



## Leaf Yield Quality of Swiss Chard *Beta Vulgaris* Spp. Cicla as Influenced by Ammonium: Nitrate Ratio and Organic Fertilizer

Zahra Abed Al-Majeed Noori <sup>1</sup> , Fouad A. Salman <sup>2\*</sup> 

<sup>1</sup> Department of Horticulture and Landscape, Faculty of Agriculture, University of Kufa, Iraq.  
E-mail: zahraaa.heeda@student.uokufa.edu.iq

<sup>2\*</sup> Department of Horticulture and Landscape, Faculty of Agriculture, University of Kufa, Iraq.  
E-mail: fouad.alibraheemi@uokufa.edu.iq

### Abstract

The experiment was conducted to study the effect of NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> ratio and spraying with seaweed extract on the quality indicators of Swiss chard. Nitrate or ammonium fertilization was used alone at a concentration of 400 mg L<sup>-1</sup>, or a balanced combination of both at a ratio of 200:200 mg L<sup>-1</sup> in combination with spraying with SWE extract at levels of 0, 3, 6 g L<sup>-1</sup>. Generally, the results showed that the studied quality indicators were affected differently by different fertilization levels and combinations. As, the results showed that the interaction of NH<sub>4</sub><sup>+</sup>:NO<sub>3</sub><sup>-</sup> ratio (200:200) and seaweed extract at a concentration of 6 g L<sup>-1</sup> led to the highest significant increase in leaf content of ascorbic acid to 44.92 mg 100 g<sup>-1</sup> fresh weight and protein to 6.62%. However, other qualitative indicators, leaf content of oxalic acid 87.85 mg/100 g<sup>-1</sup>, oxalic acid/calcium ratio 1.73, and number of calcium oxalate crystals 265.13 crystals, recorded the highest values in the nitrate 400:0 ammonium treatment in combination with the presence of SWE at a concentration of 3 g/L<sup>-1</sup>.

### Keywords:

*Chemical fertilization, vegetable, organic cultivation, leaf yield.*

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### Introduction

Chard (*Beta vulgaris* var. cicla), in the family Chenopodiaceae, is considered a leafy vegetable crop, widely cultivated in Europe and Asia, and widely used in the Levant and the Middle East (Pešević et al., 2019). The leaves of Swiss chard contain chemicals like iron, copper, calcium, phosphorus, sodium, and potassium. Flavonoids, carotenoids, folic acid, vitamins B and C, syringic acid, and flavonoids (Rivelli & Libutti, 2022). They also have a lot of fibre (up to 1.2 mg. 100 g<sup>-1</sup> fresh weight). It is very important to fertilise your yard so

\*Corresponding Author: Fouad A. Salman, E-mail: fouad.alibraheemi@uokufa.edu.iq

that the plants can get the food they need to grow. Plants need these chemicals to stay alive. Plants need nutrients to do the chemical and physical things they need to do to stay healthy. The most important part of matter is nitrogen. an important macronutrient that plants need a lot of. Plants need between 2% and 5% of their dry matter weight to be made up of it (Dzida et al., 2012).

Plants can get nitrogen from either their leaves (ammonium  $\text{NH}_4^+$ ) or their roots (nitrate  $\text{NO}_3^-$ ). It is called absorption when nitrates and ammonium join with amino acids, proteins, and nucleic acids in plant cells while they are being made (Adriani et al., 2023). A certain amount of ammonia and minerals get into the structure of protoplasm. Organic manure, on the other hand, is an important part of healthy farming and is good for both people and animals (Shi et al., 2024). It also helps plants grow by making the soil better and making it easier for roots to get water and food. When these things are added to the earth in their organic form, organic fertilisers change the amounts of many of these things. These things give plants the things they need, like nitrogen, potassium, and phosphorus. And it works to make the dirt better chemically and physically, making it a great place for many germs to live and work. Small living things called microorganisms help break down nutrients in the dirt so that plants can easily get them. Dlamini et al., (2020) say that this is how plants get minerals all the time during their own lives. This will make the high return factors and growth signs better. The point of the study was to find out how important the ratio of ammonium to nitrate is when nitrogen is added to the soil and seaweed extract is sprayed on plants to see how that affects the quality and nutrients of the spinach leaves (Cefola & Pace, 2015; Alborji, 2014).

## Materials and Methods

It was sprayed on chard leaves that had the amount of ammonium to nitrate in the manure changed. What happened to the quality of the leaves? The test took place in the field on January 25, 2023, at the University of Kufa's Agricultural Research Station (Ensikat et al., 2023). There should be 25 cm of space between lines and 15 cm of space between plants. There should also be 50 cm of space between each trial unit and 1 m of space between each block. Lots of times, as needed, water was slowly added to the plant. They did all the service work the right way so the crop could grow. Two things were looked at in the test. In the first one, 400 mg L<sup>-1</sup> of nitrogen manure was added to see what would happen. This could come in the form of nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), or a mix of the two at a level of 200 mg L<sup>-1</sup>. There wasn't any growth in another way to keep an eye on things. Three different amounts of kelp extract were sprayed on the plants: 0.3, 0.6, and 3 grammes per litre. This extract had 12% kelp extract, 20% alginic acid, and 12%  $\text{K}_2\text{O}$ . It also had 50% organic matter. During the growth season, the plants were sprayed twice. The first time, after four real leaves had grown; the second time, two weeks after the first. They were also given water that had been cleaned. A 10-liter backpack sprayer was used to cover the whole area with the spray. Every answer came 48 hours after the last one. There were three areas with treatments, and each area had 12 experimental units. There were a total of 36 experimental units. With a Random Complete Block plan, the plan was a 4x3 factorial test. The Genesest tool and Duncan's Multiples Range Test were used to compare the means at a 0.05 chance level. This showed how different they were in important ways.

## Qualitative Parameters Under Study

They used a 490 nm spectrophotometer (Herbert et al., 1971) to find out how many carbs were in 1 gramme of dry leaf matter. 0.1N NaOH was used to measure oxalic acid (mg. 100 g<sup>-1</sup>) after two or three drops of a 1% phenolphthalein indicator solution (A.O.A.C., 1990) were added. Efani et al. also found out how much calcium and oxalic acid were in the leaves. To find out how much ascorbic acid was in the leaves (in mg. 100 g<sup>-1</sup> fresh weight), they used the dye 2,6-Dichlorophenol indophenol. They were told to do this by (A.O.A.C., 1970). The

Kjeldahl method with Micro Kjeldahl was used to find out how much protein was in the leaves. The fresh weight was used to find the protein percentage. The way we did things was based on what (Cataldo et al., 1975) said should be done. It was found that the leaves had mg.g-1 of nitrates in their dry weight. Putting the work into sections was another part. This was done to make a list and a picture of the calcium oxalate crystals that were found in the cell vacuoles of the leaf blade cells after each treatment. Webb (1999) says that the fifth leaf from the plant's growth tip was taken (Haynes, 1980). We cut up some leaf samples so that they would fit in formedalin. We then mixed acetic acid and alcohol together and used a strong light microscope to count how many calcium oxalate crystals were in each part (Costa et al., 2009).

## Data Analysis

We used GenStat, a computer program made by VSN International, to look at the data (VSN International, 2009). At or below the 0.05 level, Duncan's multiple range tests were used to see if the changes were indeed important. ANOVA was used to see how the groups had changed over time (Al-Rawi & Khalaf Allah, 1980).

## Results and Discussion

If you look at Table 1, you can see how much ammonium and nitrate were in the leaves changes a lot. There would be more carbs and proteins in the leaves if the amounts of nitrate and ammonium were equal (200: 200). The difference between the amounts or the different rates of fertilisation made this stand out more. At an amount of 6 g. L-1, SWE put on the plants made the leaves have the most carbs, protein, and ascorbic acid. The method that mixed 200:200 nitrate: ammonium balanced fertiliser with 6 g L-1 SWE worked well for most plants. This is why the plants made the most FW (44.92 mg. 100g-1), proteins (6.62%), and carbs (1.295 mg. g-1). The group that had ascorbic acid had more than 1.013 mg g-1, which is 1.49%, or 30.03 mg 100g-1 FW.

Table 1. Effect of ammonium to nitrate ratio and spraying with SWE on chard *Beta vulgaris* var. *cicla* leaf content of some nutrients

Treatments		Leaf content of:			
Ammonium: Nitrate mg. L <sup>-1</sup>	SWE g. L <sup>-1</sup>	Carbohydrates mg g <sup>-1</sup> DW	Protein (%)	Nitrate mg g <sup>-1</sup> DW	Ascorbic acid mg 100g <sup>-1</sup> FW
<b>Control</b>	0	1.013 c	1.49	122.60	30.03
	3	1.037 c	2.09	124.30	32.13
	6	1.180 bc	3.59	129.22	38.11
<b>NO<sub>3</sub><sup>-1</sup> 400</b>	0	1.026 c	2.76	124.83	39.29
	3	1.062 bc	2.54	125.31	38.94
	6	1.549 a	3.18	135.40	41.50
<b>NH<sub>4</sub><sup>+1</sup> 400</b>	0	1.058 c	2.08	145.22	32.41
	3	1.177 bc	2.48	137.74	35.88
	6	1.261 b	4.78	141.56	38.59
<b>NO<sub>3</sub><sup>-1</sup>:NH<sub>4</sub><sup>+1</sup> 200:200</b>	0	1.180 bc	4.63	130.50	41.35
	3	1.203 b	4.58	130.04	41.17
	6	1.295 b	6.62	141.12	44.92

\*Values are means of 3 replications, means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ( $P \leq 0.05$ )

When ammonium wasn't present, the treatment with 400 mg/L of nitrate had the most leaf nitrate. Though, it wasn't very different from the amount found in the 200:200 balanced fertilisation methods. A rise

in the amount of nitrate in the leaves was seen as the amount of SWE used went up. There was the most of it when the quantity was 6 mg L<sup>-1</sup>. The leaves had 141.56 mg L<sup>-1</sup> of nitrate after 6 g L<sup>-1</sup> SWE and 400 mg L<sup>-1</sup> nitrate were added to them.

The treatment with 400 nitrate and no ammonium worked better than the one with 200:200 ammonium to nitrate that was changed to nitrogen. The 400 nitrate to 0 ammonium treatment had more oxalic acid, more calcium oxalate crystals, and longer ratios of oxalic acid to calcium in the leaf tissue. When 3 g L<sup>-1</sup> SWE was sprayed on, the results were even better: 85.69 mg 100 g<sup>-1</sup> and 1.64, compared to 38.39 mg 100 g<sup>-1</sup> and 1.17 in the control treatment. Effect of ammonium to nitrate ratio and spraying with SWE on chard *Beta vulgaris* var. *cicla* leaf content of some qualitative parameters shown in Table 2.

Table 2. Effect of ammonium to nitrate ratio and spraying with SWE on chard *Beta vulgaris* var. *cicla* leaf content of some qualitative parameters

Treatments		Leaf content		
Ammonium: Nitrate mg. L <sup>-1</sup>	SWE g. L <sup>-1</sup>	Oxalic acid mg 100g <sup>-1</sup>	Oxalic: Calcium Ratio	No. of calcium oxalate crystals
<b>Control</b>	0	38.39 f	1.17 e	161.10 bc
	3	52.15 e	1.41 b	141.73 c
	6	58.49 d	1.61 ab	201.14 b
<b>NO<sub>3</sub><sup>-1</sup> 400</b>	0	61.48 d	1.44 b	148.06 c
	3	68.90 d	1.54 ab	117.40 d
	6	72.60 c	1.57 ab	157.33 c
<b>NH<sub>4</sub><sup>+1</sup> 400</b>	0	86.79 a	1.42 b	284.15 a
	3	87.85 a	1.73 a	265.13 ab
	6	85.69 a	1.64 ab	211.77 b
<b>NO<sub>3</sub><sup>-1</sup>: NH<sub>4</sub><sup>+1</sup> 200:200</b>	0	72.69 bc	1.35c	187.09 bc
	3	74.32 bc	1.34c	143.35 c
	6	78.04 b	1.37 c	152.00 c

\*Values are means of 3 replications, means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ( $P \leq 0.05$ ).

There was more oxalic acid in the leaves of plants that had nitrogen added to them in the form of nitrate NO<sub>3</sub><sup>-</sup>. This might be because nitrate speeds up the process of changing vitamin C (ascorbic acid) into dehydro ascorbic acid, which is needed to make carboxylic acids (Wang, 2008). Oxalic acid is what this chemical turns into overtime. This is the reason why plants whose leaves had nitrate NO<sub>3</sub><sup>-</sup> added to them had less ascorbic acid. It's also possible that when the nitrate level went up, the plant made more of the chemical Glyoxylate. Glyoxylate is one of the main things that are used to make oxalic acid (Gomez-Espinoza et al., 2021). That area around the cell might become acidic if there is more of this acid inside it (Erfani et al., 2007). This could be bad for the cell. Say that cells store this acid in the cell vacuole, connect it to calcium, and turn it into calcium oxalate crystals that water can't break down. This is why there were more calcium oxalate crystals in plant cells that had nitrate added to them. At this point, plants that had been treated with nitrate had more oxalic acid than calcium than plants that had been treated with ammonium.

If you spread kelp extract, the quality marks might get better because it makes the fertilisers have more nutrients. Sapkota et al., (2021) say that the plant's leaves grow faster and photosynthesis works better because it gets better food. Because of this, more man-made nutrients are often made and stored (Hailay & Haymanot, 2019). These nutrients help the body do many important things, like store carbs longer and make more of them. This is one of the most important rules for making metabolic drugs, according to (Al-Ibraheem & Salman,

2020). These are the building blocks that plants need to make proteins, amino acids, vitamins, and organic acids. Researchers before this one, (Murga-Orrillo et al., 2019; Apahidean et al., 2024), also discovered that giving chard plants organic fertilisers and seaweed extract made most of the quality measures better.

## Conclusions

This study concluded that the ratio of  $\text{NH}_4^+:\text{NO}_3^-$  had a clear effect on the qualitative indicators of Swiss chard leaves. It was found that the leaf content of nitrate, oxalic acid and oxalic acid/calcium ratio increased when using nitrate fertilization in the absence of ammonium (400 nitrate: 0 ammonium). This generally had an effect on increasing the number of calcium oxalate crystals in the leaf tissue. While, applying foliar seaweed extract had an effect on improving most of the qualitative and chemical indicators.

## Author Contributions

All Authors contributed equally.

## Conflict of Interest

The authors declared that no conflict of interest.

## References

- A.O.A.C. (1970). Official Method of Analysis 11<sup>th</sup> Edn., Association of the Official Analytical Chemistry, Washington, D.C. U.S.A. pp. 101.
- A.O.A.C. (1990). Official Methods of Analysis. 15<sup>th</sup> Edn., Association of the Official Analytical Chemistry, Washington, D.C. U.S.A. pp. 200-210.
- Adriani, D., Dewi, R., Saleh, L., Heryadi, D. Y., Sarie, F., Sudipa, I. G. I., & Rahim, R. (2023). Using Distance Measure to Perform Optimal Mapping with the K-Medoids Method on Medicinal Plants, Aromatics, and Spices Export. *Journal of Wireless Mobile Networks, Ubiquitous Computing, and Dependable Applications*, 14(3), 103-111. <https://doi.org/10.58346/JOWUA.2023.I3.008>
- Alborji, B. (2014). Feed water system's optimization in thermal power plants (case study) by vector control inverters. *International Academic Journal of Science and Engineering*, 1(2), 122-132.
- Al-Ibraheem, N. A., & Salman, F. A. (2020). Effect of nitrogen sources and zeolite applications on the nitrate content and nitrate reductase activity of lettuce (*Lactuca sativa* L.) leaves. *International Journal of Agricultural & Statistical Sciences*, 16.
- Al-Rawi, K. M., & Khalaf Allah, A. M. (1980). Design and analysis of agricultural experiments. *El Mousel Univ., Iraq*, 19, 487.
- Apahidean, A. I., Hoza, G., Carbutar, M., Bei, M., Dinu, M., Cojocaru, A., & Rozsa, S. (2024). The influence of cultivar and organic fertilizations on plant growth, production and quality of swiss chard, in western romania. *Scientific Papers. Series B. Horticulture*, 68(1), 392-398.

- Cataldo, D. A., Maroon, M., Schrader, L. E., & Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in soil science and plant analysis*, 6(1), 71-80. <https://doi.org/10.1080/00103627509366547>
- Cefola, M., & Pace, B. (2015). Application of oxalic acid to preserve the overall quality of rocket and baby spinach leaves during storage. *Journal of food processing and preservation*, 39(6), 2523-2532. <https://doi.org/10.1111/jfpp.12502>
- Costa, L. M. D., Tronto, J., Constantino, V. R. L., Fonseca, M. K. A., Oliveira, A. P., & Costa, M. R. D. (2009). Extraction and concentration of biogenic calcium oxalate from plant leaves. *Revista Brasileira de Ciência do Solo*, 33, 729-733. <https://doi.org/10.1590/S0100-06832009000300025>
- Dlamini, C., Masarirambi, M. T., Wahome, P. K., & Oseni, T. O. (2020). The Effects of Chicken Manure Application Rates on Growth and Yield of Swiss Chard (*Beta vulgaris* var. *cicla* L.). *Asian Journal of Advances in Agricultural Research*, 12(4), 12-19. <https://doi.org/10.9734/ajaar/2020/v12i430088>
- Dzida, K., Jarosz, Z., Michałojć, Z., & Nurzyńska-Wierdak, R. (2012). The influence of diversified nitrogen and liming fertilization on the yield and biological value of lettuce. *Acta Scientiarum Polonorum. Hortorum Cultus*, 11(3), 239-246.
- Ensikat, H. J., Malekhosseini, M., Rust, J., & Weigend, M. (2023). Visualisation of calcium oxalate crystal macropatterns in plant leaves using an improved fast preparation method. *Journal of Microscopy*, 290(3), 168-177. <https://doi.org/10.1111/jmi.13187>
- Erfani, F., Hassandokht, M. R., Jabbari, A., & Barzegar, M. (2007). Effect of cultivar on chemical composition of some Iranian spinach. *Pakistan Journal of Biological Sciences: PJBS*, 10(4), 602-606. <https://doi.org/10.3923/pjbs.2007.602.606>
- Gomez-Espinoza, O., Gonzalez-Ramirez, D., Mendez-Gomez, J., Guillen-Watson, R., Medaglia-Mata, A. & Bravo, L.A. (2021). *Calcium Oxalate Crystals in Leaves of the Extremophile Plant Colobanthus quitensis (Kunth)Bartl. (Caryophyllaceae)*. *Plants*, 10: 1-12. <https://doi.org/10.3390/plants10091787>
- Hailay, G., & Haymanot, A. (2019). The response of Swiss chard (*Beta vulgaris* L.) to nitrogen levels and intra-row spacing in Debre Berhan Central Ethiopia. *Journal of Horticulture and Postharvest Research*, 2(2), 105-116. <https://doi.org/10.22077/jhpr.2019.2099.1041>
- Haynes, R. J. (1980). A comparison of two modified Kjeldahl digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. *Communications in Soil Science and Plant Analysis*, 11(5), 459-467. <https://doi.org/10.1080/00103628009367053>
- Herbert, D., Philips, P. J., & Strange, R. E. (1971). Chemical análisis of Microbial cells. *Methods in Microbiology*. Norris, JR, and Ribbons, DW, Eds, 5.
- Murga-Orrillo, H., Irigoín-Aguilar, J. M., Hilaes-Vargas, S., Bardales-Lozano, R. M., & Lobo, F. D. A. (2019). Fertilizers and organic covers, slow release nutrient sources in the production of multiple harvest Swiss chard. *Revista de Ciências Agroveterinárias*, 18(3), 380-383.

- Pešević, D., Knežević, N., & Marković, M. (2019). Quality Assessment of Vegetable Oil Effluent Discharged into Sava River. *Archives for Technical Sciences*, 2(21), 85–93. <https://doi.org/10.7251/afts.2019.1121.085P>
- Rivelli, A. R., & Libutti, A. (2022). Effect of biochar and inorganic or organic fertilizer co-application on soil properties, plant growth and nutrient content in Swiss chard. *Agronomy*, 12(9), 2089. <https://doi.org/10.3390/agronomy12092089>
- Sapkota, A., Sharma, M. D., Giri, H. N., Shrestha, B., & Panday, D. (2021). Effect of organic and inorganic sources of nitrogen on growth, yield, and quality of beetroot varieties in Nepal. *Nitrogen*, 2(3), 378-391. <https://doi.org/10.3390/nitrogen2030026>
- Shi, S., Caluyo, F., Hernandez, R., Sarmiento, J., & Rosales, C. A. (2024). Automatic Classification and Identification of Plant Disease Identification by Using a Convolutional Neural Network. *Natural and Engineering Sciences*, 9(2), 184-197. <https://doi.org/10.28978/nesciences.1569560>
- VSN International (2009). GenStat for Windows 12th Edition. *VSN International*, Hemel Hempstead, UK.
- Wang, Y. T. (2008). High NO<sub>3</sub>-N to NH<sub>4</sub>-N ratios promote growth and flowering of a hybrid *Phalaenopsis* grown in two root substrates. *Hortscience*, 43(2), 350-353. <https://doi.org/10.21273/HORTSCI.43.2.350>
- Webb, M. A. (1999). Cell-mediated crystallization of calcium oxalate in plants. *The plant cell*, 11(4), 751-761. <https://doi.org/10.1105/tpc.11.4.751>