RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 60 Issue 1: 62-68 (January 2026)

Influence of Foliar Nano and Organic Fertilizers on Some Growth Traits and Nitrate Aggregation in Swiss Chard (*Beta vulgaris* var. *cicla* L.)

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10.18805/IJARe.AF-958

ABSTRACT

Background: Fertilization serves an essential function in improving crop growth during both the vegetative and reproductive phases. Contemporary trends have increasingly focused on the utilization of nano and organic fertilizers as a substitute for conventional fertilizers.

Methods: The current study was implemented at the experimentation field located in the University of Kufa during the growing season 2022-2023 to assess the effect of foliar applications with nano-fertilizer and organic fertilization on certain growth traits and nitrate accumulation in Swiss chard plants. A factorial experiment with three replicates following a completely randomized block design was carried out, where the first factor included three doses of the nano-fertilizer (0, 2, 4) $ml.L^{-1}$ while the second factor included three doses of organic fertilizer (0, 10, 20) $g.L^{-1}$.

Result: Our results showed that foliar application of nano-fertilizer, organic fertilization and the interaction between them significantly affected growth and chemical characteristics. The highest averages were observed from the interaction between the nano-fertilizer at (4 $ml.L^{-1}$) and the organic fertilizer at (20 $g.L^{-1}$) regarding plant height, number of leaves, fresh and dry weights of the sheet, total chlorophyll in the leaves and vitamin C in the leaves, while a decrease in the nitrate content of the leaves. In contrast, the control treatment exhibited the lowest values for the most characteristics.

Key words: Growth traits, Nano-fertilizer, Nitrate, Organic fertilization, Spray, Swiss chard.

INTRODUCTION

The Swiss chard crop is one of the important winter crops in the world including Iraq due to its significant nutritional benefits. It is considered one of the oldest types of vegetables, known to both the Greeks and Romans and has been recognized since 300 BC. Its roots were initially employed for therapeutic applications and later its green leaves were used more widely for their medicinal benefits. Swiss chard is abundant in nutrients, amino acids, minerals, vitamins, fibers and carbs and it is used to naturally color food products and treat various diseases (Mustafa, 2010). It is affected by many factors, most notably fertilization *via* exogenous application, as most plants possess the capability to absorb nutrients when subjected to spraying on the foliage *via* the symplast and apoplast pathways effectively. Hence, foliar nutrition has become a good method for supplying plants with nutrients, in addition to being an effective way to transfer nutrients more efficiently and quickly within the plant (Al-Jawari, 2002; Kuepper, 2003, Alhasnawi *et al.,* 2025). Nowadays, nanotechnology represents one of the pioneering techniques that has demonstrated its efficacy within the agriculture industry to address numerous challenges. In recent years, attention has shifted towards using modern technologies in the field of plant nutrition, with one of the latest techniques being nanotechnology. Studies indicate that the use of nano fertilizers leads to increased efficiency in nutrient utilization and decreases potential adverse effects when applied in the conventional form (Monreal *et al.,* 2016).

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How to cite this article: Thajeel, Z.H. and Al-Bayati, A.S. (2026). Influence of Foliar Nano and Organic Fertilizers on Some Growth Traits and Nitrate Aggregation in Swiss Chard (*Beta vulgaris* var. *cicla* L.). *Indian Journal of Agricultural Research.* **60(1):** 62-68. doi: 10.18805/IJARe.AF-958.

Submitted: 30-05-2025 **Accepted:** 21-08-2025 **Online:** 12-01-2026

The nano fertilizer contains nitrogen, which plays a significant role in the formation of vital organic compounds involved in dynamic processes within the plant, the structure of nucleic acids and the chlorophyll molecule essential for photosynthesis and respiration. It also contributes to the construction of plant tissues, crop dry matter and overall yield (Glotra *et al.,* 2023). Additionally, it includes organic materials that are a rich source of nitrogen, phosphorus and some micronutrients, as well as humic and fulvic acids that contain active groups constituting 30% of the total humus. Organic matter in the soil lowers soil temperature, reduces the impact of salinity, increases the availability of many nutrients and stimulates plant growth (Hamayun *et al.,* 2011). The nano fertilizer

also contains amino acids that play a role in increasing the chlorophyll capacity of the leaves, as they are a source of nitrogen and contribute to building biosynthetic pathways (Hildebrandt *et al.,* 2015). Al-Saidi *et al.* (2022) found in their research on fenugreek during the 2020-2021 agricultural season that spraying the nano fertilizer (NPK) at a dose of 2 $g.L^{-1}$ had a substantial impact on all indicators of vegetative growth of the plant, likewise plant height and leaf number, as to the absolute control.

To achieve optimal growth and production of plants, essential nutrients must be readily available in sufficient quantities and in a balanced ratio to yield good harvests, particularly for the health-beneficial Swiss chard. Consequently, there has been increased interest among specialists in producing and improving vegetable plants by utilizing the best cultivation practices, particularly in organic fertilization, which plays a fundamental role in plant growth by activating several internal biological activities, such as promoting root growth and enhancing their nutrient absorption capacity, as well as improving the process of carbon metabolism. Moreover, organic fertilizers significantly contribute to enhancing the physicochemical and biological properties of the soil (Muslit and Muslih, 2012; Al-Bayati and Majeed, 2022). Saeed and Abdul Rahman (2016) found that organic fertilizer significantly improved the morphological growth traits of the Swiss chard plants, increasing plant height and leaf number in comparison with the unfertilized plants. Further more, Al-Khikani (2022) indicated that spraying organic fertilizer on broccoli significantly affected vegetative and chemical growth traits, increasing the following growth characteristics: plant height, the vegetative mass dry weight, collective chlorophyll in the leaves, vitamin C and nitrate concentration in contrast to the untreated plants (control), which yielded the minimum means.

The current investigation sought to assess the impacts of foliar applications with nano and organic fertilizers on the growth and quality traits of Swiss chard plants.

MATERIALS AND METHODS

Field experiment details

The field trial was implemented at the experimentation field located in the University of Kufa, Iraq during the 2022-2023 growing season on Swiss chard. The experimental soil was analyzed at the Soil Science and Water Resource Department, Faculty of Agriculture, University of Kufa (Table 1). The seeds were sown on 23/10/2022 after conducting a germination rate test, which reached 96%. The treatment was set up in a factorial experiment with three replicates following a completely randomized block design. The first factor comprised three doses of the nano-fertilizer (0, 2, 4) $ml.L^{-1}$ while the second factor included three doses of organic fertilizer (0, 10, 20) $g.L^{-1}$. The seeds were planted in rows with a spacing of 30 cm between plants and a depth of 2 cm (Matloub *et al.,* 1989). A foliar application of nano-fertilizer was carried out on 29/11/2022, consisting of two applications: The first spray was applied a month from planting, then leaving two-week intervals for the second spray. The organic fertilizer was sprayed on 6/12/2022, consisting of two applications separated by 15 days (Al-Bayati *et al.,* 2023), while the unsprayed plants as a control were treated with distilled water only. The prepared doses of each fertilizer were mixed with a surfactant to reduce surface tension. The spraying was performed early in the morning until complete wetting and the treatments were separated by barriers to avoid contamination between different treatments. The composition of each fertilizer is described in (Table 2 and 3).

Vegetative growth traits

Plant height of Swiss chard (cm) was determined from three plants selected randomly and then taking the average. The total number of leaves in a single plant was also counted from three random plants and then averaged. Shoot fresh weight (g) was taken using a scale, then the average was calculated. Shoot dry weight (g) for the sampled plants was measured using a sensitive scale and then the average was calculated.

Chemical and quality traits

Collective chlorophyll pigment in the fresh green leaves (mg.100 g^{-1} f. w.) was determined as described by Goodwin (1976). Nitrate content (mg.100 g^{-1} d. w.) was estimated separately in both the leaf's photosynthetic surface and leafstalks according to the method of Cataldo *et al.* (1975). Vitamin C content in leaves (mg.100 g^{-1} f. w.) was estimated as described by Abbas and Abbas (1992).

Statistical procedure

Statistical data analysis was done through a two-way ANOVA procedure with the help of the statistical software GenStat 12th edition. The comparison of treatment means was executed employing the least significant difference (LSD) test at the probability level of 0.05.

Table 1: Physicochemical analyses of the experiment soil sampled pre-planting.

Clay $g.kg^{-1}$	Silt $g.kg^{-1}$	Sand $g.kg^{-1}$	Soil texture	pH	ECd $S.m^{-1}$	O.M.%	$Nmg.L^{-1}$	$Pmg.L^{-1}$	$Kmg.L^{-1}$	$Camg.L^{-1}$
60	68	872	Sandy-loam soil	7.31	1.56	0.99	41.60	61.41	88.88	3.50

Table 2: Compositions of nano fertilizer applied in the experiment.

Composition	Organic matter %	Amino acids %	N%	Organic carbon %	pH
Value	50	29	2	20	5-8

RESULTS AND DISCUSSION

Effect of both fertilizers on the vegetative growth of Swiss chard

The findings in Table (4) indicate that foliar spray with a nano fertilizer at a dose of 4 ml.L^{-1} significantly affected the following growth characteristics (plant height, number of leaves, shoot fresh weight and shoot dry weight), yielding values of (44.30 cm, 18.99 leaves.$plant^{-1}$, 225.91 g and 63.23 g) in contrast to the non-fertilized plants, which showed the minimum averages of (41.70 cm, 14.11 leaves. $plant^{-1}$, 195.31 g and 58.8 g), respectively. This effect is ascribed to the act of nano fertilizer that is capable of either delivering nutrients to the plant or assisting in the transport or absorption of available nutrients, which leads to enhanced plant growth (Benzon *et al.,* 2015). Furthermore, nano fertilizers have distinct characteristics compared to conventional fertilizers as they have proven effective in increasing plant growth when used with various crops due to their small size and increased surface area. This results in an enlarged absorption surface and thereby improves the efficiency of nutrient utilization through specific mechanisms, leading to increased photosynthesis and nutrient production in the plant and consequently enhancing vegetative growth indicators (Siddiqi and Husen, 2017; Sohair *et al.,* 2018). Once nano fertilizers enter the plant, they associate with transporters such as aquaporins and endocytosis, as well as ionic channels, subsequently stimulating water absorption and encouraging plant growth (Schwab *et al.,* 2015).

This increase can also be attributed to the role of the elements within the composition of the nano-fertilizer, particularly amino acids, which are molecular building blocks containing a primary amino group (NH_2) as well as a carboxylic acid group (COOH). These amino acids, playing a role in protein synthesis, act as biostimulants, quickly absorbed and transported within various parts of the plant due to their direct effect. They are also involved in the formation of nucleotides, vitamins and growth hormones, making them crucial compounds of living matter and protoplasm. In a study by Shafeek *et al.* (2020), they found that the application of nitrogen fertilization and total amino acid foliar spraying on spinach significantly increased plant height, leaf number and dry weight of the vegetative part, against the absolute control. Bonasia *et al.* (2022) reported that applying three concentrations of protein hydrolysates to spinach significantly influenced vegetative growth indicators, increasing leaf area and dry weight as to the unsprayed plants, which yielded the minimum means. Nouri (2024) demonstrated that spraying nitrogen fertilizer on Swiss chard significantly affected vegetative growth traits, such as plant height and number of leaves, in comparison to the absolute control. This was approved by Al-Araji (2023) in carrot plants when sprayed with

Table 3: Compositions of organic fertilizer applied in the experiment.

Composition	O.M.%	Total N%	Organic N%	Soluble P_2O_5%	K_2O%	MgO%	CaO%	Cl%
Value	88	62	4.3	3.23	3.20	1.00	2.10	0.50

Table 4: Effect of foliar application of nano-fertilizer and organic fertilization and their interaction on some vegetative growth traits of swiss chard plants.

Treatments			Plant height (cm)	Leaf No. per plant	Shoot fresh weight (g)	Shoot dry weight (g)
Nano fertilizer		0	41.70	14.11	195.31	58.80
		2	43.30	16.00	213.41	61.80
		4	44.30	18.99	225.91	63.23
LSD $_{0.05}$			2.41	1.68	11.80	2.02
Organic fertilizer		0	38.70	14.44	202.81	58.80
		10	44.13	16.66	212.21	61.50
		20	46.60	18.00	219.73	63.76
LSD $_{0.05}$			2.41	1.68	11.80	2.02
Nano fertilizer ×	0	0	37.60	12.76	175.02	56.10
Organic fertilizer		10	42.20	14.67	198.12	59.30
		20	45.40	15.00	212.81	61.90
	2	0	38.20	14.30	213.12	58.70
		10	44.40	16.00	210.33	62.00
		20	47.10	17.67	217.02	64.69
	4	0	40.30	16.33	220.29	61.80
		10	45.80	19.33	228.18	63.20
		20	47.30	21.33	229.27	64.70
LSD $_{0.05}$			7.37	2.91	22.03	5.11

chamomile extract and nano potassium at a dose of 2 g.L^{-1}, which significantly influenced the measured traits likewise (plant height, number of leaves and dry weight of the vegetative part) against to the untreated plants, which yielded the minimum averages.

It is evident from Table (4) that spraying organic fertilizer at a dose of 20 g.L^{-1} significantly affected the following growth characteristics (plant height, number of leaves, shoot fresh weight and shoot dry weight), recorded (46.60 cm, 18.00 leaves plant^{-1}, 219.73 g and 63.76 g), consecutively. However, the untreated plants (control) showed lower averages of (38.70 cm, 14.44 leaves.plant^{-1}, 202.81 g and 58.80 g), consecutively. This is attributed to the role of organic fertilizers in enhancing the roots' activity in absorbing water and nutrients, particularly important nutrients such as nitrogen, phosphorus and potassium, which contribute to increased vegetative growth. This may also be related to the presence of nitrogen in organic fertilizers that stimulates the production of auxin indole-3-acetic acid (IAA) in plants, as nitrogen is a crucial element for building the amino acid tryptophan, which forms the basic units for synthesizing IAA which is known for its role in promoting cell division and elongation (Ramesh *et al.,* 2024). Moreover, nitrogen influences the increased activity of apical meristems, leading to enhanced cell division and elongation due to the availability of essential materials required by the plant in protein synthesis, along with some enzymatic co-factors NAD and NADP that incorporate nitrogen into their structure (Alrayes, 1987). The positive effect of potassium may be attributed to its role as an activator in protein metabolism and the enzymes associated with carbohydrate metabolism due to its involvement in protoplasmic construction processes (Abu Dahi and Al-Younis, 1988), which subsequently impacts water and nutrient absorption, thus promoting vegetative growth. Magnesium plays two vital roles in the biological processes of plants: Photosynthesis and carbohydrate production and it is involved in chlorophyll composition. This element is crucial for transferring carbohydrates from leaves to stems; it is also responsible for oil metabolism and the movement of phosphorus from the soil while maintaining enzyme activity since it is an integral part of their structure (Al-Nuaimi, 1999). Al-Wahili (2020) reported that phosphorus and tryptophan spraying significantly affected the vegetative growth parameters and the yield of active compounds in coriander, including plant height and the dry weight of the vegetative part, as to the absolute control, which gave the minimum values.

It can be observed from the same table that the combined treatment of (4 ml.L^{-1} of nano fertilizer) and (20 g.L^{-1} of organic fertilizer) had a pronounced significant effect on the vegetative growth characteristics (plant height, number of leaves, shoot fresh weight and shoot dry weight) reaching (47.30 cm, 21.33 leaves.plant^{-1}, 229.27 g and 64.70 g), consecutively. Contrarily, the absolute control yielded the minimum values of these traits (37.60 cm, 12.76 leaves plant^{-1}, 175.02 g and 56.10 g), consecutively.

Table 5: Effect of foliar application of nano-fertilizer and organic fertilization and their interaction on some chemical and quality traits of swiss chard plants.

Treatments			Nitrate content in leaves (mg.100 g^{-1} d. w.)	Nitrate content in leaf stalks (mg.100 g^{-1} d. w.)	Total chlorophyll in leaves (mg.100 g^{-1} f. w.)	Vitamin C content in leaves (mg.100 g^{-1} f. w.)
Nano fertilizer		0	44.13	50.81	108.66	1.26
		2	46.44	49.59	110.90	1.47
		4	48.52	49.91	117.80	1.71
LSD $_{0.05}$			0.99	N.S.	5.10	0.07
Organic fertilizer		0	45.70	49.31	104.90	1.38
		10	45.70	50.73	108.80	1.46
		20	47.70	50.27	123.70	1.59
LSD $_{0.05}$			0.99	N.S.	5.10	0.07
Nano fertilizer ×	0	0	44.40	49.73	102.60	1.04
Organic fertilizer		10	44.20	50.50	105.20	1.26
		20	54.50	52.20	118.20	1.50
	2	0	44.40	48.50	103.50	1.42
		10	45.43	51.77	106.30	1.70
		20	47.27	48.50	123.10	1.30
	4	0	43.60	49.70	108.80	1.69
		10	49.70	49.93	115.10	1.44
		20	43.80	50.10	129.70	1.99
LSD $_{0.05}$			1.72	N.S.	14.30	0.19

N.S. indicates a non-significant effect @ p>0.05.

Effect of both fertilizers on the chemical and quality traits of swiss chard

Table (5) shows that application with exogenous nano fertilizer at a dose of 4 ml.L^{-1} significantly affected the chemical and qualitative traits (nitrate content in leaves, collective chlorophyll content in leaves and vitamin C content in leaves), reaching (48.52 mg.100 g^{-1} d. w., 117.80 mg.100 g^{-1} f. w. and 1.71 mg.100 g^{-1} f. w.) as to the non-treated plants providing the minimum values of (44.13 mg.100 g^{-1} d. w., 108.66 mg.100 g^{-1} f. w. and 1.26 mg.100 g^{-1} f. w.). This increase can be ascribed to the role of the elements in the constituent of the nano fertilizer, particularly N and amino acids, which play a vital role in enhancing the plant's absorption of major nutrients such as phosphorus and potassium, thereby increasing water and nutrient uptake. Amino acids serve as a source of nitrogen necessary for building proteins that promote plant growth and are involved in the biosynthesis of vitamin C and the structure of chlorophyll molecules (Al-Sahaf, 1989). Also, Shafeek *et al.* (2020) reported that the effect of nitrogen fertilization and foliar application of total amino acids on spinach plants, significantly affected the chemical traits, as it increased the total chlorophyll content in the leaves and decreased the nitrate level compared to the absolute control. Bonasia *et al.* (2022) stated that applying three concentrations of protein hydrolysates on spinach plants significantly improved the chemical characteristics, likewise the collective chlorophyll content in the leaves and the vitamin C content, in comparison with the absolute control, which gave the minimum values. Nouri (2024) indicated that applying nitrogen fertilizer to Swiss chard significantly affected the chemical indicators by increasing leaf chlorophyll and reducing the nitrate level as to the absolute control. This was corroborated by Al-Araji (2023) on carrot plants, where the application of chamomile extract and nanoscale potassium at a dose of 2 g.L^{-1} substantially affected the chemical traits by increasing the total chlorophyll rate and vitamin C in comparison to the unsprayed plants.

The results of Table (5) display that the application of organic fertilizer at a dose of 20 g.L^{-1} had significant differences in the chemical and qualitative traits (nitrate content in leaves, collective chlorophyll content in leaves and vitamin C content in leaves) by (47.7 mg.100 g^{-1} d. w., 123.70 mg.100 g^{-1} f. w. and 1.59 mg.100 g^{-1} f. w.) consecutively. Conversely, the absolute control yielded lower values (45.70 mg.100 g^{-1} d. w., 104.90 mg.100 g^{-1} f. w. and 1.38 mg.100 g^{-1} f. w.). The superior performance of the organic fertilizer treatments in terms of leaf chemical traits is ascribed to the fertilizer content such as nitrogen, phosphorus, potassium and magnesium, which are readily available for direct absorption by plants. Moreover, these nutrients play a vital role as they are involved in various physiological and biological activities related to photosynthesis. Notably, nitrogen is a key element in chlorophyll formation and its increased availability and absorption due to the influence of organic fertilizers has led to higher chlorophyll content in the leaves (Al-Sahaf, 1989; Al-Samarrai, 1989). The decrease in nitrate levels in the leaves may be attributed to the role of organic fertilizers, which supply the plant with nitrogen in a balanced manner (NH_4+ + NO_3-), allowing for good plant growth without accumulating any substances beyond permissible limits (Abu Rayyan, 2010). Alternatively, it may be ascribed to the role of humic acids in enhancing biological and enzymatic processes, among which is the enzyme nitrate reductase, which is responsible for the assimilation of nitrate and its reduction to nitrite and then to ammonia. This ammonia binds with keto acids to form the amino acids necessary for protein synthesis, thereby reducing its negative impact. This may explain the decrease in nitrate concentration, which is in agreement with the findings of (Saloum, 2016; Shubber, 2023).

The dual interaction between the nano fertilizer and the organic fertilizer in Table (5) indicates that the dose at (4 ml.L^{-1} of nano fertilizer) and (10 g.L^{-1} of organic fertilizer) augmented nitrate accumulation in the leaves (49.70 mg.100 g^{-1} d. w.) as to the combination of (4 ml.L^{-1} of nano fertilizer) and (0 g.L^{-1} of organic fertilizer) which gave the least values of (43.60 mg.100 g^{-1} d. w.). Furthermore, the dual interaction between (4 ml.L^{-1} of nano fertilizer) and (20 g.L^{-1} of organic fertilizer) had a significant impact on the total chlorophyll and the vitamin C, reaching (129.70 mg.100 g^{-1} f. w. and 1.99 mg.100 g^{-1} f. w.). In contrast, the untreated plants (control) gave the lowest averages for these traits of (102.60 mg.100 g^{-1} f. w. and 1.04 mg.100 g^{-1} f. w.), consecutively.

CONCLUSION

The findings of the study exhibited that the foliar application of nano fertilizer and/or organic fertilizer significantly affected the growth of Swiss chard in terms of physiological and chemical traits. Spraying the nano fertilizer at a dose of 4 ml.L^{-1} individually or with a combination of organic fertilizer at a dose of 20 g.L^{-1} notably improved vegetative growth and quality characteristics. This improvement is attributed to the ability of both fertilizers to enhance nutrient absorption and stimulate physiological processes in the plant, such as photosynthetic capacity and increased nutrients and essential bioactive compounds, which positively reflects on overall growth.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the staff of the Department of Horticulture, Faculty of Agriculture, University of Kufa, Iraq for their valuable help and technical assistance in conducting this research.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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