between total phenolics in collard leaves grown in conventional and organic systems, with mean content values between 173 and 244 mg gallic acid 100g^{-1}. Most of the total antioxidant activity of *Brassica* spp. is associated with phenolic compounds, especially flavonoids, which provide protection against free radicals [30]. Silva et al. (2010) [32] reviewed the available literature on methods for evaluating phenolic compounds and carotenoids and concluded that the continued intake of foods rich in these compounds is associated with the prevention of several degenerative diseases.

Figure 1 shows that the flavonoid content of collard leaves increased with the use of organic compost and the rate of 240 kg ha^{-1} of S topdressing. Collard plants grown in soil with gypsum showed a linear decrease in the flavonoid content with the increase of S rates. No influence of the soil amendment with organic compost + gypsum and S rates was observed on the flavonoid contents in collard plants. As the environmental conditions and the organic compost rates were the same, the presence of nitrogen in the ammonium sulfate associated with macronutrients P, K and Mg in the organic compound may have favored the production of flavonoids in collards, as the presence of calcium sulfate (gypsum) associated with ammonium sulfate did not favor the production of flavonoids [58, 59]. De Pascale (2007) [33] points out that sulfur is essential in the biosynthesis of secondary metabolites with high nutritional value that normally accumulate in *Brassica* species, which was observed here only in the treatment with organic compost.
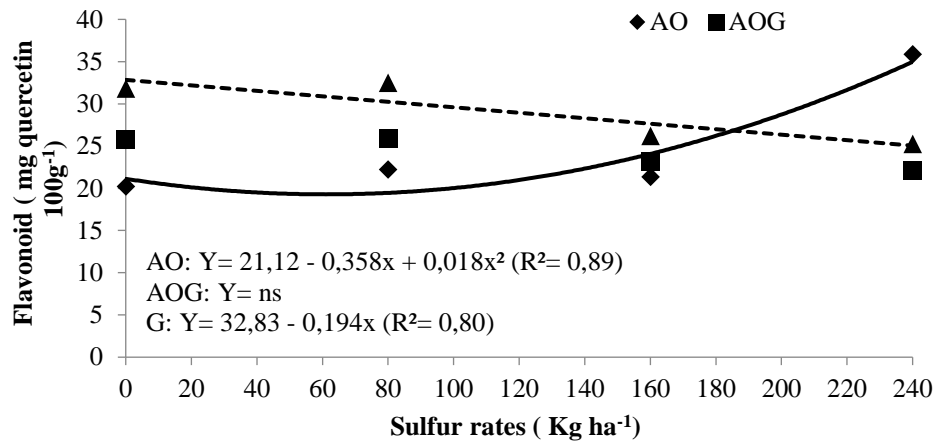
AO: Y= 21,12 - 0,358x + 0,018x² (R²= 0,89)
AOG: Y= ns
G: Y= 32,83 - 0,194x (R²= 0,80)

Figure 1: Flavonoid contents (mg quercetin 100g⁻¹) in commercial collard grown in different soil amendments and four rates of topdress-applied sulfur. São Manuel-SP, 2016.

The factors soil amendments and topdress sulfur rates were not significant for reducing sugars, soluble solids, pH, titratable acidity, fiber, and protein, neither was the interaction between these factors significant by the F test at a 0.05 significance level (Table 2). The polynomial regression analysis also found no significant effect of S rates on the characteristics evaluated.

There were no significant changes in the percentage of reducing sugars, with an overall mean of 1.74% (Table 2). Due to the lack of sucrose in the collard leaves analyzed, the total sugar content was considered equal to that of the reducing sugar content. In this case, the analysis took into account only the monosaccharides, glucose and fructose present in the leaves. These values can be considered low when compared with the reducing sugar content of 4% in minimally processed cabbage [34] and 3.45% to 3.86% in cauliflower inflorescence at rates of topdress-applied potassium [35]. Chitarra and Chitarra (2006) [36] state that the variation in the content of these sugars in vegetables ranges between 2% and 5%, hence the values in collards were lower than those in most vegetables.

Table 2: Reducing sugars (RS), soluble solids (SS), pH, titratable acidity (TA), fiber, and protein in commercial collards grown in different soil amendments and four rates of topdress-applied sulfur. São Manuel-SP, 2016.

Soil amendment	RS (%)	SS (ºBrix)	pH	TA (%)	Fiber (%)	Protein (%)
Organic compost (O.C.)	1.80	9.19	6.23	0.25	1.87	2.78
Gypsum (G)	1.81	9.16	6.22	0.25	1.99	2.83
O.C. + G	1.61	9.50	6.27	0.25	1.93	2.84
S rates (kg ha⁻¹)	**RS (%)**	**SS (ºBrix)**	**pH**	**TA (%)**	**Fiber (%)**	**Protein (%)**
0	1.70	8.92	6.25	0.24	2.01	2.73
80	1.72	9.21	6.25	0.25	2.06	2.81
160	1.80	9.58	6.23	0.27	1.73	2.81
240	1.75	9.42	6.22	0.24	1.91	2.92
Overall mean	1.74	9.28	6.24	0.25	1.93	2.82
C.V.1 (%)	16.77	9.26	2.36	28.35	16.38	9.47
C.V.2 (%)	15.93	9.96	1.95	21.34	16.65	10.17
F(soil.a)	2.35 ns	0.78 ns	0.43 ns	0.06 ns	0.60 ns	0.24 ns
F(S)	0.30 ns	1.16 ns	0.30 ns	0.51 ns	2.43 ns	0.88 ns
F(soil.a x S)	1.50 ns	0.64 ns	0.92 ns	1.03 ns	1.38 ns	0.88 ns

ns not significant at by the F test at a 0.05 significance level.

The different soil amendments and topdress sulfur rates had no influence on soluble solids contents of commercial collards, with an overall mean of 9.28 ºBrix (Table 2). Lower values were found by Sanches et al. (2016) [37] in curly 'butter collard' (7.8 °Brix), by Salata et al. (2014) [34] in minimally processed cabbage immediately after harvest (5.65 °Brix), and by Godoy et al. (2012) [35] in cauliflower (6.58 °Brix). This trait can be influenced by various production factors such as genetic material, soil type, climate, and crop cultural practices.

Soil amendments and topdress sulfur rates caused no significant changes in pH and titratable acidity in the collard leaves (Table 2), with an overall mean of pH = 6.24 and titratable acidity = 0.25%. Sanches et al. (2016) [37] reported lower pH (5.20) and higher titratable acidity (0.31%) values for curly 'butter collard', which is an evidence of consistency in the results, i.e., higher acidity with lower pH. Pereira et al. (2015) [38] evaluated collards produced in an organic system and reported pH of 5.6 and titratable acidity of 0.20%. Acidity is known to quantify the presence of organic acids in vegetables, and acidity is generally low in vegetables.

The mean fiber content was 1.93% (Table 2), with no influence from the factors soil preparation and topdress sulfur rates. In the vegetable nutrient composition table organized by Luengo et al. (2011) [39], 'butter collard' appears with 1.3% of fibers. However, the Brazilian Food Composition Table presents a higher content thereof for raw collard leaves, with 3.1% of fibers [4].

The mean percentage of protein (2.82%) in collard leaves was not significantly influenced by the treatments (Table 2). This result is very close to the data presented in the Brazilian Food Composition Table, which reports protein values of 2.9% for raw collard leaves [4] and 2.83% for braised collard leaves [3]. These protein contents compared with those of vegetables such as zucchini (0.6 to 1.1%), lettuce (0.6 to 1.7%), carrot (1.3%), cauliflower (1.9%), bell pepper (1.0 to 1.2%), cabbage (0.9 to 1.9%), and tomato (1.1%) show that collard greens have a significant value, behind only another *Brassica*: broccoli (3.6%) [4].

The applied soil amendments and sulfur rates did not affect the levels of chlorophyll *a* and *b*, carotenoids, or anthocyanins (Table 3). Chlorophyll *a* and *b* contents had an overall mean of 10.06 mg 100 g^{-1} and 3.48 mg 100 g^{-1}, respectively (Table 3). Von Elbe (2000) [40] observed that chlorophylls *a* and *b* have a 3:1 ratio in nature, which is very close to the 2.9:1 ratio found in the present study.

The concentrations of chlorophyll *a* and *b* (Table 3) were lower than those found by Padula et al. (2006) [41], in broccoli leaves, of 15.50 mg 100 g^{-1} chlorophyll *a* and 9.16 mg 100 g^{-1} chlorophyll *b*.

As can be seen in Table 3, the use of sulfur combined with the different soil amendments had no significant effect on the carotenoid and anthocyanin contents in collard leaves, with means of 2.96 and 0.70 mg 100 g^{-1}, respectively. This study found a chlorophyll:carotenoid ratio of 4.6:1, which is close to the 5:1 ratio reported by Von Elbe and Schwartz (1996) [43]. Carvalho et al. (2006) [44] mentioned that the body converts carotenoids into vitamin A, and these natural pigments can be found in yellow-orange and dark green leafy vegetables, such as the collard species in this study. Rodriguez-Amaya, Kimura and Amaya-Farfan (2008) [45] found 3.40 mg 100 g^{-1} of total carotenoids in collard leaves, and higher values, expressed in mg 100 g^{-1}, in carrots (5.60), squash (9.90), watercress (10.70), common chicory (12.0), and arugula (13.30).

Taiz and Zeiger (2009) [42] discuss that carotenoids – yellow or orange colored accessory pigments often hidden by chlorophyll – can provide the photosynthetic system with photoprotection against singlet oxygen ($^1O_2$*) toxicity, which can destroy compounds such as lipids. In this sense, Wahid (2007) [46] reported that these pigments prevent oxidative damage caused by stress. Anthocyanins, which belong to the flavonoid group, are characterized by their antioxidant, anti-inflammatory and anticarcinogenic properties [47, 44].

The analysis of variance (Table 3) showed no significant effect of topdress sulfur rates on the chlorophyll index, which ranged from 53.98 to 58.65 SPAD units, with an overall mean of 56.2 SPAD units. Conversely, significantly higher indices were found for the use of organic compost alone compared with the application of organic compost +gypsum in the soil preparation before transplantation (Table 3).

Table 3: Contents of chlorophyll a and b, carotenoids, anthocyanins, and the SPAD index in commercial collard leaves grown in different soil amendments and four rates of topdress-applied sulfur. São Manuel-SP, 2016.

Soil amendment	Chlorophyll		carotenoids	anthocyanins	SPAD
	a	*b*			
	(mg 100 g^{-1})		(mg 100 g^{-1})		(unid.)
Organic compost (O.C.)	9.55	3.37	2.80	0.69	57.3 a[1]
Gypsum (G)	9.66	3.51	2.93	0.75	56.3 ab
O.C. + G	10.96	3.55	3.14	0.65	54.9 b
S rates (kg ha^{-1})	Chlorophyll		carotenoids	anthocyanins	SPAD
	a	*b*			
	(mg 100g^{-1})		(mg 100g^{-1})		(unid.)
0	10.04	3.51	2.91	0.63	55.2
80	9.66	3.47	2.87	0.70	56.8
160	10.30	3.66	3.07	0.70	56.3
240	10.23	3.27	2.97	0.76	56.3
Overall mean	10.06	3.48	2.96	0.70	56.2
C.V.1 (%)	35.45	25.77	24.95	20.82	3.09
C.V.2 (%)	31.29	24.03	21.66	45.27	6.05
F(soil.a)	0.77^{ns}	0.17^{ns}	0.86^{ns}	1.80^{ns}	7.94**
F (S)	0.10^{ns}	0.46^{ns}	0.22^{ns}	0.37^{ns}	0.48^{ns}
F(soil.a x S)	1.12^{ns}	1.82^{ns}	1.49^{ns}	0.27^{ns}	0.54^{ns}

^{ns} not significant, ** significant by F test at the 0.01 significance level.
[1]Means followed by the same letter in the column do not differ statistically by the Tukey's test at the 0.05 significance level.

The high concentration of Ca^{2+} in the soil (28 mmolc dm^{-3}), coupled with the use of gypsum, organic compost, topdress-applied potassium, and low CEC, may have displaced Mg^{2+} in the exchange sites, which preferably leached in the soil in the form of Mg^{2+} ions or formed ionic pairs with SO_4^{2-} [48, 49, 50, 51]. Lower Mg levels in collards may result in less intense green leaves, thus reducing the SPAD index values. However, no symptoms of Mg deficiency were observed for the leaves in all treatments.

Soares et al. (2017) [52] found that topdress-applied sulfur in the form of ammonium sulfate increased green color intensity in arugula leaves, which, according to the authors, favored the product quality, attracting consumers looking for greener and more vigorous leaves. Sediyama et al. (2009) [53] observed a linear increase in the relative chlorophyll index (SPAD) in okra leaves with increased rates of swine biofertilizer.

Henz and Mattos (2008) [54] point out that food and nutrition professionals recommend leafy vegetables with brighter and greener color. However, in this study, no visible differences were found in leaf color as a function of S rates. Moreover, all the plants in the experiment received S directly or indirectly at planting, either via organic compost and/or gypsum – what varied was the amount of topdressing.

Moreira and Vidigal (2009) [55] reported 53.5 SPAD units for cabbage hybrid Shutoku fertilized with 278 kg ha^{-1} N, rate that led to the highest fresh biomass of cabbage heads. Increased applications of nitrogen and organic fertilizers in zucchini, okra and tomatoes resulted in increased the chlorophyll content and/or the SPAD indices in the leaves thereof [53, 56, 57]. Consequently, N influences leaf color and the SPAD index. Nevertheless, all the treatments received the same nitrogen topdressing rate.

4. CONCLUSIONS

The application of organic compost alone and gypsum alone during soil preparation provides the highest content of total phenolic compounds in collard greens.

The application of organic compost combined with a topdressing of sulfur from ammonium sulfate during soil preparation increases the flavonoid content in collard greens.

The application of gypsum during soil preparation increases the antioxidant activity of collards greens. However, when with organic compost, the content of phenolic compounds decreases.

Soil preparations with rates of topdress-applied sulfur, and with compost or gypsum, or the combination of the two methods, have no effect on the physicochemical characteristics and the pigment content of commercial collard greens.

The application of organic compost combined with gypsum during soil preparation decreases the SPAD index in commercial collard leaves. The SPAD indices reaches the highest values when organic compost alone is applied to the soil.

Rates of topdress-applied sulfur have no effect on the relative chlorophyll index (SPAD) in commercial collard greens.

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