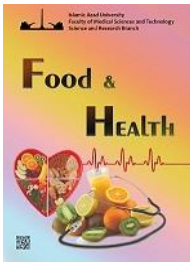

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Evaluation the impact of selenium-enrichment and some organic matter on morphophysiological and essential oil in *Anethum graveolens* L.

Parviz samavatipour [1], Vahid Abdossi [1*], Reza Salehi [2], Saeed Samavat [3], Alireza Ladan Moghadam [4]

[1] Department of Horticulture Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Horticultural Sciences, Campus of Agriculture and Natural Resources, University of Tehran, 31587-77871, Karaj, Iran
[3] Department of Soil Fertility and Plant Nutrition, Soil and Water Research Institute (SWRI), Agricultural Research, Education and Extension Organization, Karaj, Iran
[4] Department of Horticultural Science, Garmsar Branch, Islamic Azad University, Garmsar, Iran

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ABSTRACT

Recently, the role of selenium (Se) as an antioxidant has generated wide interest in humans and animal-based on its presence in antioxidant defense systems. Unfortunately, people in many countries fail to get enough selenium. Dill (*Anethum graveolens* L.) is one of the medicinal plants belonging to the family *Apiaceae*. Any kind of herbal drug is influenced by many factors. Genetic, pre-harvest (season of harvest, soil, type of water), and post-harvest factors (storage, temperature, moisture) can affect total phenolic, flavonoid, and antioxidant capacity as well as other elements and compounds. The aim of this study was to investigate the effect of selenium trace in combination with and acids on the enrichment of dill. The experiment was conducted in a completely randomized design with three levels of acid (0, 50, and 150 mmol/L) and selenium application at 5 levels (0, 6, 8, 12 and 16 mg/L). The results of this experiment showed that the effect of selenium at different level of acids was significant. So that shoot fresh and dry weight, chlorophyll, essential oils, selenium content, and antioxidant enzymes were affected by increasing treatment level. The results indicated that (50 mg/L acid+12 mg/L sodium selenate) increased and improved some morphophysiological traits and essential oils including α-Pinene, β-Myrecene, α-Phellandene, and Carvone.

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1. Introduction

Dill (*Anethum graveolens* L.) is one of the medicinal plants belonging to the family of Apiaceae, which is eaten as a fresh and dried vegetable. This is the only species of this genus that is planted in Iran. Its three main constituents of herbal essential oils are Carvone, Flandron, and Limonene. Dill was analyzed in Iran obtained higher quality in essential oils while the lowest was with other countries. *Anethum graveolens* is used as an ingredient in gripe water, given to relieve colic pain in babies and flatulence in young children. The seed is aromatic, carminative, diuretic, galactagogue, stimulant, and stomachic. The essential oil in the seed relieves intestinal spasms and griping, helping to settle colic (1). Selenium (Se) is an element found in antioxidant defense systems and hormone balance that has recently been identified as a key ingredient for human and animal health. Selenium deficiency has directly affected human health and cause more than 40 types of deficiency-related diseases such as cancer, liver disease, cardiovascular disease and cataracts (2). Plant foods are the major sources of Se in most countries, so it enters the food chain through plants. Therefore, selenium-enriched production is one of the most effective ways to deal with the deficiency of selenium. For example, Finland started to add Se in fertilizer in 1984 to reduce the occurrence of Se deficiency in the population, through a policy established by the Ministry of Agriculture and Forestry. Recent researches showed Se content of the soil in the southern and central region of Iran was the moderate levels compared to the northern area, with the lowest rates. There are various methods for increasing selenium in plants, such as adding selenium to soil, seeds and foliar application on leaves (3). Selenium has been shown to play a positive effect on plant growth, antioxidant mechanism, and stress. The role of Se in the plant depends mainly on its concentration. Regarding the

[*]Corresponding author: Department of Horticultural Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
E-mail address: abdusivahid@gmail.com (Vahid Abdossi).

destructive effects of metals on the environment and the health of living organisms, acids are used as a modifier to increase the number of metals absorbed by the plants in the soil. Fulvic acids as an organic acid mainly derived from humus and other natural sources, which have a significant effect on neutralizing soil pH, solubility, and absorption of nutrients availability that led to increased biomass (4). Researchers have found that the use of acids at low doses at toxic concentrations of metal such as Se, by absorbing these elements, have reinforcing effects on growth factors such as leaf area and shoot dry weight. Humic acids are a mixture of weak aliphatic chains and aromatic organic acids that can be soluble in water at all pH (acidic, neutral, and alkaline). As this molecule enters the plant, it can bring microelements from the plant's surface into the tissue. It is a key ingredient in high-performance foliar applications. Humic acid addition with micro-chelate elements has a great influence on the quality of the product (5). However, modifiers, through chelators enhance nutrients uptake in tissue, and thereby improve the growth of the plant. Leaf vacuoles are major reservoirs for the accumulation and storage of metals (6). The results of the investigation on *Setaria italica* showed that the highest plant height, panicle length, and grain yield per plant were observed in the treatment of organic acid spraying and the highest chlorophyll leaf index in Humic acid application (7). In another study, the researcher investigated that Selenium at a concentration of 10 mg /l can increase the physiological and functional parameters (8). The results indicated that in *Brassica napus* using Humic and Fulvic acids at low concentrations increased the plant tolerance to copper toxicity (9). Due to the Se deficient areas of Iran Se enriched with vegetables are necessary. This issue has been considered in some developed countries and achieved some success by introducing selenium into their native plants, but in our country, this problem has been less addressed and almost no action has been taken in this regard. Since different physiological traits related to selenium (Se) have not been discussed in detail on dill, the aim of this study was to evaluate the selenium-enrichment along with acids on phytomorphological, essential oil, (Se) accumulation, and enzymatic activity of *Anethum graveolens* L. On the other hand, we investigated to approach optimum selenium enrichment that has been exploited as organize cultivation.

2. Materials and methods

The available genotype seeds were obtained from Isfahan Seed Pakan Company. Seeds were sterilized for 5-7 minutes with commercial sodium hypochlorite 5% and then rinsed with distilled water. After soil testing, they were cultured in pots (16 cm-diameter and volume=4000 cm^3) in a greenhouse at Tehran municipality. During germination and plant establishment, regular irrigation, weed control, and fertilization were performed according to soil requirements. The treatments were applied after complete plant deployment and germination of leaves. Four weeks after the application of treatments, samples were taken from the plants. The experiment was conducted in a completely randomized design with different levels of sodium selenate at concentrations (0, 6, 8, 12, and 16 mg/L) and soil application of Humic and Fulvic acids at concentrations of (0, 15, and 50 mmol/L) in 3 replications. Subsequently, it was transferred to the laboratory to evaluate the samples. Fresh weight of shoot and root was recorded by digital balance accuracy of 0.1 g. Shoot and root were weighed and placed in the oven at 60 $^{\circ}$C for 72 hours and were re-weighed by a digital scale. Chlorophyll content was measured as explained (10). 0.5 g leaf was calculated. The material was homogenized in a homogenizer by the addition of 10 ml of 80% acetone. The acetone extract containing all chloroplast was centrifuged at 2500 rpm for 5 minutes. This obtained extract was diluted by adding 9 ml of 80% acetone per ml of the extract. It was read on a spectrophotometer at 645nm and 663nm. The activity of guaiacol peroxidase was expressed in the method previously described (11). 50mM potassium phosphate buffer, 10 mM hydrogen peroxide, and 10µ of crude extract. Detecting the rate of enzyme activity was defined by monitoring the release of hydrogen peroxide (H_2O_2) at 240nm**.** Malondialdehyde (MDA) level was considered after the end of treatment. For measurement of MDA content, 3ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added to a 1ml aliquot of the supernatant. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. The tube was centrifuged at 10,000×g for 10min, and then the absorbance of the supernatant was read at 532nm. The value for the nonspecific absorption at 600nm was subtracted from the 532nm reading. The concentration of MDA was calculated using MDA's extinction coefficient of 155mM/cm (12). To identify the essential oil of this plant, the mass spectrometer attached to the chromatograph gas was used (13). The analysis was performed on data using SPSS 16. Comparisons were made using one-way analysis of variance [ANOVA] and Duncan's multiple range tests. Differences were considered to be significant at $p \leq 0.05$.

3. Results and discussion

Significant differences were found among the acids and Se in shoot fresh and dry weight parameter ($p \leq 0.01$) (Table 1). Plants growing in Fulvic acid 50 mg/L + 12 mg/L Sodium selenate with (145 g) had more shoot fresh weight than plants growing in others. Similar results were obtained in shoot dry weight in Dill plant treated with Fulvic acids 50 mg/L + 12 mg/L Sodium (Fig. 1 a-b). Organic compounds such as organic acid play a very important role in increasing moisture storage capacity, soil cationic exchange, plant resistance to drought, salinity, and soil balance, and has resulted in the conversion of nutrients such as phosphorus to formable absorption for plants. The application of Fulvic acid on Impatiens walleriana has shown a significant effect on fresh and dry weight (14 -15). Also, acid spray in low concentrations stimulates growth and increases the fresh and dry weight of tomatoes when compared to higher concentrations (16). Garbera treated with Fulvic acid showed early flowering and recorded higher fresh and dry weight of the shoots. Fulvic acid promotes improving the root system and as a chelating agent plays an important role in the

Table 1. Analysis of variance of traits in *Anethum graveolens* L.

SOV	DF	Shoot length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	GPX	Malondialdehyde	Selenium concentration in shoot	Selenium concentration in root	Chlorophyll	Ion leakage
Selenium concentration	4	19.92ns	0.02ns	0.01ns	0.06ns	0.16 ns	0.34ns	0.17ns	178.01 ns	0.22ns	2.13ns	0.28 ns
Acids concentration	2	168.18**	0.84**	0.73**	0.32**	12.02**	19.14**	0.54**	223.22**	18.76**	3.43**	11.20**
Type of acids	1	34.04*	0.03*	0.19*	0.03*	0.31*	0.43*	0.07*	78.2*	0.43*	0.07*	0.20*
Selenium concentration × Acids concentration	8	0.601*	0.125*	0.08*	0.01ns	0.08ns	0.05ns	0.01*	22.33*	0.08ns	0.32*	0.07*
Selenium concentration × Type of acids	4	13.61*	0.045*	12.13*	9.34ns	13.5ns	11.3ns	12.87ns	0.78**	16.11ns	11.03ns	12.14*
Selenium concentration × Acids concentration × Type of acids	8	321.80*	0.13*	0.21*	0.04*	0.20*	0.40*	0.04*	0.25*	0.40*	0.25*	13.1*
error	-	14.2	0.012	0.06	0.02	0.07	0.09	0.02	0.02	2.18	2.45	1.98
CV	-	12.73	13.2	12.14	7.45	11.04	21.5	3.54	14.22	11	1.38	13

*, **: Significant at the 5% and 1% probability levels, respectively; 'ns' is not significant.

formation of composite complexes. It allows for nutrients and minerals such as calcium, iron, magnesium, selenium, and manganese to be absorbed readily, stimulating improved plant growth and healthy roots (17). ANOVA indicated that the effects of acids and Se on the measured traits were significant for chlorophyll content, enzyme activity (Table 1). The highest amount of chlorophyll rose in Fulvic acids 50 mg /l + 12 mg/L Sodium selenate: 19.64 mg/g F.W. Results showed that the use of treatments at different concentrations increased the dry matter content compared to the control (Fig. 2-c). Fulvic acid promotes improving the root system and as a chelating agent plays an important role in the formation of composite complexes. It allows for nutrients and minerals such as calcium, iron, magnesium, zinc, and selenium to be absorbed

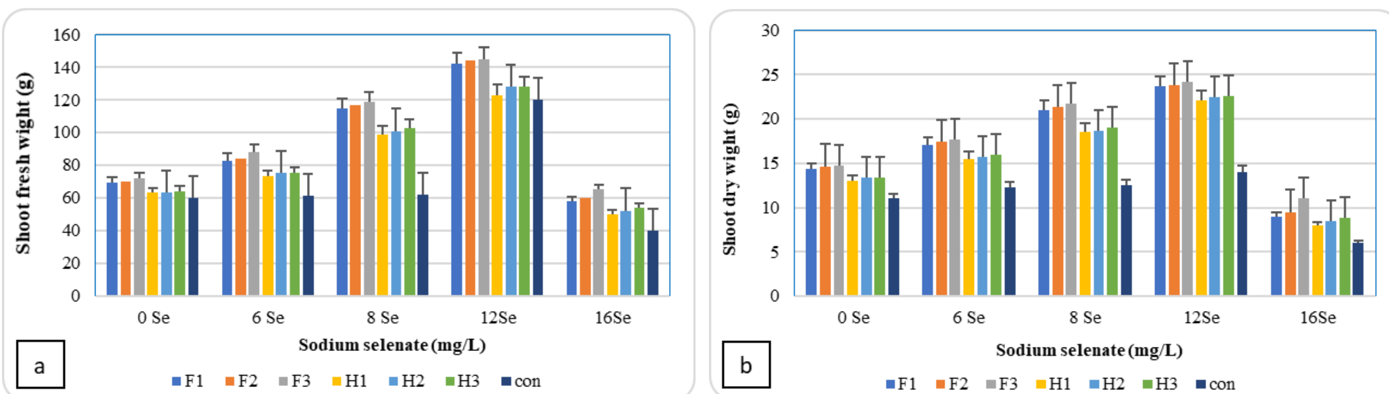

Fig. (1 a-b). Changes in shoo/root fresh weight to selenium trace supplemented with Fulvic acids and Humic acids; F1:Fulvic acids (0 mmol/L), F2:Fulvic acids,(15mmol/L), F3:Fulvic acids (50 mmol/L), H1:Humic acids (0 mmol/L), H2:Humic acids,(15mmol/L), H3:Humic acids (50 mmol/L); [error bar indicate standard deviation].

readily, stimulating improved plant growth and healthy roots. It leads to the availability of many essential nutrients for the plant and in the process of metabolism, production, and transfer of energy in plants, and increases the growth parameters such as plant height, protein increase, chlorophyll increase, grain yield, and other quantitative and qualitative traits in crops (18-19). A greenhouse study by the researcher on the chlorophyll content of leaves in wheat showed that Fulvic acid significantly increased leaf chlorophyll content (20). Highest increase in guaiacol peroxidase activity recorded in plants treated with Fulvic acid 50 mg /L + 12 mg /L Sodium selenate (16.5 µmol/g FW.min); while minimum guaiacol peroxidase was related to control treatment an average of 2.2 µmol/g FW.min (Fig. 2d). The rate of Malondialdehyde (MDA) concentration in plants treated by Fulvic acids 50 mg /L + 12 mg/L Sodium selenate (2.5 µmol/g FW.min) (Fig. 3e). The results of the study were agreed with (21). They found that Selenium acts as an antioxidant. Its content in Azolla plants increased significantly with increasing Se concentrations in the culture media up to 5 ppm. This indicated that Azolla plants were a good accumulator for Se. Selenium accumulation determined changes in Azolla biomass, doubling time and relative growth rates. Treatment of Azolla plants with low concentrations of Se (1 ppm) resulted in a significant increase in biomass. This was accompanied by a reduction in hydrogen peroxide and malondialdehyde (MDA) contents; the decrease percentages were 78% and 60%, respectively at 1 ppm Se in comparison with the control. At higher Se concentrations (>5 ppm), there was a significant increase in H_2O_2 and MDA contents, these increases were 3.2- and 2.8-fold at 10 ppm Se in comparison to control, respectively. The acids and Se had a significant difference in adsorption of Se at 1 % level.

Selenium level in shoot reached in maximum with Fulvic acids 50 mg /L + 12 mg/L Sodium selenite (8.8 mg/kg DW); when the minimum belongs control, with averaged (0.3 mg/kg DW) (Fig. 3f).

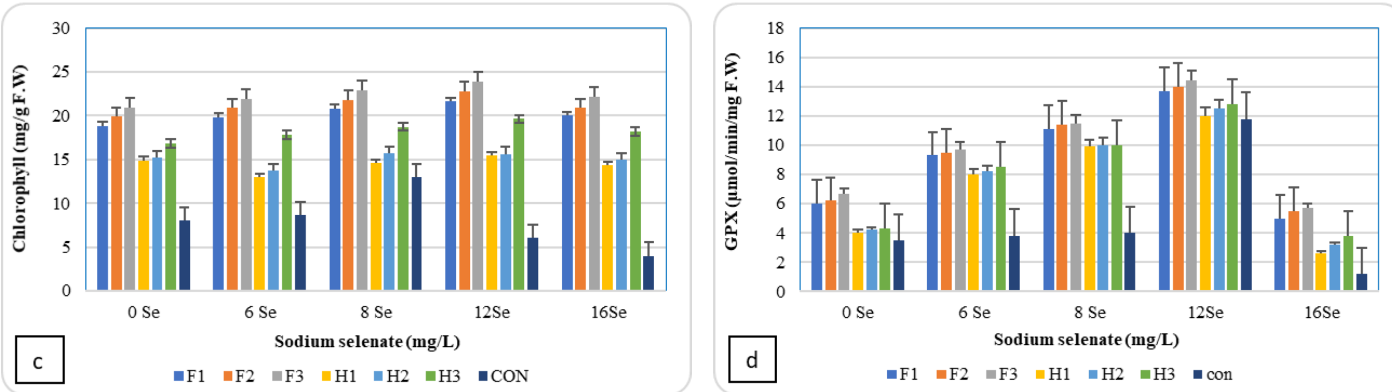

Fig. (2 c-d). Changes in chlorophyll/ GPX to selenium trace supplemented with Fulvic acids and Humic acids; F1: Fulvic acids (0 mmol/L), F2: Fulvic acids, (15mmol/L), F3: Fulvic acids (50 mmol/L), H1: Humic acids (0 mmol/L), H2: Humic acids,(15mmol/ L), H3: Humic acids (50 mmol/L); [error bar indicate standard].

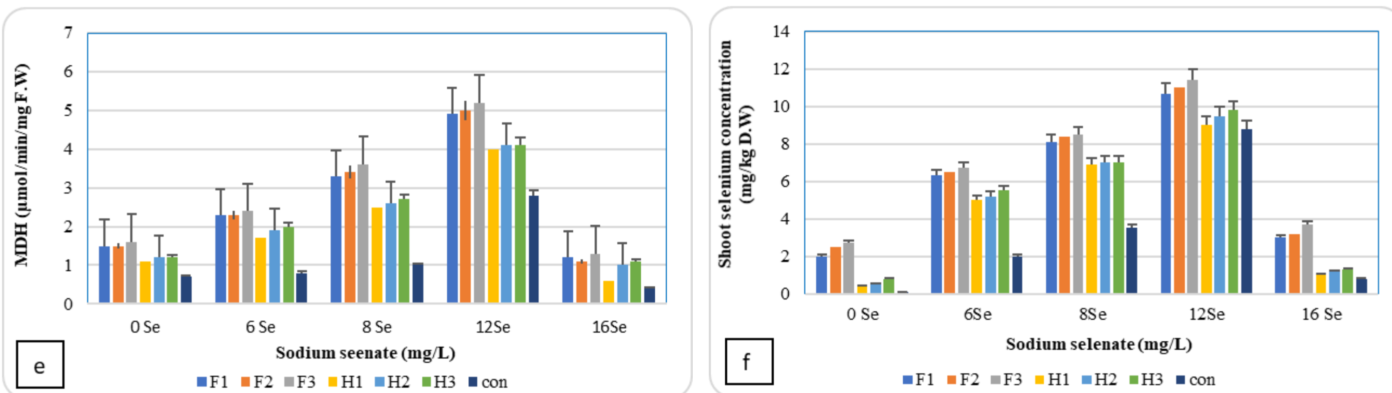

Fig. (3 e-f). Changes in Malondialdehyde / shoot selenium concentration to selenium trace supplemented with Fulvic acids and Humic acids; F1: Fulvic acids (0 mmol/L), F2: Fulvic acids, (15mmol/L), F3: Fulvic acids (50 mmol/L), H1: Humic acids (0 mmol/L), H2: Humic acids,(15mmol/L), H3: Humic acids (50 mmol/L); [error bar indicate standard deviation].

The same result was observed in root Selenium concentration, which was (8.1 mg/kg DW), in (Fulvic acids 50 mg/L + 12 mg/L Sodium selenate) and (0.1 mg/kg DW) in control (Fig. 4g). The chemical form of selenium in the soil is largely controlled by the redox potential and soil pH. Soil and plant management in seleniferous areas must take into account soil types and the genetic tolerance by plants of high selenium and salt concentrations. For example, plants will tolerate more selenium on high-sulfate soils than on low sulfate soils. Some plants, such as alfalfa, are very sensitive and will show signs of damage at low soil selenium concentrations while others, such as saltbush, may accumulate thousands of milligrams per kilogram of selenium without damage. Some arid and semiarid soils may need to be managed by prudent irrigation practices to reduce selenium and salinity to acceptable levels (22). A very important point to note in the enrichment of selenium is that the borderline between the toxicity and selenium deficiency is narrow. It depends on the chemical form of

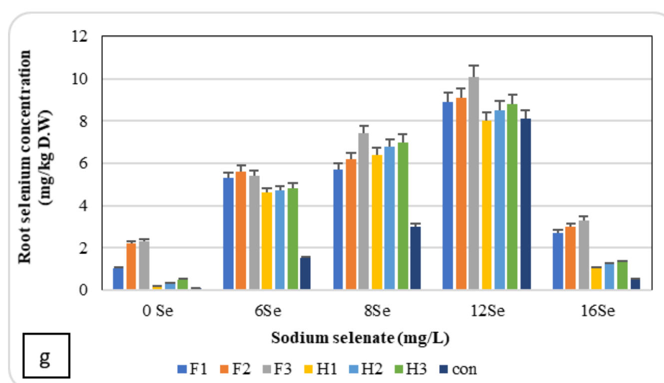

Fig. (4 g). Changes in root selenium concentration to selenium trace supplemented with Fulvic acids and Humic acids; F1: Fulvic acids (0 mmol/L), F2: Fulvic acids, (15mmol/L), F3: Fulvic acids (50 mmol/L), H1:Humic acids (0 mmol/L), H2: Humic acids,(15mmol/ L), H3:Humic acids (50 mmol/L); [error bar indicate standard deviation].

selenium. According to the experiment by (23) the low level of Se-treated plants showed greater growth parameters in spinach while, higher levels of selenium dramatically reduced these growth factors. Using GC and GC-MS 33 constituents were found 16 compounds in Table 2. Results indicated that selenium along with acids increased some major oil components include α-Pinene, β-Myrecene, α-Phellandene, and Carvone. The effectiveness of dill essential oil is demonstrated by the acids and Selenium amounts. Results also showed that essential oil obtained from dill under treatment with acids and Selenium exhibited a dose-dependent increase. Selenium along with acids increased α-Pinene (3.129%) significantly compared to control plants.

Table 2. Chemical composition (%) of the essential oil of *Anethum graveolens* L.

Compound	Oil (%)	KI
Thujene	0.121	974
α-Pinene	3.129	991
Β-Myrecene	1.183	1023
α-Phellandene	48.94	1027
Β- Phellandene	0.99	1030
Dill	0.45	1036
Trans-Dihydrocarvone	0.76	1049
Carvone	2.23	1057
Germacrene-D	0.23	1112
Apiol	0.45	1129
Trans-Limone oxide	0.65	1325
Limonene	0.54	1293
Sabinol	0.07	1325
N-Eicsonae	0.09	1383
3-Methyl henicosane	0.07	1470
Eicsonae	0.05	1522
Terpineol	0.06	1635

Comparison of average showed that the application of mild treatment acids 50 mg/L + 12 mg/L Sodium selenate significantly increased the β-Myrecene rate (1.183%), if that high Selenium (12 mg/L) led to a reduction in this combination (0.01%). Severe Selenium level resulted in a Carvone 2.23% compared to the treatment with acids (74.83%). The mean comparison showed that the highest percentage of α-Phellandene (48.94%) was observed in treatment with acids and Selenium. These responses are similar to those obtained by (24) with spinach (*Spinacia oleracea* L.) plants (25) with ryegrass (26) with tobacco and (27) with lettuce as well as with potato (28). At lower concentrations, selenium stimulated growth, on the other hand, at high doses act as pro-oxidant, reducing yields and inducing metabolic disturbances. High Se levels may inhibit photosynthesis, impair nutrient uptake and transport (29). Any kind of herbal drug is influenced by many factors. Genetic, pre-harvest (season of harvest, soil, type of water), and post-harvest factors (storage, temperature, moisture) can affect total phenolic, flavonoid, and antioxidant capacity as well as other elements and compounds (30). This study indicated that used mixtures of selenium and organic substance (fulvic acids) in the soil led to increases morphological, essential oils, and enzymatic activities. Only low concentrations of applied Selenium led to improve growth.

4. Conclusion

In conclusion, results showed that Dill could be grown successfully on a low concentration of Selenium because its essential oil yield and antioxidant compounds increased under the combination of substance and Selenium but severe these elements significantly decreased Dill growth, photosynthesis rate, and essential oil yield.

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