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The effect of nitrogen and selenium on some phytochemical characteristics and allicin of garlic leaf

Masoomeh Amerian[1*], Mahmud KhoramiVafa[2], Amir Palangi[1], Gholamreza Gohari[3] and Georgia Ntatsi[4]

[1]Department of Horticultural Sciences and Engineering, Faculty of Agricultural Sciences and Engineering, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran.
[2]Plant Production and Genetics Department, Faculty of Agriculture Science and Engineering, Razi University, Kermanshah, Iran.
[3]Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh, Iran.
[4]Department of Crop Science, Laboratory of Vegetable Crops, Agricultural University of Athens, Greece.

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***Corresponding author:**

Department of Horticultural Sciences and Engineering, Faculty of Agricultural Sciences and Engineering, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran.

Email: masoomehamerian@yahoo.com

ABSTRACT

Purpose: This research has investigated the effect of different levels of nitrogen and selenium on some growth and physiological characteristics of garlic leaves. **Research method:** This research was done as a factorial in the form of randomized complete blocks in 3 replications. The first factor included four levels of nitrogen (0, 50, 100, and 150 kg ha^{-1}) and the second factor included three levels of selenium (0, 5, and 10 mgL^{-1}). **Findings:** In all four nitrogen levels, with increasing selenium concentration, plant height (69.66 cm), fresh weight (10.66 g m^{2}), and dry weight (51.33 g m^{2}) of leaf increased. The highest amount of photosynthetic pigments was observed in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate. Nitrogen and selenium increased antioxidant capacity (45.69 µmol g^{-1}FW), total phenol (295.60 mg 100 g^{-1}FW) and ascorbic acid (18.30 mg 100 g^{-1}FW). Contrary to selenium, nitrogen increased the amount of allicin in garlic leaf, and the highest amount of allicin (0.33 mgmL^{-1}) was in the treatment of 150 kg ha^{-1} of nitrogen along with 0 mgL^{-1} of sodium selenate. The maximum plant height and wet and dry weight of the leaf were observed in the treatments of selenium and nitrogen, which shows the positive effect of both elements on increasing the amount of chlorophyll synthesis and, as a result increasing the amount of photosynthesis and carbon fixation, which ultimately will have, increasing the growth rate of garlic plant. **Research limitations:** None were found to report. **Originality/Value:** As a result, the treatment of 150 kg ha^{-1} of nitrogen along with 10 mgL^{-1} of sodium selenate is recommended to increase the antioxidant compounds of garlic leaf, which is a good source of these compounds and selenium in early spring, which also plays an essential role in human health.

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the Alliaceae family and is the second most widely used *Allium* next to onion (Amarakoon & Jayasekara, 2017). Garlic possesses anti-microbial, anti-carcinogenic and anti-mutagenic properties. Garlic has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Garlic has played important dietary and medicinal roles throughout the history. Allicin ((R, S)-diallyldisulfid-S-oxide), one of the sulfur compounds from garlic, is formed by the action of the enzyme alliinase on alliin. It possesses antioxidant activity and is shown to cause a variety of actions potentially useful for human health. Allicin exhibits hypolipidemic, antiplatelet, and procirculatory effects. Moreover, it demonstrates antibacterial, anticancer, and chemopreventive activities (Efiong et al., 2020). The commonly used edible organ is the clove, while the leaves and stems are discarded during technological processing. Nevertheless, the cultivation of garlic for bunching, using the young leaves of the plant, has become increasingly popular in recent years (Adem & Tadesse, 2014). Garlic leaf consumption is an alternative to broadening the available options in the fresh vegetable market, especially in the early spring. In this case the edible young leaves in the early stage of growth and the undivided cloves are used for food, which have many medicinal properties (Jedrszczyk et al., 2018). According to research, nutritional value, protein content, sulfur compounds related to taste and phenolic compounds found in young leaves are comparable to cloves (Garg & Sekhon, 2016). Considering the many and valuable compounds that exist in garlic and its different parts, the expansion of consumption of different parts of garlic, it is essential to research the amount of chemical fertilizers used (Kumari et al., 2023).

Nitrogen (N) is an essential element for plant growth, and the availability of nitrogen is essential for the vegetative growth of agronomic and horticultural. Nitrate is one of the primary forms of nitrogen that plays an essential role in many plant metabolic processes. After water, nitrogen is one of the most important factors for plant growth. Nitrogen deficiency is associated with a decrease in the biomass of aerial organs and the leaf surface index and leads to a decrease in photosynthesis and dry matter production. At the same time, excessive nitrogen consumption in the long term will cause diseases, environmental problems, destruction of soil structure and decrease in production. Unfortunately, the use of nitrogen fertilizer in agricultural production is increasing. About 50-70% of the nitrogen consumed in the soil is not available and is not absorbed by the plant (Lei et al., 2022). Nitrogen plays a structural role in proteins and nucleic acids and is an essential component of the protein molecule. The amount of nitrogen the plant absorbs depends on the type of soil, environment, and plant species. In the stages of vegetative growth, the nitrogen requirement of garlic is essential and appropriate levels of nitrogen lead to solid growth and leaf development (Kevlani et al., 2023). Nitrogen affects the height, fresh and dry weight of the plant, stem diameter and the number of garlic leaf (Ibraheim, 2022).

Selenium (Se) is an essential microelement in human and animal nutrition, has important roles including regulation of blood lipid metabolism and potent antioxidant activity. It also protects against chronic diseases such as heart disease and cancer. The recommended intake of selenium for adults is 25 to 34 µg day^{-1} and for children 6 to 22 µg day^{-1} (Patnaik et al., 2023). Vegetables are important sources of selenium for humans, but the amount of selenium in the soil of some areas is low, which reduces the amount of selenium in vegetables. Selenium deficiency in the diet is a worldwide problem. As a result, there is an excellent demand for plants and foods enriched with selenium. Vegetables play an essential role in the human diet, so consuming selenium-enriched vegetables is an effective and safe way to compensate for selenium deficiency (Pérez et al., 2019). In plants, using appropriate levels of selenium improves the antioxidant capacity of the plant, and leads to an increase in the growth rate and yield of the product. Selenium delays senescence and increases the efficiency of nitrogen use and nitrogen metabolism (Zhang et al., 2023), but little research has on the interaction between nitrogen and selenium. In garlic cloves, selenium increased the amount of photosynthetic pigments, nutrient absorption, antioxidant enzyme activity and nitrogen metabolism (Poldma et al., 2011 & Xia et al., 2012). Therefore, the purpose of this research is to investigate the effect of different levels of nitrogen and selenium on some morphological and physiological characteristics of garlic leaf, which has for the first time.

MATERIALS AND METHODS

This study conducted in Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran, in year 2023, which has 34.3176°N, 47.0869°E and it is elevation averages about 1,350 meters above sea level. Average rainfall and temperature during the growing season were 36.8 mm and 11.8 ºC, respectively. This research carried out as a factorial experiment based of a randomized complete block design with two factors of different levels of nitrogen and selenium in three replications on landrace garlic of Kermanshah. The first factor included four levels of nitrogen (0, 50, 100 and 150 kg ha^{-1}) (Hashemi Jozani et al., 2020) and the second factor included three levels of selenium (0, 5 and 10 mg L^{-1} of sodium selenate) (Veisialiakbari et al., 2020). Nitrogen fertilizer from the source of urea (46% nitrogen) added to the soil in two stages, one at the same time as cultivation (October) and the other at the stage of 2 leafy garlic (December). The amount of fertilizers related to each treatment added to each plot in a strip form at the designated times and irrigation was done immediately. Selenium foliar spraying in the form of sodium selenate salt done manually in the evening, and simultaneously with nitrogen top-dressing fertilizer. After preparing the land, plots (experimental units) with dimensions of 3 × 2 m^2 were considered. Cloves were planted in rows at a depth of 5 cm in the first half of November. The distance between planting rows was 30 cm, and between plants on the row was 10 cm. immediately after the planting of the cloves, furrow irrigation carried out. The subsequent irrigations were according to the custom of the region and according to the weather conditions, the amount of rainfall, the soil conditions and environment temperature done. Before conducting the experimenting, samples were taken from the field soil up to a depth of 30 cm to analyze the soil and determine its physical and chemical characteristics, and the test results are presented in Table 1.

In early April, leaves were sampled to determine the studied traits after removing marginal effects from the surface of 0.4 m^2. The samples were taken to the laboratory to measure some morphological traits (plant height, fresh and dry weight of leaf and number of leaf) and physiological (photosynthetic pigments, total phenol, ascorbic acid, antioxidant activity, total protein and total carbohydrate) and allicin.

Table 1. Physical and chemical characteristics of experimental field soil at a depth of 30 cm.

Soil texture	pH	Electrical conductivity (dS m^{-1})	Nitrogen (%)	Phosphorus (mg Kg^{-1})	Potassium (mg Kg^{-1})
Silty-Loamy	7.7	0.87	0.11	13	280

Photosynthetic pigments

To measure chlorophylls and carotenoids, one g of garlic leaf tissue was ground in a mortar containing 10 ml of 80% acetone and then passed through filter paper. The filtered solution was used to measure photosynthetic pigments with a spectrophotometric device according to the following equations (1-4) (Lichtenthaler, 1987; Lichtenthaler &Buschmann, 2001).

$$\text{Chl a (mg L}^{-1}\text{)} = (12.7 \times A663) - (2.69 \times A645) \quad (1)$$

$$\text{Chl b (mg L}^{-1}\text{)} = (25.8 \times A645) - (4.68 \times A663) \quad (2)$$

$$\text{Chl total (mg L}^{-1}\text{)} = (20.21 \times A645) + (8.02 \times A633) \quad (3)$$

$$\text{Car (mg L}^{-1}\text{)} = \frac{[(100 \times A470 - 2.27 \times Chla - 81.4\ Chlb]}{227} \quad (4)$$

Total phenol

To measure the phenolic content of the leaves, first 0.5 g of leaf samples were utterly crushed in 4 ml ethanol (80%) and a homogeneous solution prepared. After 20 min of centrifugation at 9000 rpm, a clear supernatant solution removed. Afterward, 1200 µl of 7% sodium carbonate and 1.0 ml Folin (10%) mixed with homogenate (300 µl) then placed in a dark place for 20 min. After the required time (20 min), the absorption rate measured by a spectrophotometer at 725 nm (Model Kerry 100, Varian, America) (Singleton & Rossi, 1965).

Ascorbic acid

The ascorbic acid concentration of garlic leaf extract measured based on the color reduction of 6,2-dichlorophenol indophenol by ascorbic acid (Kapur et al., 2012). In this method, one gram of fresh garlic leaf tissue homogenized with 3 ml of metaphosphoric acid (1%). After half an hour, the above samples centrifuged a temperature of 4°C and at a speed of 6000 rpm for 15 min and50 µl removed from the solution and 200 µl of 6,2-dichlorophenol indophenol 50 µmol added to it. The absorbance of the samples read at a wavelength of 520 nm with a spectrophotometer (Model Kerry 100, Varian, America).

Total carbohydrate

Half a gram of garlic leaf tissue ground and crushed with liquid nitrogen in a Chinese mortar. Then, 5 ml of 95% ethanol was immediately added to it and shaken vigorously. The upper part of the resulting solution separated and its sediments washed twice with 5 ml of 70% ethanol and their upper phase added to the previously collected supernatant. The obtained solution centrifuged at 3500 rpm for 10 min. After separating the liquid and solid phase, the liquid part kept inside the refrigerator at a temperature of 4°C (Irigoyen et al., 1992). To measure total carbohydrate, 0.1 mL of alcoholic extract added to 3 mL of freshly prepared anthrone (150 mg anthrone + 100 mL 72% sulfuric acid), then placed in a boiling water bath for 10 min. At this time, a colored substance formed and glucose standards prepared from 0 to 0.1 µm ml^{-1}. Finally, the light absorption of standard solutions and samples read with a spectrophotometer (model Kerry 100, Varian, America) a wavelength of 625 nm (Paquin & Lechasser, 1979).

Total soluble protein

Leaf soluble protein measured based on Bradford's (1976) colorimetric method using albumin as a standard. The total soluble proteins absorbance recording was done at 595 nm by spectrophotometer.

Antioxidant capacity

The antioxidant capacity of garlic leaf measured by the DPPH method. In this method, the neutralizing activity of 2-2 diphenyl-1-picrylhydrazyl radicals determined by the methanolic extract by spectrophotometric method at a wavelength of 515 nm, which follows Lambert's law and the reduction of its absorption, has a linear relationship with the amount of antioxidant. The more the antioxidant substance added, the more DPPH consumed and the purple color tends to yellow. The DPPH is a purple compound that quickly becomes a radical due to the presence of phenyl groups in its structure, and is a source of free radicals. This compound changes color from purple to yellow by taking an electron from the antioxidant compound (D'Abrosca et al., 2007). DPPH radical neutralization activity was calculated based on the following formula. DPPH radical neutralization percentage formula, in this formula (5); AC: Absorbed DPPH radical without any antioxidant as a control As: Absorbed DPPH with the extract, and methanol used as a blank.

$$\text{DPPH} = [\frac{(\text{AC}-\text{AS})}{\text{AC}}] \times 100 \qquad (5)$$

Allicin

The outer skin of the garlic cloves peeled and crushed in a garlic press. The pressed garlic was then collected in a beaker, and mixed thoroughly. 700-900 mg of the pressed mash weighed and transferred to a 50 ml centrifuge tube. Using a volumetric pipette, 25 ml of cold water delivered to the sample and immediately capped and shaken vigorously for 30 seconds. Heat transfer from hands avoided by holding the tube cap while shaking. An additional 25 ml of cold water added and shaken for 30 more seconds to dilute and mix the solution. Each sample is filtered through 0.45µm glass filter into High – performance liquid chromatography (HPLC) vial and capped for injection. Allicin content extracted and determined according to (Hoppe et al., 1996).

Statistical analyses

Data analyzed with SAS (9.1) statistical software. Mean comparisons performed with Duncan's multiple range tests at the 1% significance level.

RESULTS

Growth characteristics of garlic plant

Nitrogen and selenium significantly affected the growth characteristics of the garlic (Table 2). The interaction between these two factors had a significant effect ($p \leq 0.05$) on plant height, fresh and dry leaf weight. The interaction between nitrogen and selenium had non-significant effect on leaf number (Table 2).

Table 2. Analysis of variance of nitrogen and selenium treatments on some growth characteristics of garlic plant.

		Mean square			
S.O.V	df	Plant height	Number of leaf	Leaf fresh weight	Leaf dry weight
Block	2	9.25**	0.25*	19.19**	1.25**
Nitrogen	3	277.44**	6.33**	1868.32**	69.18**
Selenium	2	65.33**	0.58**	174.52**	9.28**
Nitrogen×Selenium	6	8.66**	0.13ns	15.82**	1.51**
Error	22	1.61	0.06	1.43	0.16
C.V (%)	-	2.14	3.19	4.20	8.10

ns, * and **: Non-significant, Significant at the 5% and 1% probability levels, respectively.

Table 3. Mean comparison of the effect of different levels of nitrogen and selenium on some growth characteristics of garlic plant.

Nitrogen (Kg ha^{-1})	Selenium (mg L^{-1})	Plant fresh weigh (cm)	Leaf fresh weight (g m^2)	Leaf dry weight (g m^2)
0	0	48.00^{i}	2.00^{i}	11.00^{j}
	5	53.33^{h}	2.50^{hi}	14.66^{j}
	10	55.00^{gh}	2.80^{gh}	16.66^{i}
50	0	56.66f^{g}	3.16^{gh}	19.66^{h}
	5	58.33^{ef}	3.43^{fg}	20.66^{gh}
	10	60.00^{de}	3.96^{f}	22.00^{g}
100	0	60.00^{de}	4.80^{e}	26.00^{f}
	5	61.00^{cd}	5.60^{d}	32.33^{e}
	10	61.33^{cd}	6.43^{c}	38.33^{d}
150	0	62.66^{bc}	6.86^{c}	41.33^{c}
	5	64.00^{b}	8.66^{b}	48.33^{b}
	10	69.66^{a}	10.66^{a}	51.33^{a}

Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

In all four nitrogen levels, plant height, fresh and dry leaf weight increased with increasing selenium concentration. The maximum plant height (69.66 cm), and fresh weight (10.66 g m^2) and dry weight (51.33 g m^2) of leaf observed in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate, which had significantly of different with the control treatment (Table 3).

According to the obtained results, nitrogen and selenium had a positive effect on the number of leaf, and the highest number of leaf was observed at the highest levels of nitrogen (9.11) and selenium (8.41), which were significantly different with the control (Fig. 1a and b).

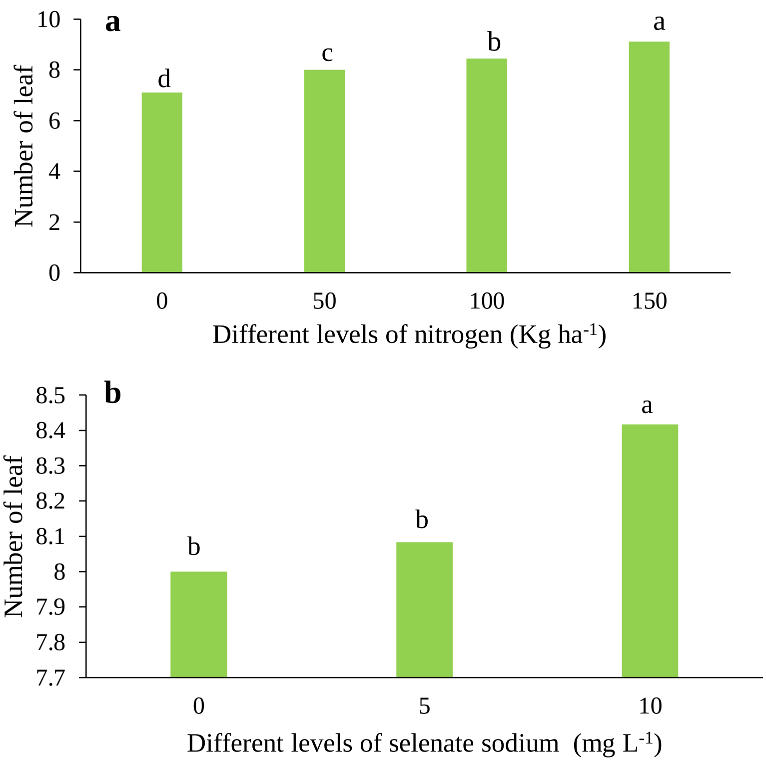

Fig. 1. The effect of different levels of nitrogen (a) and selenium (b) on number of garlic leaf.

Table 4. Analysis of variance of nitrogen and selenium treatments on photosynthetic pigments of garlic leaf.

		Mean square			
S.O.V	df	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Block	2	0.0007**	0.00012**	0.0028**	0.00034*
Nitrogen	3	0.1095**	0.02774**	0.1819**	0.02501**
Selenium	2	0.0094**	0.00107**	0.0199**	0.00203**
Nitrogen× Selenium	6	0.0004**	0.00008**	0.0005*	0.00026*
Error	22	0.00008	0.000020	0.0002	0.00008
C.V (%)	-	1.61	2.50	2.31	3.93

* and **: Significant at the 5% and 1% probability levels respectively.

Photosynthetic pigments

The results showed that nitrogen and selenium significantly affected the photosynthetic pigments in garlic leaves. Also, the interaction between two factors was significant on chlorophyll a, chlorophyll b ($p \leq 0.01$), total chlorophyll, and carotenoid ($p \leq 0.05$) (Table 4).

Nitrogen and selenium had a positive effect on the photosynthetic pigments. The highest amount of chlorophyll a (0.70 mg g^{-1} FW), chlorophyll b (0.2490 mg g^{-1} FW), and total chlorophyll (0.9113 mg g^{-1} FW) in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate observed and the lowest of the photosynthetic pigments were in the control treatment. At the concentration of 50, 100 and 150 kg ha^{-1} of nitrogen, significant of difference was not observed in the carotenoid content of garlic leaf at the levels of 5 and 10 mg L^{-1} of sodium selenate (Table 5).

Physiological traits

According to the results (Table 6), nitrogen, selenium and the interaction between nitrogen and selenium significantly affected at 1% probability level on total phenol, ascorbic acid, antioxidant capacity, total soluble protein and allicin leaf. While the interaction between two factors on total soluble protein and total carbohydrate was non-significant (Table 6).

Table 5. Mean comparison of the effect of different levels of nitrogen and selenium on photosynthetic pigments of garlic leaf.

Nitrogen ($Kgha^{-1}$)	Selenium (mgL^{-1})	Chlorophyll a (mgg^{-1} FW)	Chlorophyll b (mgg^{-1} FW)	Total chlorophyll (mgg^{-1} FW)	Carotenoid (mgg^{-1} FW)
0	0	0.4033^{k}	0.1133^{i}	0.4973^{g}	0.1366^{h}
	5	0.4373^{j}	0.1236^{h}	0.5650^{f}	0.1646^{g}
	10	0.4566^{i}	0.1273^{h}	0.5923^{f}	0.1813^{f}
50	0	0.4786^{h}	0.1360^{g}	0.6300^{e}	0.2063^{e}
	5	0.5000^{g}	0.1466^{f}	0.6566^{e}	0.2326^{d}
	10	0.5600^{f}	0.1670^{e}	0.7200^{d}	0.2380^{d}
100	0	0.5933^{e}	0.2160^{d}	0.7566^{c}	0.2600^{c}
	5	0.6310^{d}	0.2213^{cd}	0.7833^{c}	0.2600^{c}
	10	0.6376^{d}	0.2253^{c}	0.8366^{b}	0.2623^{c}
150	0	0.6550^{c}	0.2276^{b}c	0.8516^{b}	0.2690^{bc}
	5	0.6843^{b}	0.2346^{b}	0.8663^{b}	0.2813^{ab}
	10	0.7000^{a}	0.2490^{a}	0.9113^{a}	0.2930^{a}

Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

With the increase in nitrogen and selenium concentration, the amount of total phenol in garlic leaf increased. A significant difference observed between the high levels of nitrogen and selenium with the control treatment. (Table 7). Selenium and nitrogen had a positive effect on the amount of ascorbic acid and antioxidant capacity of garlic leaf. The highest amount of ascorbic acid (18.30 mg 100 g^{-1} FW) and antioxidant capacity (45.69 µmol g^{-1} FW) observed in the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of selenium. The lowest amount of ascorbic acid (4.62 mg 100 g^{-1} FW) and antioxidant capacity (12.44 µmol g^{-1} FW) was in the control treatment (Table 7).

Table 6. Analysis of variance of nitrogen and selenium treatments on some antioxidant compounds of garlic leaf.

S.O.V	df	Mean					
		Total phenol	Ascorbic acid	Antioxidant capacity	Total soluble	Total carbohydrate	Allicin
Block	2	251.12^{ns}	2.37**	9.73**	5.50**	9.65**	0.00016**
Nitrogen	3	20839.247**	156.55**	767.80**	514.93**	432.09**	0.01712**
Selenium	2	1148.46**	24.99**	120.86**	29.98**	56.14**	0.00138**
Nitrogen× Selenium	6	379.34**	1.88**	14.51**	0.66^{ns}	2.20^{ns}	0.00002**
Error	22	757.21	0.20	1.42	0.45	0.99	0.000006
C.V (%)	-	12.29	4.33	4.25	2.34	1.72	1.01

ns, * and **: Non-significant, Significant at the 5% and 1% probability levels, respectively.

Table 7. Mean comparison of the effect of different levels of nitrogen and selenium on some antioxidant compounds of garlic leaf

Nitrogen (Kg ha^{-1})	Selenium (mg L^{-1})	Total phenol (mg 100 g^{-1} FW)	Ascorbic acid (mg 100 g^{-1} FW)	Antioxidant capacity (µ mol g^{-1} FW)
0	0	174.98^{ef}	4.62^{i}	12.44^{j}
	5	147.87^{f}	6.00^{h}	18.28^{i}
	10	172.33^{ef}	7.24^{g}	21.64^{h}
50	0	196.00^{def}	7.48^{g}	24.20^{g}
	5	214.33^{cde}	8.00^{g}	25.83^{fg}
	10	220.76^{cde}	9.94^{f}	27.42^{ef}
100	0	231.14^{bcd}	11.36^{e}	28.16^{e}
	5	240.51^{bcd}	12.06^{de}	29.25^{de}
	10	250.27^{abc}	12.78^{cd}	30.59^{d}
150	0	262.00^{abc}	13.46^{c}	35.15^{c}
	5	279.00^{ab}	14.72^{b}	38.42^{b}
	10	295.60^{a}	18.30^{a}	45.69^{a}

Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

Based on the obtained results (Fig. 2), the amount of total soluble protein and total carbohydrate in garlic leaf increased with nitrogen content. So that their highest and lowest amount was observed at the level of 150 kg ha^{-1} of nitrogen and control, respectively. Selenium, like nitrogen, had a positive effect on the amount of total soluble protein and total carbohydrate in garlic leaf. With increasing selenium concentration, the amount of total soluble protein (30.40 g 100 g^{-1} DW) and of total carbohydrate (60.05 g 100 g^{-1} DW) increased in garlic leaf compared to the control treatment (Fig. 2).

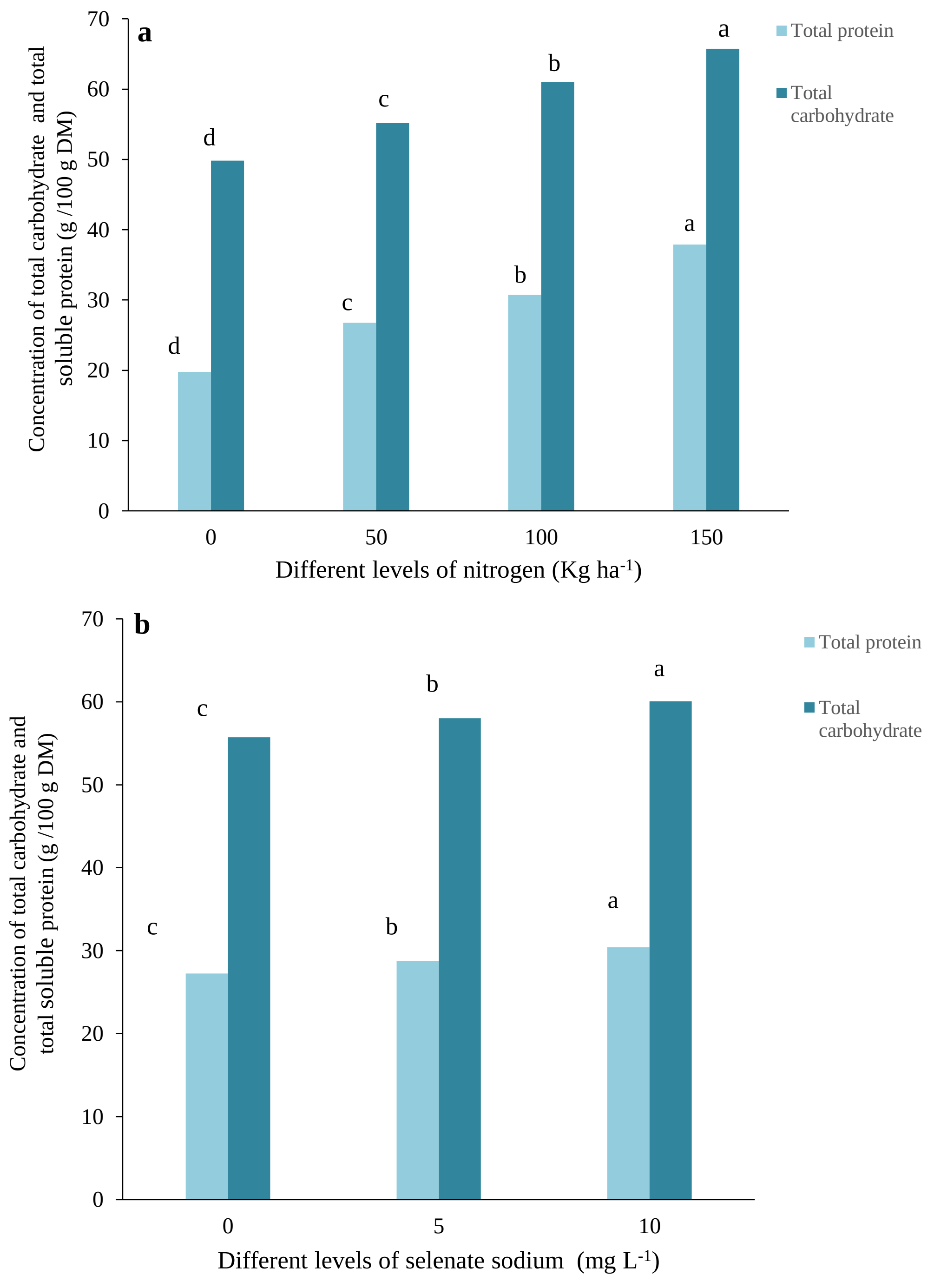

Fig. 2. The effect of different nitrogen levels (a) selenium (b) on total carbohydrate and total soluble protein.

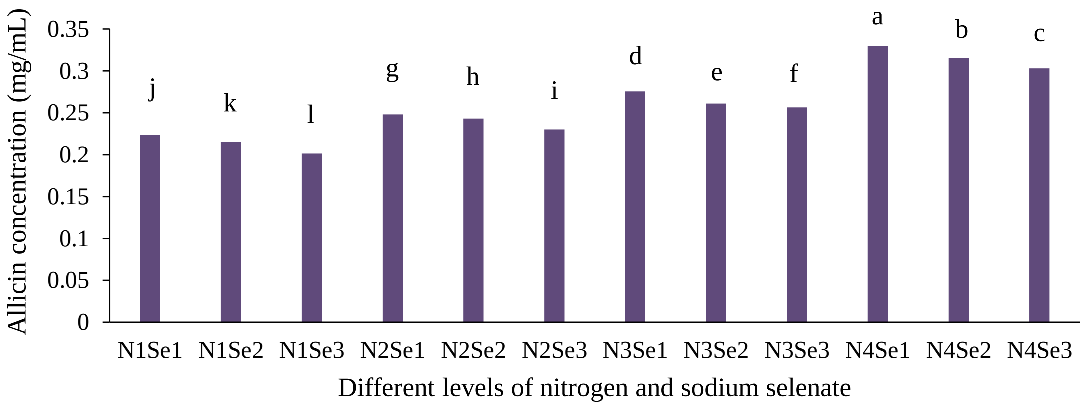

Fig. 3. The effect of different levels of nitrogen and selenium on allicin garlic leaf. N_1, N_2, N_3 and N_4: 0, 50, 100 and 150 Kg ha^{-1} nitrogen, respectively- Se_1, Se_2 and Se_3: 0, 5 and 10 mg L^{-1} sodium selenate, respectively.

Unlike selenium, nitrogen had a positive effect on the amount of allicin in garlic leaf (Fig. 3). In all four levels of nitrogen, the amount of allicin in garlic leaf decreased with increasing selenium concentration. The highest amount of allicin (0.33 mg mL^{-1}) observed in the treatment of 150 kg ha^{-1} of nitrogen along with 0 mg L^{-1}of sodium selenate, which was significantly different from the control treatment (Fig. 3).

DISCUSSION

According to the numerous reports on the chemical composition of garlic cloves, the central part of the garlic plant that used, the information on the nutritional and medicinal value in its other organs, especially the leaf, is only available in a few reports. The results have shown that garlic leaves can be an excellent substitute for garlic cloves, especially in early spring when there is a shortage of good-quality garlic cloves and fresh garlic is not yet ready to harvest. Harvesting of young leaves can be started from February 10 and continue until March 11. Also, during the investigations, the amount of phenolic and antioxidant compounds of garlic leaf is comparable to cloves and even higher (Jedrszczyk et al., 2018).

Nitrogen is the most important element required by the plant compared to other commonly used elements and plays a vital role in increasing plant yield (Mahboob et al., 2023). A decrease in soil nitrogen is associated with a decrease in the production of vegetative organs and leaf surface, which ultimately causes a decrease in photosynthesis and the production of dry matter in the plant (Alizadeh Sehzabi et al., 2007; Berenguer et al., 2009). According to the results, the amount of nitrogen used up to the level of 150 kg ha^{-1} during two stages has improved the growth characteristics of the garlic plant. The increase in the height of the garlic plant can be due to the positive effect of nitrogen on stimulating the growth of the plant, the ability of the plant to have more access to nutrients and the improvement of the water holding capacity of the soil. At the same time, applying nitrogen can increase the capacity of photosynthesis and vegetative growth of plant organs (Leesawatwong & Rerkasem, 2003). Nitrogen increases the synthesis of auxin and cytokinin hormones, which is associated with an increase in cell division and cell length, leading to an increase in leaf fresh weight, internode length, and plant height (Wajid et al., 2007). It can be said that the optimal management of fertilizer levels has played an essential role in increasing the growth characteristics of garlic, which is in accordance with the results of the study conducted on garlic by Singh and Sharma (2023). Selenium increased the growth characteristics of garlic compared to the control treatment (Table 2), which is by the results obtained in onion (*Allium cepa* L.) (Hamed-Far et al., 2022). This, increase can be attributed to the positive effect of selenium on chlorophyll synthesis, carbon fixation, starch synthesis, and stimulation of cell division in meristem cells (Bakhtiari et al., 2023). Selenium in low levels stimulates growth and increases plant growth and photosynthetic activity. However, at high levels, it causes leaf chlorosis, and necrosis, reduced growth and even death before the plant matures. There is also a direct relationship between selenium concentration and glutathione peroxidase activity, which leads to a delay in senescence and increased plant growth (Moulick et al., 2023). Therefore, the concentration of 20 mg L^{-1} of sodium selenate recommended to increase the growth characteristics of the garlic plant. Of course, it is worth noting that garlic is one of the plants that accumulate selenium and is part of the selenophorous plants. As a result, it can convert mineral selenium into an organic form, especially on its sulfur compounds, so the problem of poisoning does not occur (Hamed-Far et al., 2023).

There are few reports on the interaction between nitrogen and selenium. According to the obtained results, the interaction between nitrogen and selenium improved the growth characteristics of the garlic plant, consistent with the results of Amerian et al. (2018) and Veisi-Ali Akbari et al. (2020) in onion. According to the research conducted in lettuce (*Lactuca sativa* L.), selenium increased nitrogen metabolism, which was associated with increased nitrogen efficiency and yield. Selenium at low levels of nitrogen can increase the growth characteristics of lettuce by increasing the amount of chlorophyll and nitrogen metabolism (Bian et al., 2020), which is according to the results obtained from the research. In the present research, with increasing concentrations of nitrogen and selenium, the amount of photosynthetic pigments in garlic leaf increased (Table 5). Chlorophyll is a pigment whose primary responsibility is to receive light energy for photosynthesis. The chlorophyll molecule consists of two parts (a porphyrin head and a long hydrocarbon with a phytol sequence). Porphyrin consists of four nitrogen-containing pyrrole rings arranged in a ring. The complement of the chlorophyll molecule is a magnesium ion that forms a chelate with four nitrogen atoms in the center of the ring (Ahmadi et al., 2004). Chlorophyll as a place to absorb light and synthesize substances necessary for the growth, and development of plants depends on nitrogen. Nitrogen deficiency causes yellowing of old leaves and finally plant growth stops (Gao et al., 2023). In fact, nitrogen plays an essential role in the structure of chlorophyll, which leads to an increase in photosynthetic substances (Rahmani et al., 2008), which is associated with an increase in plant growth (Table 3). Selenium also increases the amount of chlorophyll in leaves by protecting chloroplast enzymes, increasing the biosynthesis of photosynthetic pigments and the efficiency of photosystem II (Safaryazdi et al., 2012). Selenium is involved in the path of chlorophyll biosynthesis and the interaction of selenium with enzymes containing sulfhydryl-SH group, 5-aminolevuinic acid dehydratase and deaminase porfobilinogen is effective in the amount of leaf chlorophyll (Nowaka et al., 2004). By increasing the amount of carotenoid and reducing oxidative stress, selenium increases the amount of sugars in plants, which provides the energy needed by the plant and causes the plant's survival (Khan et al., 2023). Carotenoids are antioxidant pigments that limit chlorophyll peroxidation and chloroplast degradation. Selenium in the appropriate concentration increases the amount of carotenoid, which leads to an increase in chlorophyll and the efficiency of photosynthesis (Tufail et al., 2023). Selenium can increase carotenoids by improving plant metabolism under normal conditions (Feng & Wei, 2012). An increase in the amount of chlorophyll pigments at a concentration of 3 mg L^{-1} has been reported in okra (*Abelmoschus esculentus* L.) (Ali et al., 2023) and pepper (*Capsicum annuum* L.) (Hassan et al., 2023). Nitrogen and selenium had a positive effect on the photosynthetic pigments of garlic leaf, which could be due to the increase in the synthesis of enzymes related to chlorophyll synthesis (Li et al., 2023).

Nitrogen is one of the most essential elements in the synthesis of proteins, so an increase in nitrogen to a certain extent is associated with an increase in protein, which leads to an increase in plants height and the number of leaves (Chong et al., 2023). Selenium, with its antioxidant properties, increases enzymes and antioxidant compounds and supports all types of proteins by increasing the clearance of free radicals (Cunha et al., 2023). In potato plant (*Solanum tuberosum* L.), different levels of nitrogen and selenium led to an increase in carbohydrates and protein (Li et al., 2023).

Polyphenols are antioxidant compounds and can absorb free radicals. These compounds also play an essential role in the human body, such as protecting the body against various cancers (Torabi-Toran-Pashtoshti et al., 2022). Considering the benefits of these compounds and their presence in plants, it seems necessary to investigate natural sources to find antioxidant and phenolic compounds. In Iran, the consumption of vegetables is high, which, besides providing fiber, vitamins and minerals, can be a good source of phenolic and antioxidant compounds. Garlic has high antioxidant activity due to having phenolic compounds in its structure. Garlic is a rich source of phytochemicals, including antioxidant compounds such as phenols, flavonoids, and allicin, which play an essential role in human health (Pascual-Teresa et al., 2010). Environmental conditions and fertilizer management play an essential role in the compounds found in different organs of garlic, including antioxidants, phenol and flavonoids. The presence of these potent antioxidants in garlic increases human health and resistance to oxidative stress (Rahman et al. 2012). Nitrogen fertilizer management (levels of 150 and 250 kg ha^{-1}) in onion has led to an increase in compounds (total phenol, ascorbic acid, etc.) and antioxidant enzymes (Barrales-Heredia et al., 2023). With the application of selenium, the increase in total phenol related to the increase in the activity of the enzyme phenylalanine ammonia-lyase (Elguera et al., 2013; Abdalla & Mühling, 2023). The increase in the antioxidant activity of the plant under the influence of selenium may be due to the increase in phenolic compounds, the effect of selenium on glutathione redox metabolism. The enzymes involved in this metabolism and, the direct antioxidant the effect of selenium and its organic metabolites (Rad et al., 2019).

Plant nutrition, including medicinal plants, is one in the most essential influencing factors in metabolism, and the amount of secondary metabolites. Allicin is the main component of the biologically active compounds of garlic, which is the result of the degradation of alliine by alliinase. The leaf is the primary site of synthesis of cysteine sulfoxide (alliine), from where it transferred to other parts of the plant such as the clove (Nguyen et al., 2022). According to the obtained results, the amount of allicin in garlic leaf increased with the increase of nitrogen concentration (Fig. 3).

CONCLUSION

The results of this research indicates that the management of nitrogen and selenium fertilizers can play an important role in the growth characteristics, photosynthetic pigments and antioxidant compounds of garlic leaf. Since selenium affects nitrogen metabolism, as a result, simultaneous application of sodium selenate with nitrogen had a vital role in improving some growth and physiological characteristics of garlic plants. The maximum plant height and wet and dry weight of the leaf observed in the treatments of selenium and nitrogen, which shows the positive effect of both elements on increasing the amount of chlorophyll synthesis and, as a result, increasing the amount of photosynthesis and carbon fixation, which ultimately will have increases the growth rate of garlic plant. As a result, the treatment of 150 kg ha^{-1} of nitrogen along with 10 mg L^{-1} of sodium selenate is recommended to increase the antioxidant compounds of garlic leaf, which is a good source of these compounds and selenium in early spring, which also plays an essential role in human health.

Conflict of interest

The authors have no conflict of interest to report.

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