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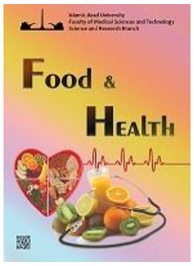

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Investigation of different selenium sources on phytochemical characteristics of *Echium amoenum* fisch. & C.A. Mey. as a medicinal herb of Iran

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A B S T R A C T

The aim of this research was to determine the effect of selenium forms (sodium selenate and sodium selenite) at (2, 4, 8 and 16 mg/l) level on the phytochemical attributes of *Echium amoenum* (*Vipers bugloss)* as factorial randomized complete block design. Selenium sources were added in four steps: 2 true leaves stage, 10 leaves, 2 weeks and 1 week before flowering. The traits were evaluated in different stages of flowering (beginning, full flowering, and end of flowering). Results showed that selenium sources significantly affected the qualitative characteristics of the *E. amoenum*. The highest photosynthetic pigments were obtained by 4 mg/l sodium selenite at beginning of flowering. When the plants were sprayed with 4 mg/l sodium selenate and harvested at beginning of flowering, higher total alkaloid contents in leaves and petals were observed compared to the other treatments. When the plants were sprayed with 8 mg/l sodium selenate and harvested at end of flowering, higher total phenols and flavonoids contents, soluble sugars content was observed compared to the other treatments. Moreover, foliar application of selenium sources significantly increased the content of this element in the petals. Sodium selenate was more effective than sodium selenite in increasing the content of this element. Generally, the results showed selenium sources significantly improved phytochemical properties of *E. amoenum*.

1. Introduction

Selenium (Se) is essential element for human and animal health due to the activation of antioxidant defense systems. In addition, Se shows a narrow range between deficiency (<40 µg/day) and toxicity (>400 µg/day) in human nutrition (1). Its deficiency can lead to lethal diseases for humans such as cancer, thyroid, liver and cardiovascular diseases (2). Selenium chemistry is complex and chemical properties there of resembles to the sulfur and is found in multiple oxidative forms. Selenate (SeO_3^{2}) is stable under the alkaline and oxidative conditions and is capable to be easily dissolved and absorbed by the plants. Selenite (SeO_3^{2-}) is soluble and toxic but is easily reduced to the elemental selenium (Se^0). Elemental selenium as elemental sulfur is found in various chemical forms. Normal mineral form of the selenium is selenite and selenate while selenomethionine (SeMet) is of organic forms of this element. Nevertheless, volatile forms of the said element are available. Selenium at low concentration is an essential element, at least 25 human selenopolypepties and enzymes-containing selenocysteine for human and animal body. In the environment, it exists as ionic selenite (Na_2SeO_3) and selenate, solid-state Se, selenocysteine and selenomethionine (3). The plants play an exclusive role in recirculation and releasing the selenium from the soil to the food chain. Selenium concentration available in agricultural products is dependent on the selenium content available in the soil and its biological accessibility. Selenium accessibility in soil is limited and the content there of is almost low. The plant's roots absorb selenium in the form of selenate and seleniteions from the soil. Absorption and accumulation the selenium by the plants depends on the chemical forms, concentration and factors such as pH, salinity, calcium carbonate content and plants capability. Selenium is chiefly

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absorbed from the soil by the plants as selenate and selenite and emphasis is mostly on the selenate, because selenite accessibility is lower in soils (4). In the previous studies the beneficial effects of selenium on seed germination (5), enhancing crop yield (6) and increasing the antioxidative capacity (7) of the plants are reported. A study by (8) indicated that the aerial parts of mung bean compared to the roots thereof were more influenced by the sodium selenate application. *Echium amoenum* (*Vipers bugloss*) is a medical plant from Boraginaceae family with thick roots and leaves of dovetail and rosette. It is a biennial or perennial herb indigenous to the narrow zone of northern part of Iran and Caucasus, where it grows at an altitude ranging from 60-2200 m. *Echium amoenum* includes secondary metabolites such as anthocyanins, tannins, mucilage, flavonoids and alkaloids (9). The presence of rosmarinic acid in medicinal plants such as *E. amoenum* has beneficial and health promoting effects. This medicinal plant has long been used as a tonic, tranquillizer, diaphoretic, a remedy for cough, sore throat and pneumonia in traditional medicine of Iran (10). A search through the literature stated that the petals of *E.amoenum* have anthocyanidine (13%), flavonoid aglycons (0.15%) and trace amount of alkaloids (5, 6), a clear lemon-yellow volatile oil (0.05%) with δ-cadinene (24.25%) as the major component (7). *E. amoenum* has anxiolytic effect in mice (8, 9) and has the capacity to increase the cellular immune response (10). Because the decoct of its dry plants are used in traditional medicine, the aim of this study was to investigate the effect of different stages of flowering and sodium selenate and sodium selenite applications on some phytochemical properties of the *Echium amoenum* Fisch. & Mey.

2. Materials and methods

The purpose of this research was to determine the effect of different selenium concentrations (2, 4, 8 and 16 mg/l) and forms (sodium selenate and sodium selenite) on phytochemical quality of *Echium amoenum* Fisch. & Mey in the Behshahr, Mazandaran, Iran. The experiment was done as Factorial Randomized Complete Block Design (RCBD) with 9 treatments and 3 replications. Selenium sources were added in four steps in the form of foliar application: 2 true leaves stage, 10 leaves, and 2 weeks before flowering and one week before flowering. After sufficient growth of the plants, the traits were evaluated in different stages of flowering (beginning, full flowering and end of flowering). In this study, several growth parameters of plants were evaluated, including chlorophyll and carotenoid content, total phenols and flavonoids contents, soluble sugars content, petal anthocyanin, total alkaloid contents in leaves and petals and selenium content in petals. 100 milligrams of flower tissue in fractions were placed in a vial containing 7 mL DMSO, and chlorophyll was extracted into the fluid without grinding at 60°C by incubating for 25 minutes. The extract was transferred to a new tube and was increased to a total volume of 10 mL with DMSO. Measurements of the chlorophyll and carotenoid contents were performed using the spectrophotometric method at a wavelength of 645, 663 and 470 nm against a blank DMSO (11). In order to extract the samples to measure biochemical factors, methanol 70% was used at a ratio of 5:1 (volume-weight). Measuring the total phenols was performed according to the Folin's reagent method and the use of gallic acid as standard by using a spectrophotometer at the wavelength of 765 nm (12). The method proposed by (13) was used for measuring the total flavonoid by means of a spectrophotometer at a wavelength of 510 nm through a standard curve of quercetin. The soluble sugars content was read at a wavelength of 490 nm using a spectrophotometer (Jenway 7305 UV–vis spectrophotometer, England) according to the phenol-sulfuric acid method, while glucose (Sigma-Aldrich, USA) was used as standard (14). pH differential method was used for measuring the total anthocyanin contents in petal. Initially, two buffer samples were prepared using of potassium chloride (pH=1, 0.025M) and sodium acetate (pH=4.5, 0.4M) solutions. An amount of 1 ml from extract was separately mixed with 4 ml from buffers and then the absorption in 510 and 700 nm was measured using of spectrophotometer (15). Total alkaloid contents in leaves and petals were read at a wavelength of 258 nm using a spectrophotometer, while Atropine (Fluka Art. 11320, Merck, Germany) was used as standard (16). For measurement of selenium content in petals, initially 1g from dried petals samples were digested in 5 ml mixture of nitric acid and concentrated per chloric acid (with volume rate of 4:1) at temperature 130˚C for an hour. After cooling, 5 ml of concentrated hydrochloric acid were added and heated for 20 minutes at a temperature of 115˚C. Finally, atomic spectrometry (ICP-OES spectrometer Integra XL2, GBC Australia) was used to determine of selenium contents in petals (17). Data were analyzed by using Duncan's multiple range test ($p \leq 0.05$) by SAS, version 9.4.

3. Results and discussion

In this research, selenium sources and flowering stages had significant effect on some morphological and biochemical properties of *Echium amoenum*. The effects of selenium and flower stage linked with physiological and biochemical plant properties i.e., the changes in photosynthetic pigments, lipid peroxidation, total phenols, total flavonoids and total soluble sugars (3). Significant differences were found among interaction between flowering stage and Selenium in Chlorophyll b, soluable suger, flovonoid, phenol, flovonoid, Alkaloid's leaf, Alkaloid's petals and Selenium's petal parameter; whereas there are no significant different in Chlorophyll a, total Chlorophyll, carotenoid and anthocyanine ($p \leq 0.05$) (Table 1). In this research, foliar application 4 mg/l sodium selenite significantly increased the amount of chlorophyll a. This treatment increased the amount of this pigment to 1.82 mg/g fresh weight. In addition to that, the lowest amount of chlorophyll a, was for the foliar application of the plants with 16 mg/l sodium selenite treatment (Fig. 1-a). The amount of chlorophyll b was affected by both application 4 mg/l sodium selenite at beginning stage as maximum content was observed 0.338 mg/g fresh weight (Fig. 1-b). Moreover,

Table1. The amount of elements in flowering stage and selenium forms.

SOV	DF	Chlorophyll a	Chlorophyll b	Total Chlorophyll	carotenoid	Anthocyanine	Soluable suger	flovonoid	Alkaloid' s leaf	Alkaloid' s petal	Selenium' s petal	Ion leakage
Rep.	2	0.001	0.001	0.011	0.005	0.001	0.27	1.11	3.36	0.06	0.0001	0.28 ns
Flowering stage	2	0.0001ns	0.008**	0.010*	0.161**	0.0003*	4.67**	310.05**	754.9**	5.4**	0.005**	11.20**
Selenium (Se) forms	7	0.311**	0.010**	0.428**	2.42**	0.004**	32.06**	175.05**	26.57**	0.23***	0.003**	0.20*
Selenium (Se) forms × flowering stage	14	0.001ns	0.00022**	0.0013ns	0.009ns	0.001ns	0.33*	2.64**	31.11**	0.25**	0.00019**	0.07*
error	-	0.002	0.00057	0.0027	0.0215	0.0001	0. 17	0.67	3.48	0.05	0.00001	1.98
CV	-	3.17	2.99	3.1	1.96	3.59	11.24	9.53	4.91	7.88	6.37	13

the form and concentration of selenium had significant effect on the amount of total chlorophyll in *Echium amoenum*. The highest amount of total chlorophyll was observed after 4 mg/l sodium selenite treatment and then 8 mg/l sodium selenite (Fig. 1-c). At the present study, the anthocyanin content (TAC) was affected by the flowering stages. Moreover, the amount of total anthocyanin in flowers was changed after the application of forms and different selenium concentrations. After the application of 2 and 8 mg/l sodium selenite, the highest amount of total anthocyanin was achieved by 0.327 and 0.329 mg/g fresh weight, respectively. In addition, the lowest amount of total anthocyanin in the plant (0.262 mg/l fresh weight) was related to 16 mg/l sodium selenite treatment (Fig. 1-d). In the present research, the content of carotenoid pigmentation in flowers was impacted by the flowering stages. Average total carotenoid content at the beginning, full flowering and end of flowering was 6.29, 6.13 and 6.28 mg/g fresh weight, respectively.

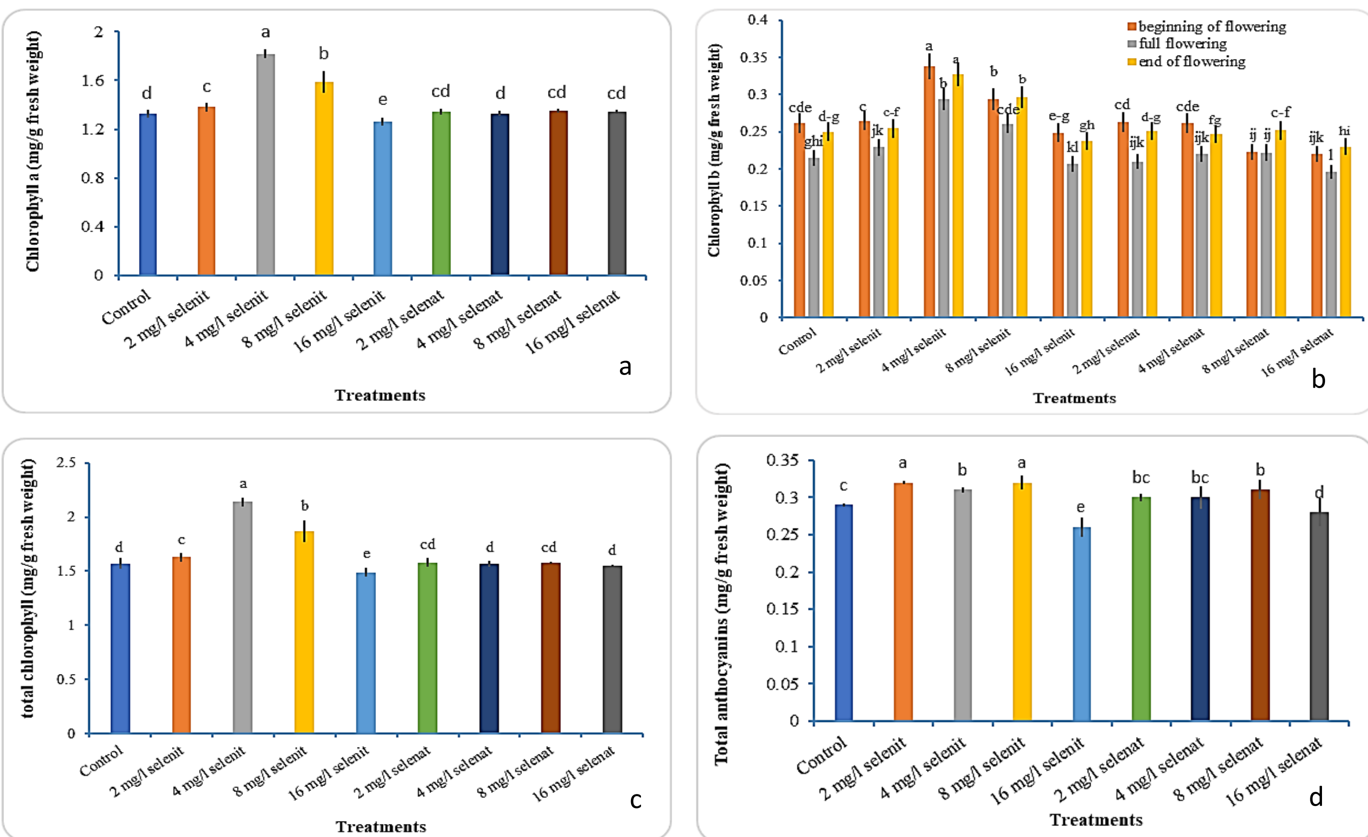

Fig. 1(a-d). The effect of selenium sources and flowering stages on chlorophyll a, chlorophyll b, total chlorophyll and anthocyanin of leaves of *Echium amoenum.*

There was no significant difference between the harvested flowers at the beginning and end of flowering in terms of the total carotenoid content (Fig. 2-a). Furthermore, the form and concentration of selenium had significant effect on carotenoid content of *Echium amoenum*. The highest amount of total carotenoid with an average of 7.35 mg/g fresh weight was observed after 4 mg/l sodium selenite treatment which showed a significant difference with other experimental treatments (Fig. 2-b). In a study conducted by (18) different selenium concentrations improved the morphological characteristics and increased photosynthetic pigments in spinach plant. The effects of the selenium foliar application on improving the growing performance can be the result of increasing the starch accumulation in chloroplasts (6) and protecting the cell content such as pigments i.e., anthocyanin (19). Several studies have confirmed an increase in chlorophyll content in plants after selenium application (20). Because the iron element is involved in increasing chlorophyll biosynthesis, selenium is likely to increase the content of this pigment in the plant by increasing iron absorption (21). In a research conducted on rice, the application of selenium low amount resulted in increasing the photosynthesis rate and chlorophyll and carotenoid contents which is in compliance with the results of this research (22). In addition, the use of selenium had significantly increased transpiration rate, photosynthesis rate, and stomatal conductance in grain sorghum (23).

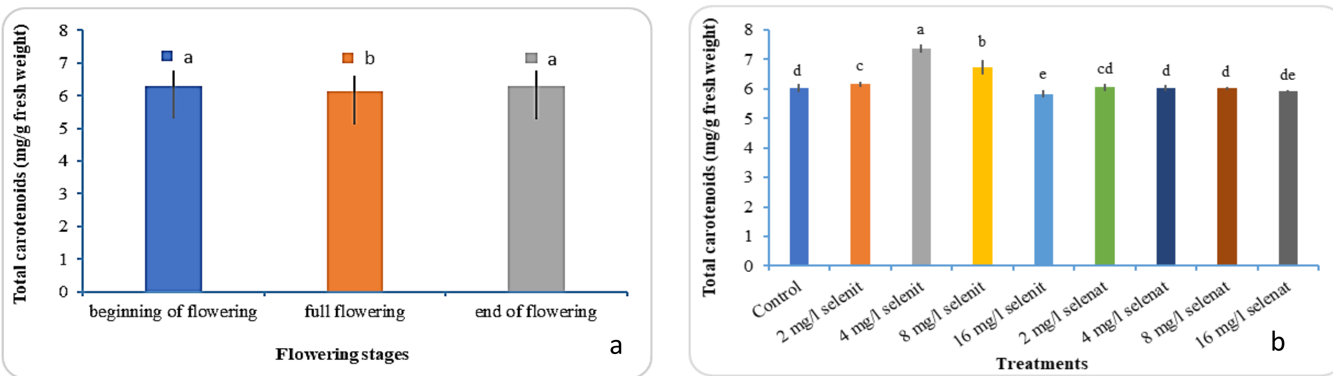

Fig. 2 (a-b). The effect of selenium sources and flowering stages on total phenols and flavonoids of *Echium amoenum*.

The amount of total phenol significantly increased from beginning to the end of flowering. Foliar application of the plants with 8 mg/l of sodium selenate and harvesting at the end of flowering significantly increased the content of the total phenol (by 32.38 mg gallic acid/g dry weight) compared to other treatments (Fig. 3-a). Total flavonoid contents had the lowest amount at the beginning of flowering and highest amount at the end of flowering. The results showed that the plants which sprayed with 8 mg/l of sodium selenate and harvested at the end of flowering stage, had the highest amount of the total flavonoid (by 21.01 mg quercetin/g dry weight) (Fig. 3-b).

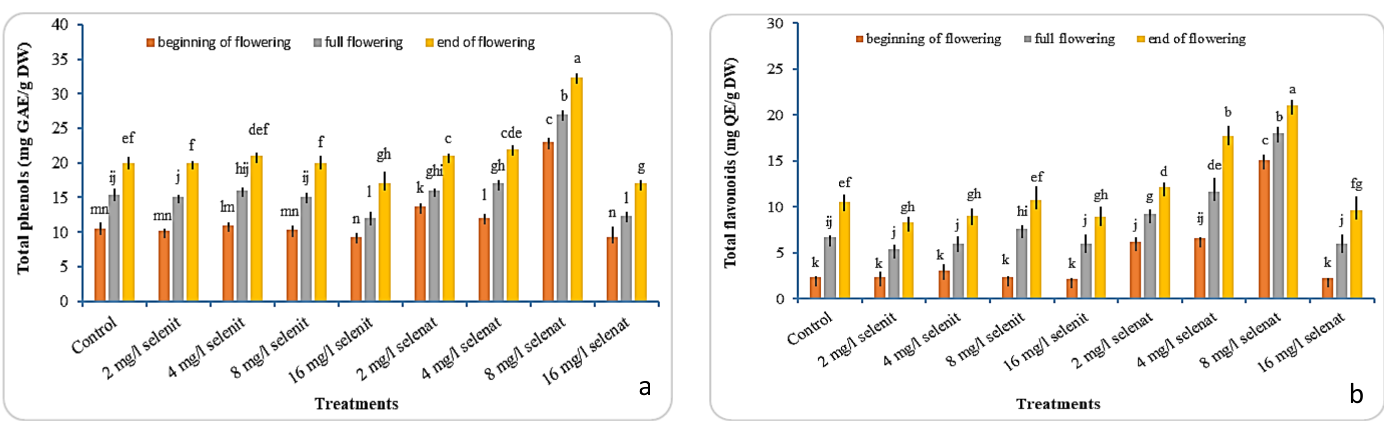

Fig. 3(a-b). The effect of selenium sources and flowering stages on total phenols and flavonoids of *Echium amoenum*.

The results of this study showed that sodium selenate and sodium selenite increases the total phenols and flavonoids contents in *Echium amoenum*. Among the experimental treatments, the application of 8 mg/l sodium selenate significantly increased the total phenols and flavonoids contents. Probably one of the reasons for the increase in the total phenols and flavonoids contents after selenium foliar application is due to the role of this element in increasing the phenylalanine ammonia lyase (PAL) activity as a key enzyme in the biosynthesis of phenolic and flavonoid compounds in plants (24). In a research, selenium foliar application significantly resulted in increasing the acid ascorbic amount of green tea leaves. Redox properties of phenolic compounds play an important role in the absorption and neutralization of free radicals (25). Phenolic compounds are also responsible for the antibacterial properties of the extracts and essential oils (26). However, the mechanism of selenium effect on increasing the amount of phenolic and flavonoid compounds in the plants remains unknown. Generally, from the beginning to the end of flowering stage, the amount of soluble sugars was increased significantly. The highest amount of soluble sugars was achieved after 8 mg/l sodium selenate application. There was no significant difference between the flowers harvested at the different stages of flowering after this treatment (Fig. 4). It is stated that the activity of starch hydrolyzing enzymes-amylases and sucrose hydrolyzing enzyme-invertase was stimulated significantly with selenium. This was associated with elevation of activities of sucrose synthesizing enzymes-

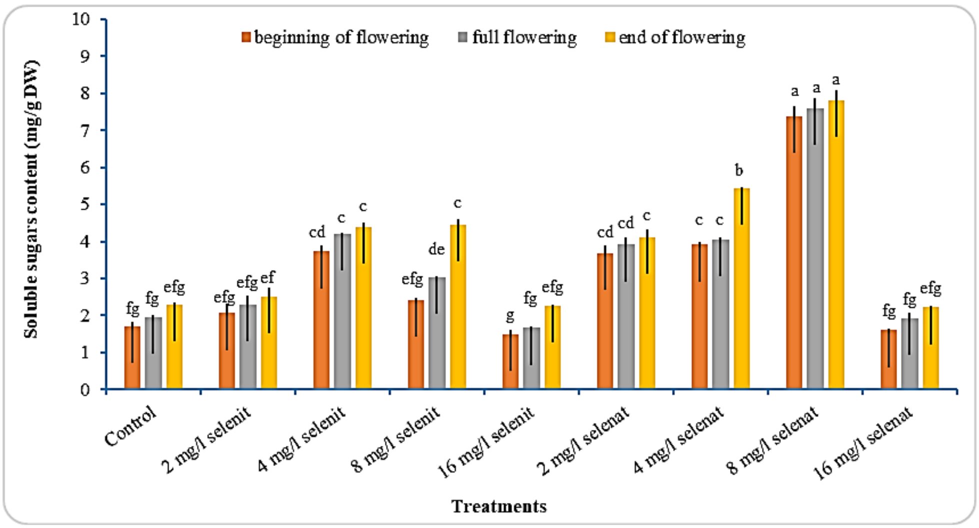

Fig. 4. The effect of selenium sources and flowering stages on soluble sugar of *Echium amoenum*.

sucrose synthase and sucrose phosphate synthase. It was concluded that increase in growth of shoots and roots by application of Se was possibly the result of up-regulation of enzymes of carbohydrate metabolism thus providing energy substrates for enhanced growth (27). The increased photosynthetic products of the plants treated by the selenium can be the consequence of increasing the number of photoreceptors and consequently more synthesis of carbohydrates for growing the organ acts as a source for selenium and carbohydrates (28).

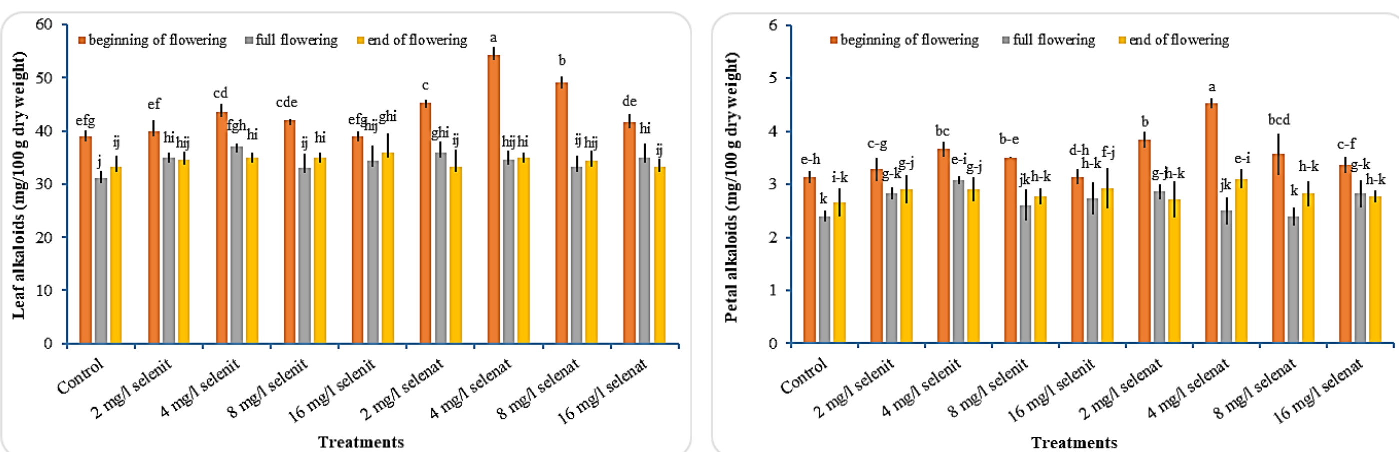

Fig. 5 (a-b). The effect of selenium sources and flowering stages on leaf and petal alkaloids of *Echium amoenum*.

Selenium can play a role on increasing the quantum efficiency of photosystem II in the plants (29). Improving the yields of the potato plants treated by the selenium has indicated that the selenium has probably been a factor to allocate more photosynthetic material to the tubers and can prolong aging by its antioxidative effects (30). In the same research, Xue et al. (31) attributed the lettuce's growing and photosynthesis indexes to the selenium role in producing the carbohydrate combinations. In a research conducted on cucumber the selenium application resulted in increasing the photosynthesis pigments which is in compliance with the results of this research (32). The results indicated that the total alkaloid contents in leaves and petals were significantly affected by the flowering stages and selenium forms. The amount of total alkaloid in leaves and petals at the beginning of the flowering was more than other stages. 4 mg/l sodium selenate foliar application significantly resulted in increasing the total alkaloid contents in leaves and petals. After the application this treatment, the amount of the total alkaloid in leaf and petal reached to 54.33 and 4.53 mg/100g dry weight (Fig. 5-a, and 5-b). Generally, by increasing the growing period of the plants, the content of selenium in the petals was reduced. The results showed that by increasing the concentration of the selenium sources used, the content of this element in petals was also increased significantly. Among the used sources, the effect of sodium selenate was more than sodium selenite. The highest content of selenium in the petals was achieved after the application of 16 mg/l sodium selenate treatment (Fig. 6).

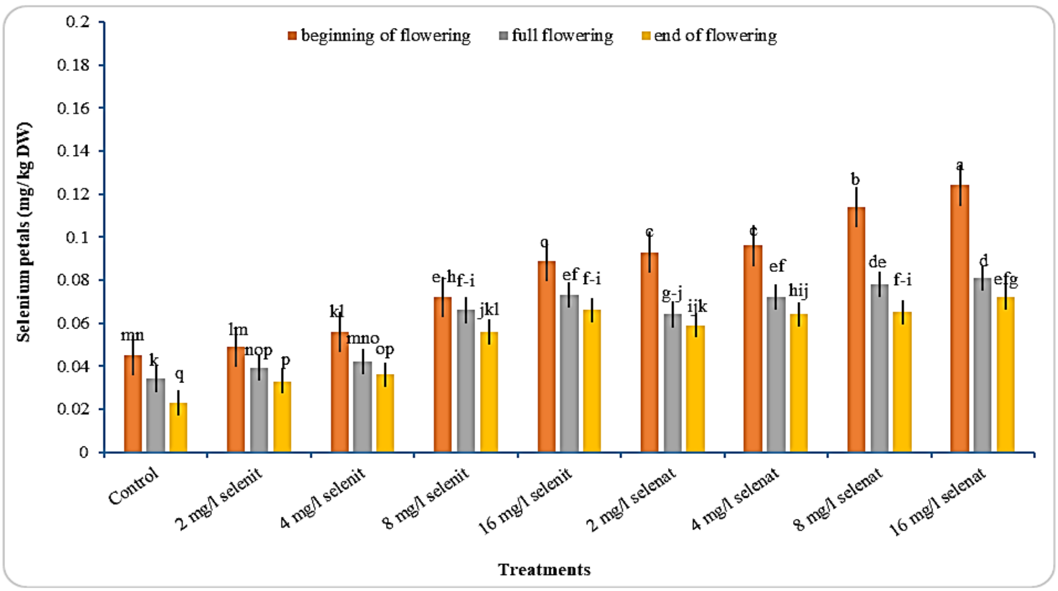

Fig. 6. The effect of selenium sources and flowering stages on selenium content of *Echium amoenum*.

The results of this research showed the application of selenium sources results in significant increasing of selenium content in petals. Meanwhile, the role of sodium selenate was more considerable. The findings of (33) on the onion showed the amount of bulb total selenium was increased by increasing the sodium selenite and sodium selenate concentrations in the nutrient solution. However, this increase was higher in plants treated with sodium selenate, which supports the results of this study. In previous studies, the use of selenium to a certain concentration increased yield and chlorophyll index and decreased electrolyte leakage in Brussels sprouts leaves. Moreover, by increasing the selenium levels, selenium concentration in the tissues was increased (34).

4. Conclusion

The results indicate that the stages of flower harvesting and selenium forms and concentrations play an important role in determining the final quality of the product. In general, the use of selenium sources to a certain concentration increased the growth characteristics and the use of higher amounts has negative effects on plant growth and development. Foliar application of plants with 4 mg/l sodium selenate significantly increased plant growth characteristics as well as total alkaloid content. The maximum amount of photosynthetic pigments in the plant was obtained in beginning of flowering stage and after foliar application of 4 mg/l of sodium selenite. When the plants were sprayed with 8 mg/l sodium selenate and harvested at end of flowering, higher total phenols and flavonoids contents, antioxidant activity and soluble sugars content was observed. Moreover, foliar application of selenium sources significantly increased the content of this element in the petals. Sodium selenate was more effective than sodium selenite in increasing the content of this element.

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