Colegio de Postgraduados

Evaluation of the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard (*Beta vulgaris* L.).

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ABSTRACT

Objective: To evaluate the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard (*B. vulgaris* L.) var. 'Rainbow' grown under greenhouse conditions.

Design/Methodology/Approach: A completely randomized design was used with three treatments and 33 repetitions: T1 (poultry manure-based organic fertilizer (T1 PM)), T2 (coffe pulp-based vermicompost (T2 V)), and T3 (chemical fertilization (T3 F)). Plant height, stem diameter, number of leaves, leaf area, fresh biomass, dry biomass, chlorophyll A, chlorophyll B, total chlorophyll, carotenoids, total polyphenols, DPPH, and yield were evaluated. An analysis of variance and Tukey's test (α=0.05) were used.

Results: For most of the variables evaluated, the best results were obtained with the poultry manure-based organic fertilizer (T1 PM) treatment. The antioxidant compounds and the quality of Swiss chard were influenced by organic fertilizers, with 0.21 mg Eq. gallic acid g^{-1} fresh tissue (ft), total chlorophyll of 19.5 μg g^{-1} (ft), and a yield of 6,150 kg·m^2.

Study Limitations/Implications: The mineral content of this vegetable was not evaluated.

Findings/Conclusions: The use of poultry manure-based organic fertilizers can be an alternative nutritional management for Swiss chard cultivation.

Keywords: Vermicompost, total polyphenols, poultry manure, DPPH.

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INTRODUCTION

Swiss chard is a green leafy vegetable with a thick stem from the Quenopodiaceae family. Very few varieties are cultivated, and they are classified according to their color, the size of their leaves and petioles, the thickness of the stem, and the quick recovery when their leaves are cut. According to Valero *et al.* (2018), based on their quality and flavor, the most well-known, cultivated, and appreciated varieties are the Blond de Lyon and the white-stem Bressane. The latter variety is valued for its fatty acids (omega 3), lutein, flavonoids, beta-carotene, and zeaxanthin, as well as its vitamins, fiber, folic acid, and mineral salts,

including Ca, Na, K, Cu, Fe, and P (SADER, 2020). According to SIAP (2020), more than 12,000 tons of Swiss chard are produced every year in Mexico.

Although it can be grown all year round, as a monoculture, it has high fertilization requirements, especially regarding nitrogen. In addition, the excessive use of pesticides, from the beginning of plant growth to the end of cultivation (Ninfali *et al.*, 2017), requires finding ecological alternatives for its production, remarkably the addition of compost — an organic fertilizer formed by the microbial degradation of waste subjected to a natural decomposition process (Spaccini and Piccolo, 2020; Ro *et al.*, 2022). The application of this type of materials has become very important, as they increase the organic matter of agricultural soils, improving their fertility, structure, and water retention and preventing erosion and degradation. Likewise, Álvaro (2019), Mamun *et al.* (2020), and Yazid *et al.* (2020) mention that, in social and economic terms, these practices encourage the search and improvement of the systematization of agricultural production activities and practices. Regarding the effect that this kind of organic fertilizers has on horticultural crops, Libutti *et al.* (2020) mention that the growth variables and chlorophyll content of Swiss chard responded positively to the organic amendment, especially when the soil was treated with animal waste compost. Therefore, the objective of this research was to evaluate the effect of organic fertilizers on the morphophysiological parameters and antioxidant compounds of Swiss chard var. 'Rainbow' grown under greenhouse conditions.

MATERIALS AND METHODS

Location of the study area

The research was carried out in a tunnel greenhouse, with an average temperature of 35 °C and a relative humidity of 55%. Six-centimeter-high Swiss chard var. 'Rainbow' seedlings, with a pair of true leaves, were used for the experiment.

Experimental design and treatment description

A completely randomized design with three treatments was used: T1 (poultry manure-based organic fertilizer (T1 PM)), T2 (coffe pulp-based vermicompost (T2 V)), and T3 (chemical fertilization (T3 F)). Each treatment had 33 repetitions, resulting in a layout of 99 experimental units.

Crop establishment

The substrate consisted of a mixture of soil and *tepezil* in a 1:1 (v/v) ratio, disinfected with Full-Gro (6 L·1 L^{-1}). The substrate was used to fill 4-kg black polyethylene bags. Before transplanting, 273 g of vermicompost (V) and 273 g of poultry manure (PM) were applied, following the recommendations of SAGARPA (2014). The control treatment (F) was fertilized with a blue granular fertilizer (10 g·plant^{-1}). The poultry manure-based organic fertilizer had the following characteristics: 400-45% organic matter; 2-3% N, P, K; 6-8% Ca; 1-1.6% Mg; 0.4-1% Fe; 400-700 ppm Zn, 60-90 ppm Cu; 450-800 ppm Mn; 40-100 ppm B; and 24-28% organic carbon. The commercial vermicompost (TerraNova Lombricultores, Xalapa, Veracruz, Mexico) produced from coffee pulp (V) contained: 84% organic matter; pH 7.4; 0.108% organic P; 0.25% total P; 3.99% total N;

2.14% total K; 1.72% total C; 0.8% total Mg; 12:21 C:N ratio; 10.5% fulvic acids; and 15.1% humic acids.

Variables evaluated and statistical analysis

The following agronomic traits were evaluated at 45 days after transplanting (DAT): plant height (cm), with a Truper® flexometer; stem diameter (mm), with a Steren® graduated vernier; number of leaves (unit); leaf area (cm^2) in the Photoshop® software; fresh biomass (g), with a Truper® BASE-5EP digital scale; and dry biomass (g). The samples were dried in a Robert Shaw® oven at 75 °C for 7 days; each sample was then weighed on a Denver Instrument® APX 200 analytical balance. Variables related to compounds with antioxidant activity (chlorophyll A, chlorophyll B, total chlorophyll, and carotenoids ($\mu g\ g^{-1}$ tf)) were determined according to the 80% acetone extraction method, following Song *et al.* (2021), who used a UV-VIS® spectrophotometer (Shimadzu UV-1800), at 645, 663, and 440 nm wavelengths. Total polyphenols (mg▪ EAG g^{-1} tf) were determined with the method developed by Singleton and Rossi (1965); meanwhile, antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Brand-Williams *et al.* (1995). Finally, yield was measured in m^2. The assumptions of normality and homoscedasticity for the resulting data were verified following the criteria of Shapiro-Wilk (1965) and Hartley (1950). An analysis of variance (ANOVA) and Tukey's Least significant difference (LSD) test were performed, with a 5% significance level (α=0.05), using the Statistica software version 10.0 (StatSoft Inc., 2011) for Windows.

RESULTS AND DISCUSSION

Morphophysiological variables

The ANOVA showed significant differences for the variables evaluated (Tukey, P≤0.05). Table 1 shows that poultry manure-based fertilization was a better treatment than chemical fertilization (T3 F) regarding plant height, stem diameter, number of leaves, leaf area, fresh biomass, and dry biomass, with 6.28, 58.12, 77.44, 2.5, 109, and 111.47% increases, respectively. These results could be related to the contribution of macro and microelements (*e.g.*, a high content of nitrogen, phosphorus, and potassium) that the poultry manure-based fertilizer makes to the soil, as well as their influence on the improvement of soil characteristics (*e.g.*, the structure and texture of the substrate). Therefore, it enables a better absorption of nutrients and, consequently, a greater total biomass. These results match the findings of Cabaleiro *et al.* (2017), who applied different doses of dehydrated and granular broiler litter (BL) to lettuce crops, during the spring and winter cycles, and proved that fertilization with any dose of BL guarantees lettuce production, both regarding the fresh weight and number of commercial lettuces; these results are like those obtained with mineral fertilizers.

Chlorophyll content

According to the analysis of variance, the chlorophyll A content of Swiss chard leaves showed significant differences (P≤0.05) (Table 2). The T3 F with 12.1 $\mu g▪g^{-1}$ and the T1 PM with 11.9 $\mu g▪g^{-1}$ were statistically equal. Similarly, the best content of chlorophyll

Table 1. Morpho-physiological.

Variables evaluated	Mean Square Error	F	CV	T1 PM	T2 V	T3 F
Plant height (cm)	0.52	5.66	10.12	71±1.0a	55.20±4.87b	68.66±0.57a
Stem diameter (mm)	0.52	5.55	10.08	23.70±0.45a	22.20±0.43a	16.93±1.72b
Number of leaves	0.44	4.55	9.33	18.33±0.57a	10.33±1.52b	12.66±0.57b
Leaf area (cm^2)	0.33	4.23	8.55	56.45±0.62a	38.04±0.57c	44.62±0.99b
Total fresh biomass (g)	0.55	6.02	11.11	384.66±23.54a	186±8.0b	247±45.73b
Total dry biomass (g)	0.57	6.21	11.2	25.25±0.45a	11.94±0.96b	12.68±0.32b

Values with the same letters within columns are statistically equal (Tukey, P≤0.05). F: calculated, CV: coefficients of variation, and ±Standard deviation.

B and total chlorophyll were recorded by the T3 F (7.6 $\mu g \cdot g^{-1}$ and 19.8 $\mu g \cdot g^{-1}$) and T1 PM (7.2 $\mu g \cdot g^{-1}$ and 19.5 $\mu g \cdot g^{-1}$) treatments. Likewise, Moncayo-Luján *et al.* (2015) and Alvarado (2020) determined that the content of chlorophyll A, chloropyll B, and total chloropyll —in the crops of basil, lettuce, and radish, respectively— was influenced by chemical fertilization, obtaining a result similar to the treatment with organic fertilizers. This trend could be attributed to the availability of N (on which the chlorophyll content depends); this availability stimulates the photosynthetic process and therefore the synthesis of chlorophyll (Moncayo-Luján *et al.*, 2015).

Total polyphenols and antioxidant activity (DPPH)

The statistical analysis showed significant differences (P≤0.05) regarding the content of total polyphenols: the T3 F treatment had higher content (0.21 mg Eq. Gallic Acid$\cdot g^{-1}$ tf) than the other treatments (Figure 1a). This phenomenon may be related to the high N content, which at a given time can influence the presence of phenolic compounds. These results are similar to those reported by Vázquez-Vázquez *et al.* (2015), who reported that the chemical fertilization treatment showed a higher phenolic content than organic fertilizer treatments in a basil crop. Meanwhile, the antioxidant activity increased in the T1 PM treatment with 3.74 μmol E. Trolox$\cdot g^{-1}$ tf (Figure 1b). This figure may be related to the concentration of major elements (N, P, K) in this fertilizer, which matches the findings of Martínez *et al.* (2017), who mention that plant nutrition affects the antioxidant capacity and performance of plants.

Table 2. Chlorophyll content.

	Mean Square Error	F	CV	T1 PM	T2 V	T3 F
Chlorophyll A	2.64	11.97	14.33	11.9±0.57a	7.8±0.57b	12.1±0.57a
Chlorophyll B	0.51	5.86	10.27	7.2±0.25a	5.8±0.25b	7.6±0.25a
Chlorophyll T	5.45	10.26	12.54	19.5±0.87a	13.9±0.83b	19.8±0.85a

Values with the same letters within columns are statistically equal (Tukey, P≤0.05). F: calculated, CV: coefficients of variation, and ±Standard deviation.

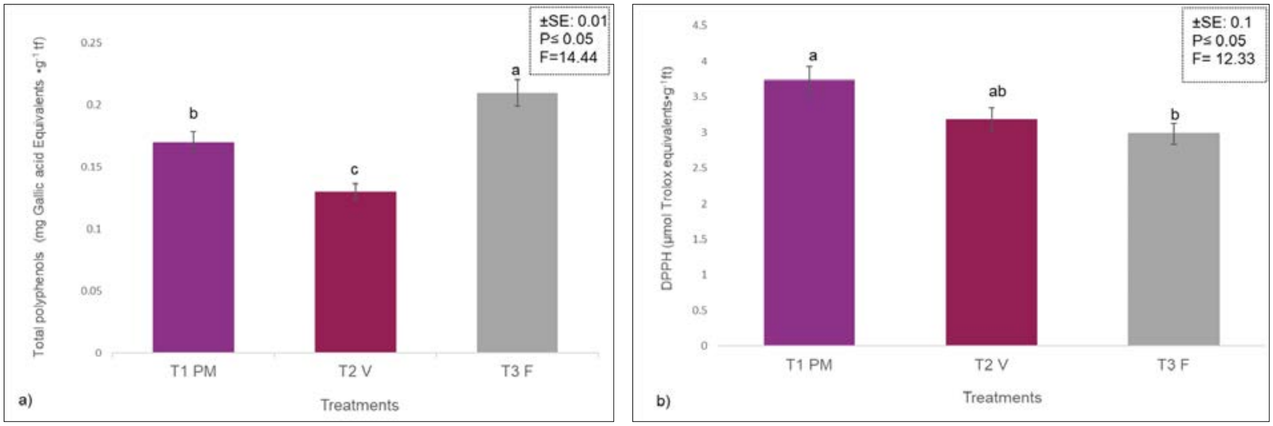

Figure 1. Polyphenol (mg Eq. Gallic acid • g^{-1} tf) a) and DPPH (μmol E. Trolox▪g^{-1} tf) b) content in Swiss chard var. 'Rainbow' at 45 DAT. Columns with the same letter are statistically equal (Tukey, P≤0.05). The vertical lines on the bars represent the standard error (±).

Yield

The highest yield for the T1 PM treatment was quantified (6,150 kg▪m^2) with an increase of 34.86% (Figure 2a and b), possibly as a consequence of the mineral content of the poultry manure-based fertilizer. These results were higher than those reported by Rodríguez *et al.* (2016) and by Huanca and Blanco (2019), who reported yields of 1,645 kg▪m^2 and 2.32 kg▪m^2, respectively.

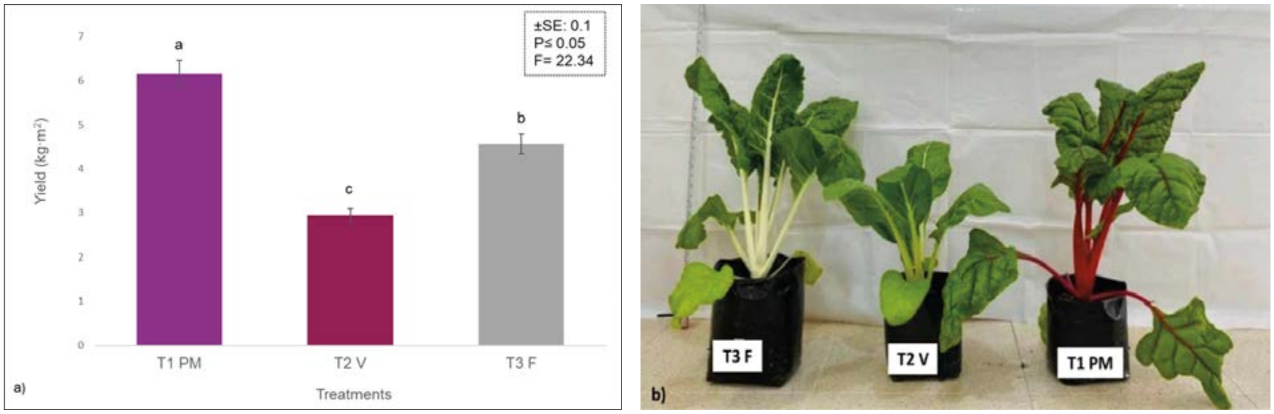

Figure 2. Yield (kg m^{2-1}) of Swiss chard var. 'Rainbow' a). Comparative effects b). Columns with the same letter are statistically equal (Tukey, P≤0.05). The vertical lines on the bars represent the standard error (±).

CONCLUSIONS

Fertilization with poultry manure-based organic fertilizer could be a viable nutritional management alternative, with a positive effect on Swiss chard var. 'Rainbow' grown under greenhouse conditions, increasing its morphophysiological variables and some antioxidant compounds.

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