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THE INFLUENCE OF CULTIVAR AND ORGANIC FERTILIZATIONS ON PLANT GROWTH, PRODUCTION AND QUALITY OF SWISS CHARD, IN WESTERN ROMANIA

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Abstract

Swiss chard (Beta vulgaris L., ssp. cicla) are grown for whole leaves or only for petiole. In Romania, chard is a less cultivated species, being present in some areas of Transylvania. Research aim was to establish how some Swiss chard cultivars behave, after fertilization with three different organic fertilizers. Plant growth, total production as well as dry matter, phenols, flavonoids, dietary fiber, carbohydrates and vitamin C content was measured. Experiment took place between 2021-2022, in an organic vegetable farm, in Săcueni, Bihor county, Romania. Three organic fertilizers were used: Lignohumate, Alcygol Z2M and Alg Green, applied 30 days after emergence, at the dose recommended by the manufacturer. Plant development, 60 days after emergence, was higher at Lucullus cultivar, fertilized with Alg Green, 54.35 cm. Production ranged from 44.67 t/ha (Carde Blanche d'Ampuis fertilized with Lignohumate) to 51.04 t/ha (Lucullus fertilized with Alg Green). Plant content in dry matter, fibers and vitamin C, was higher when plants were fertilized with Lignohumate. Phenols and flavonoids were higher at variants fertlized with Alg Green.

***Key words**: chemical composition, organic fertlizers, plant development, swiss chard.*

INTRODUCTION

Swiss chard (*Beta vulgaris* L.*,* ssp. *cicla*) is a dark green leafy vegetable (GLV) available throughout the year. Swiss chard could be planted in mid spring and again in late summer, which indicates the possibility of harvesting during a long period (Kolota et al., 2010). Chard is a commonly used food crop which is found along the shores of the Mediterranean, frequently used all over the world due to its nutritional value and delicious taste. Chard is a desirable food crop because it adapts to environments with elevated saline concentrations, and it can grow in soils with scarce availability of water (Ninfali and Angelino, 2013). Furthermore, Swiss chard is tolerant to conditions of low light and both cold and hot weather (Kolota et al., 2010).

GLVs are exceptionally low in energy but also relatively high in micronutrients and phytochemicals, which recommend GLVs for consumption in everyday diet (van Jaarsveld et al., 2014). Certain epidemiological studies promoted consumption of GLVs because these vegetables were found to protect against numerous chronic diseases caused by free radical activity (Slavin and Lloyd, 2012). Swiss chard, as one of the GLVs, is rich in phytopigments such as chlorophyll and carotenoids, flavonoids and minerals with antioxidant and immunomodulating properties (Ivanovic et al., 2019). Phytopigments improve immune, detoxication and antioxidant systems of the human body, thus indirectly helping the prevention of disease (Fiedor and Burda, 2014). Swiss chard is a very good source of vitamins C, A and B, phenolic acids (syringic, caffeic and pcoumaric), flavonoids (kaempferol, quercetin and glycosides derived from apigenin) and minerals such as iron, potassium, calcium, magnesium and manganese, which additionally contributes to the functionality of Swiss chard (Ninfali & Angelino, 2013). Swiss chard is also rich in dietary fibers, proteins and antioxidants such as alpha-lipoic acid, which is linked to lower glucose levels and increased insulin sensitivity (Ivanovic et al., 2019; Yang et al., 2014).

The plant has a thick, crunchy stalk that can be white or colorful and wide fanlike green leaves (Rana, 2016). Leaves can be consumed raw as part of a salad or cooked alone or along with the stems in a similar way as spinach (Dietitians of Canada, 2020).

In Romania, chard is a less cultivated species, being present in some areas of Transylvania but not in organic culture. Organic farming is an important and ever-growing sector of agriculture and of Romanian economy, because it can bring significant contribution to a sustainable development, increasing the economic activities, thanks to the significant added value of the organic products (Saracin & Vasile, 2015).

Factors such as climate, environmental conditions, applications of fertilizers, time of harvesting, germination, plant physiology state, all affect nutritional properties and phytochemical content of the food crops (Lombardo et al., 2017; Miceli & Miceli, 2014). The application of the optimal amounts of nitrogen fertilizers is a common farmer practice that aims to maximize the economic return and maintain environmental quality (Miceli & Miceli, 2014). The treatment of Swiss chard with different amounts of fertilizer impacted certain nutritive factors (Petrova & Mitova, 2023) but the data regarding the effects of fertilization on phytochemical content of Swiss chard are scarce.

MATERIALS AND METHODS

Experiment took place between 2021-2022, in a organic vegetable farm, in Săcueni (47.347116, 22.099245), Bihor county, Romania. In 2021, average annual temperature was 11.5^{0}C and in 2022, 12.2^{0}C. Annual rainfall recorded was 539.21 mm in 2021, respectively 516.36 mm in 2022, according to data recorded at the Săcuieni Meteorological Station. Soil on which the experiment was placed was alluvial, with a morphological profile of the Ao-AC-CN type. From a physical point of view, the soil in the experimental area has the following characteristics: clay content (0.002 mm) 16.4-19.1; total porosity value, large-51; low apparent density, 1.25 g/cmc; permeability per profile, high - 18 mm/h; useful edaphic volume, large-100. Chemically, the soil had the following characteristics: soil reaction, weakly acidic, pH 5.95-6.4, throughout the profile; humus content, small 1.14-1.51; total nitrogen content, low 0.075; mobile phosphorus content, small 12 ppm; mobile potassium content, very low 60 ppm.

Three organic fertilizers were used: Lignohumate (dose of 1 kg/ha), Alcygol Z2M (dose of 5 l/ha) and Alg Green (dose of 1 l/ha), applied 30 days after emergence. Control remained unfertilized. Three Swiss chard cultivars were tested, Lucullus, Carde Blanche d'Ampuis and Verca.

Establishment of chard culture was carried out by direct sowing on April 28 (2021) and 29 (2022). By combining the two experimental factors (cultivar x fertilizer used) resulted in 9 experimental variants, which were placed in three repetitions. Plot size was 9 m^2 (3 m × 3 m). Chard was planted into rows spaced 25 cm apart.

During the growing season, development degree and plant growth (plant height, leaf rosette diameter, number of leaves, leaf length, length and thickness of petiole) and production (total production, production of petioles) were determined. After harvesting, determinations of dry matter dry matter, betalains, flavonoids, dietary fiber, carbohydrates and vitamin C. Average data of the experimental years 2021 and 2022 is presented in this manuscript.

The edible parts of Swiss chard, leaves and steams were washed with tap water and double distilled water. They were drained completely, dried over filter paper and homogenized with domestic processor in a dark room at 25°C. Vitamin C was determined after the homogenization of fresh Swiss chard. The rest of the samples were packaged in a vacuum plastic bag and stored at −25°C until analysis.

Moisture content was determined by drying the edible parts of Swiss chard in an oven at 105°C until constant weight was obtained. The samples were incinerated in a muffle furnace at 520°C until constant weight was obtained in order to evaluate the ash content (Latimer, 2012). Dietary fibers were determined according to the Scharrer-Kurscher method (Matissek & Steiner, 2006). Total carbohydrates were calculated by subtracting the total amount of proteins, total lipids, total ash, moisture content and dietary indigestible fibers out of a hundred (Ivanovic et al., 2019).

The content of vitamin C in Swiss chard samples was measured on a Reflectometer RQflex10 Reflectoquant using Reflectoquant ascorbic acid test strips (Reflectoquant® Ascorbic Acid Test, Merck, 2016). The results were presented as mg of vitamin C in 100 g FW.

Total flavonoid content (TFC) was determined, according to the method of Sakanaka et al. (2005), using catechin as a standard flavonoid compound. A 0.5 mL of the chard extract (1 mg/mL) was taken in a test tube and 2.50 mL of distilled water and 0.15 mL of a 5 percent $NaNO_2$ solution were added. After 6 min, 0.3 mL of a 10 percent $AlCl_3$ solution was added and allowed to stand at room temperature for 5 min and then 1 mL of 1M NaOH was added to the test tube. The solution was then diluted with distilled water to make the final volume up to 5 mL. The absorbance was read at 510 nm. TFC was calculated from a calibration curve, and the result was expressed as mg of CAE per mg of the water extract. The absorbances were measured using a spectrophotometer (Varian Cary 3E UV-VIS).

Chard soluble carbohydrates were extracted based on the method described by Bartolozzi et al. in 1997. The extract was dried and transformed into trimethylsilyl ethers by treatment with pyridine, hexamethyldisilazane and trimethylchlorosilane. Soluble sugars were analyzed using a Hewlett Packard 5890 series II gas chromatograph equipped with a flame ionization detector (FID) and a HP-5MS column (30 m x 0.25 mm). The soluble sugars contents were expressed as g per 100 g of fresh weight (fw).

Total flavonoids content was determined according to (Zhishen, Mengcheng, & Jianming, 1999). An aliquot (0.5 mL) of Swiss chard solution was mixed with distilled water (2 mL) and subsequently with a $NaNO_2$ solution (5%, 0.15 mL). After 6 min, an $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min. After wards, a NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to adjust the final volume to 5 mL. Then, the blend was properly mixed and allowed to stand for 15 min. The intensity of the pink color was measured at 510 nm. (+)-Catechin was employed to prepare the standard curve and outcomes were expressed as mg of (+)-catechin equivalents (CE) per g of chard extract.

Total phenolics content was determined based on Stojovic method (Stojković et al., 2014). In fact, an aliquot of the chard ethanol extract (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L, 4 mL). The tubes of appropriate dilutions of the ethanol extract were homogenized for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then read at 765 nm. Gallic acid was employed as standard for the calibration curve, and results were expressed as mg of gallic acid equivalents (GAE) per g of chard extract. Results were expressed as a mean ± SD. Significant differences between the means, for experimental variants, were determined with two-way ANOVA and Duncan's test at $p < 0.05$.

RESULTS AND DISCUSSIONS

Growth parameters (plant height, petiole lenght and petiole width) of Swiss chard are presented in Table 1. Plant nutrition through application of fertilizers can influence growth, plant morphology, yield and quality (Ullah et al., 2016). Growth of chard plants, 30 days after emergence (DAE), averaged between 18.33 cm at Verca and 19.12 at Carde Blanche d'Ampuis. This measurement was performed before the application of organic fertilizers. Swiss chard plant growth was influenced by the fertilization with organic fertilizers. Unfertilized variants recorded lower values compared to fertilized variants at all studied cultivars. Regarding plant height 60 DAE, the highest value of 54.35 cm was registered at Lucullus fertilized with Alg. Green. Pokluda and Kuben (2002) determined plant height in 13 Swiss chard cultivars where values ranged from 42.5 cm (Charlote) to 57.9 cm (Swiss Chard-Kings).

Table 1. Influence of cultivar and organic fertilizers on plant growth (average for 2021-2022)

Variant		Plant height (cm) after:				Petiole length (cm)		Petiole width (cm)	
Cultivar	Fertilizer	30 days		60 days		60 days		60 days	
Lucullus	Unfertilized	18.77	d	48.67	J	13.42	f	2.03	i
	Lignohumate	18.81	c	53.22	B	14.81	c	3.12	f
	Alcygol Z2M	18.73	d	49.61	G	14.53	d	2.72	h
	Alg Green	18.72	d	54.35	A	15.83	a	4.05	b
Carde Blanche d'Ampuis	Unfertilized	18.65	e	49.00	J	13.54	f	2.33	i
	Lignohumate	18.71	d	51.52	F	15.26	b	4.24	a
	Alcygol Z2M	19.12	a	52.81	C	14.91	c	3.03	g
	Alg Green	18.55	f	49.25	I	15.35	b	4.02	b
Verca	Unfertilized	18.46	g	48.53	J	12.52	f	2.54	i
	Lignohumate	18.43	g	51.82	E	14.91	c	3.71	d
	Alcygol Z2M	18.63	e	49.41	H	14.22	e	3.31	e
	Alg Green	18.94	b	52.05	D	15.31	b	3.92	c
LSD P = .05		0.065		0.090		0.206		0.094	
Standard Deviation		0.045		0.062		0.142		0.065	

Means followed by same letter or symbol do not significantly differ (P = .05, Duncan's New MRT)

Petiole length 60 DAE, the highest value of 15.8 cm was registered also at Lucullus fertilized with Alg. Green. Petiole width 60 DAE was between 2.03 cm at unfertilized Lucullus variant and 4.24 at Carde Blanche d'Ampuis fertilized with Lignohumate. Similar results were obtained by Kolota et al., in 2017, when they studied Swiss chard Lucullus plants development under the effect of different doses of nitrogen.

Compared with the average of unfertilized variants, results obtained by fertilized variants were statically assured regarding al measured traits.

Swiss chard harvesting began in the second decade of July and continued until the second decade of November (in both years), with 6 harvests carried out during the growing season. Volume of harvested production increased from the beginning of the harvest period until September, after which it decreased to all experimental variants. Lowest yields were obtained by unfertilized variants. For the fertilized variants, yields varied between 44.67 t/ha at Carde Blanche d'Ampuis cultivar fertilized with Lignohumate and 51.04 t/ha at Lucullus fertilized with Alg Green (Figure 1). Compared to the average of unfertilized variants, the differences in production obtained in fertilized variants are significant (Figure 1). This values are simillar or higher to those obtained by Krishkova et al., 2022, in an experience with 13 cultivars, yields ranging from 16 t/ha to 52 t/ha. Lower yields, of 43.5 t/ha were recorded for Lucullus, in organic culture, by Murga-Orrillo et al., in 2019.

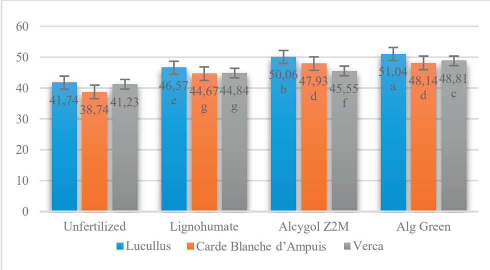

Figure 1. Swiss chard production (average 2021-2022)
Means followed by same letter or symbol do not significantly differ (P = .05, Duncan's New MRT)
LSD P = .05 0.601
Standard Deviation 0.414

Dry matter (DM) content was measured at each harvest and averages were made for the summer and autumn months. DM content was lower in unfertilized variants compared to fertilized ones, at all cultivars. For harvests done in summer, DM content of leaf petiole averaged between 7.05% for Lucullus unfertilized and 9.05% for Verca fertilized with Lignohumate (Table 2). In leaf blades, DM content was higher compared to values recorded in petiole, so that during summer harvests, values were on average between 11.88% (Lucullus unfertilized) and 13.90% (Verca fertilized with Lignohumate). For autumn harvests, DM content was higher, in leaf petiole and leaf blades compared to summer harvests. Bozokalfa et al., 2016, determined DM content of 52 Swiss chard cultivars, minimum value being 9.02%, maximum value being 18.53% and average was 10.89%. In similar experiments, Lucullus, Green White, Ribbed, Vulcan, Bresanne, Green Silver varieties had on average a DM content in leaf blades of 11.62% in summer harvested plants and 13.96% in autumn. In the leaf petiole the content was 6.93% for summer harvested plants and 8.54% autumn (Kołota et al., 2010).

Table 2. Influence of cultivar and fertlizer on swiss chard plant content in dry matter and vitamin C (average for 2021-2022)

Variant		Dry matter content (%) in:				Vitamin C content (mg/100 g f.w.)			
Cultivar	Fertilizer	Leaf petiole		Leaf blades		Leaf petiole		Leaf blades	
		Summer	Autumn	Summer	Autumn	Summer	Autumn	Summer	Autumn
Lucullus	Unfertilized	7.05	8.12	11.88	13.79	33.25	48.93	23.27	36.80
	Lignohumate	7.43	8.60	12.14	14.37	36.27	52.77	24.78	38.87
	Alcygol Z2M	7.26	8.37	12.09	14.08	35.50	50.45	23.62	38.04
	Alg Green	7.40	8.55	12.25	14.20	36.02	51.16	24.56	38.51
Carde Blanche d'Ampuis	Unfertilized	8.04	9.83	12.85	14.28	37.44	58.37	23.74	37.66
	Lignohumate	8.71	10.29	13.57	15.21	39.80	61.72	25.08	39.66
	Alcygol Z2M	8.33	10.07	13.20	14.76	39.01	60.79	24.85	39.03
	Alg Green	8.65	10.34	13.38	15.12	39.36	61.12	24.70	39.21
Verca	Unfertilized	8.72	9.83	13.12	14.17	36.51	54.30	24.00	36.22
	Lignohumate	9.05	10.45	13.90	14.70	37.92	57.42	25.12	38.42
	Alcygol Z2M	8.87	10.18	13.44	14.51	36.73	56.77	24.65	37.58
	Alg Green	9.02	10.33	13.78	14.62	37.40	57.08	25.04	38.12

Vitamin C content was lower in unfertilized variants compared to fertilized ones, at all cultivars. Vitamin C content was higher during autumn harvests in both leaf petiole and blades (Table 2). Leaf petiole also contained a higher amount of vitamin C compared to leaf blades. Similar level of vitamin C concentration in this vegetable crop within a range of 43.8 and 59.8 mg/100 g f.w., depending on the organic fertilizers, was found by Rivelli & Libutti, 2022.

In this study, dietary fiber content (DF) outcomes revealed that DF (Table 3) was between 2.45 g/100 g f.w. for Carde Blanche d'Ampuis unfertilized and 2.74 g/100 g f.w. for Lucullus fertilized with Lignohumate. Similar results were obtained by Mzoughi et al., 2019. Generally, the chemical composition of plant species differs depending on the harvest period and the growth conditions (e.g. climate, soil quality, irrigation, treatments, etc.). It is well-known that a daily intake of 7 g of plant dietary fibers is considered enough to significantly decrease the menace of cardiovascular and coronary heart diseases (Barreira et al., 2017). In this sense, the consumption of 100 g of fresh wild Swiss chard leaves would cover approximately 35% of the recommended daily dose. Thus, chard leaves can be considered an interesting source of dietary fiber and could be added to other foods to improve fiber intake (Mzoughi et al., 2019).

The consumption of vegetables that are rich in phenols and flavonoids is associated with preventions of diseases caused by oxidative stress (Ballistreri et al., 2013).

Total phenol (TPC) and flavonoid (TFC) contents are shown in Table 3. TPC varied from 18.23 to 55.78 mg of GAE/mg of extract. Sacan and Yanardag (2010) measured TPC of water extract from dry Swiss chard, and they found 31.09 mg pyrocatechol equivalent/mg extract. The difference in results may be due to the use of different equivalent, where gallic acid and pyrocatehol have different reactivity with FC reagents (Everette et al., 2010). TFC ranged from 10.21 to 14.55 mg CAE/mg of extract. The used equivalent was the same as the one used by Sacan and Yanardag (2010), and the values for TFC were in agreement with this study (11.88 mg CAE/mg of extract) (Sacan and Yanardag, 2010).

Table 3. Influence of cultivar and organic fertilizers on fiber, total phenols and total flavonoids content (average for 2021-2022)

Variant		Dietary fibers (g/100g f.w.)	Total phenols (mg GAE/g extract)	Total flavonoids (mg CE/g extract	Total carbohydrates (mg/100g f.w.)
Cultivar	Fertilizer				
Lucullus	Unfertilized	2.60	21.32	11.50	5.68
	Lignohumate	2.74	35.65	12.88	5.81
	Alcygol Z2M	2.62	44.23	13.32	6.02
	Alg Green	2.56	53.44	13.89	5.96
Carde Blanche d'Ampuis	Unfertilized	2.45	18.23	10.21	5.92
	Lignohumate	2.55	29.25	12.67	6.05
	Alcygol Z2M	2.52	34.21	13.11	6.16
	Alg Green	2.50	42.89	13.20	6.00
Verca	Unfertilized	2.56	32.18	11.02	5.80
	Lignohumate	2.68	41.12	13.21	6.02
	Alcygol Z2M	2.61	44.13	14.12	6.10
	Alg Green	2.58	55.78	14.55	5.92

However, TPC and TFC in different cultivars of Swiss chard and other vegetables vary widely and they are difficult to interpret or compare to our study, because many factors have been showed to influence TPC and TFC.

Total carbohydrates content in Swiss chard, ranged from 5.68 g/100 g f.w. at Lucullus unfertilized and to 6.16 g/100 g f.w. Carde Blanche d'Ampuis fertilized with Alcygol Z2M. These values are higher compared to 3.74 g/100 g f.w. found by the U.S Department of Agriculture (USDA, 2014) and lower compared to 6.84 g/100 g f.w. found by Ivanovic et al., 2019.

CONCLUSIONS

Swiss chard can be grown organically with good results, if organic fertilizers are applied. Growth parameters and production of Swiss chard were influenced by used cultivar and type of fertilizer. Plant height, 60 DAE, was higher at Lucullus fertilized with Alg Green (54.35 cm). This was also the case for petiole length, that reached 15.83 cm. Petiole width was higher at Carde Blance d'Ampuis (4.24 cm) when fertilized with Lignohumate. Plant content in DM, fibers and vitamin C, was higher when Swiss chard was fertilized with Lignohumate, at all studied cultivars. Swiss chard petiole DM content was higher, in the material harvested during autumn compared to the one harvested in summer. Higher DM content was registered by Verca when fertilized with Lignohumate, of 10.45% in petiole and 13.90% in leaf blades. These results are also confirmed by Colonna et al. in 2016, when they had grown Swiss chard under different light intensities and at a lower light intensity (as in autumn), the DM content is higher. Vitamin C content was lower in unfertilized variants compared to fertilized ones, at all cultivars. Vitamin C content was higher during autumn harvests at Carde Blance d'Ampuis when fertilized with Lignohumate, of 61.72 mg/100 g f.w. in petiole and 39.66 mg/100 g f.w. in leaf blades. Leaf petiole also contained a higher amount of vitamin C compared to leaf blades. Swiss chard has a good DF content, 100 g of fresh wild Swiss chard leaves would cover approximately 35% of the recommended daily dose (Mzoughi et al., 2019). High DF content was register at Lucullus fertilized with Lignohumate (2.74 g/100 g f.w.).

TPC varied from 18.23 to 55.78 mg of GAE/mg of extract. TFC ranged from 10.21 to 14.55 mg CAE/mg of extract. However, TPC and TFC in different cultivars of Swiss chard and other vegetables vary widely and they are difficult to interpret or compare to our study, because many factors have been showed to influence TPC and TFC. Total carbohydrates content was between 5.68 mg/100 g f.w. at Lucullus unfertilized and 6.16 mg/100 g f.w. at Carde Blance d'Ampuis, fertilized with Alg Green. These results were somewhat average compared to the findings of other authors and was influenced by used cultivar and fertilizer.

Results proved that chard leaves have a nutritional profile suitable to be including in modern diets. Also, chard leaves exhibit a significant content of antioxidants compounds, such as flavonoids, phenolic acids.

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