Investigation of morphological, phytochemical, and enzymatic characteristics of *Anethum graveolens* L. using selenium in combination with humic acid and fulvic acid

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**ABSTRACT**

The aim of this study was to investigate the effect of selenium trace element supplemented with fulvic acids and humic acids on some trait of *Anethum graveolens* L. This experiment was conducted in a completely randomized block design with three levels of fulvic acids and humic acids (0, 15, and 50 mmol/l) and selenium application in five levels (0, 6, 8, 12, and 16 mg/l) with three replications in the greenhouse at Tehran municipality. The results of this experiment showed that the effect of selenium at different acids on morphological traits was significant. So that the dry weight of shoot and root, plant height, ion leakage, chlorophyll, and antioxidant enzymes were affected by increasing Se, humic and fulvic acids levels. Results indicated that selenium along with acids increased some major oil components, including α-Pinene, β-Myrcene, α-Phellandrene, and Carvone.

1. INTRODUCTION

Dill (*Anethum graveolens* L.) is one of the medicinal herbs belonging to the plant family Apiaceae; this is the only species of the genus Anethum planted in Iran [1]. However, the three major components of the essential oil of dill herb are Carvone, Flandron, and Limonene. Selenium (Se) is an element, which presents as antioxidant defense systems and maintains hormone balance. It has recently been recognized as a basic substance for human and animal health and can play the important role of an antioxidant mechanism in plants [2]. However, it is toxic at high concentrations for plants and humans. Symptoms resulting from selenium toxicity in plants include growth retardation, chlorosis, fading, leaf drying, reduced protein synthesis, and premature death [3]. Regarding the destructive effects of heavy metals on the environment and the health of living organisms, acids are used as a modifier to increase the amount of heavy metals absorbed by the plants in the soil. Humic acids as an organic acid mainly derived from humus and other natural sources, which have a significant effect on neutralizing soil pH, the solubility, and absorption of nutrients availability that lead to increased biomass [4]. Researchers have found that the use of humic acids at low doses at toxic concentrations of heavy metal such as Cu, by absorbing these elements, have reinforcing effects on growth factors such as leaf area and shoot dry weight. Fulvic acids are a mixture of weak aliphatic chains and aromatic organic acids that can be soluble in water at all pHs (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 application. Fulvic acid addition with micro-chelate elements has a great influence on the quality of the product [5].

Soil modifiers, such as fulvic acids and humic acids, have the potential for the release of heavy metals in soils, uptake, and accumulation of them by the plant tissue. 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 heavy metals [6].

The results of [7] on Setaria italica showed that the highest plant height, panicle length, and grain yield per plant were observed in the treatment of humic acid spraying and the highest chlorophyll leaf index in the fulvic acid application. In another study, [8] investigated that Selenium at a concentration of 10 mg/l can increase the physiological and functional parameters. The results indicated that in Brassica napus using fulvic acids and humic acids at low concentrations increased the plant tolerance to copper toxicity [9]. The aim of this study was to evaluate the effect of selenium element along with fulvic acids and humic acids on morphological, phytochemical, and enzymatic characteristics of A. graveolens L.

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 = 4,000 cm³) in a greenhouse at Tehran municipality. During germination and plant establishment, regular irrigation, weed control, and fertilization were performed according to soil requirement. 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 as split plot in a randomized complete block design with different levels of selenium from source 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 three replications. Subsequently, it was transferred to the laboratory to evaluate the samples.

Fresh weight of the shoot and the root was recorded by digital balance accuracy of 0.1 g. Shoot and root were weighted and placed in the oven at 60°C for 72 hours and were re-weighed by a digital scale. The longest root length was measured using a ruler. Chlorophyll content was measured as explained [10]. 0.5 g leaf was calculated. The material was homogenized in a homogenizer by 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 645 and 663 nm. Activity of catalase (CAT) was expressed in the method of previously described [11]. 50mM potassium phosphate buffer, 10 mM hydrogen peroxide, and 10 µ of crude extract. Detecting the rate enzyme activity was defined by monitoring the release in hydrogen peroxide ($H_2O_2$) at 240 nm. Malondialdehyde (MDA) level was considered after the end of treatment. For measurement of MDA content, 3 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid was added to a 1 ml aliquot of the supernatant. The mixture was heated at 95°C for 30 minutes and then quickly cooled in an ice bath. The tube was centrifuged at 10,000×g for 10 minutes and then the absorbance of the supernatant was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The concentration of MDA was calculated using MDA’s extinction coefficient of 155 mM⁻¹ cm⁻¹ [12]. Electrical conductivity of the solution was measured with the EC meter. Selenium concentration of each sample was calculated with the atomic absorption Spectrometer [13]. To identify the essential oil of this plant, the mass spectrometer attached to the chromatograph gas was used [14].

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

Shoot length, shoot fresh and dry weight, root fresh and dry weight, chlorophyll content, MDA and CAT activity, ion leakage, selenium concentration in the shoot and root, and type and percentage of essential oil were tested in this study. ANOVA for different traits as influenced by Se, humic and fulvic acids level is given in Table 1.

Shoot fresh and dry weight and root fresh and dry weight were significantly affected by acid type, concentration of acids, sodium selenate concentration, and their interaction (Table 1).

The control showed a statistically significant difference with other treatments. Treatment of Dill with fulvic acids 50 mg/l + 12 mg/l sodium selenate with (145 g) was the highest and control treatment with 60 g has the lowest shoot fresh weight. The same results were obtained in shoot dry weight, so that plant treated with fulvic acids 50 mg/l + 12 mg/l sodium have more dry weight than others (Figs 1 and 2). Root fresh weight of plants growing in fulvic acids 50 mg/l + 12 mg/l sodium selenate (2.47 g) was higher than plants in control (0.6 g). Also, the ratio root dry weight was higher on plants in fulvic acids 50 mg/l + 12 mg/l sodium selenate 2.12 g than others (Figs. 3 and 4).

Organic compounds, such as humic acid and fulvic acid, as an 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 and humic acid on Impatiens walleriana has shown a significant effect on fresh and dry weight [15]. In addition, fulvic acid spray in low concentrations stimulates growth and increases the fresh and dry weight of tomato when compared to higher concentration [16]. Garbera treated with fulvic acid showed early flowering and recorded the higher fresh and dry weight of the shoots [17].

Length of the shoot, chlorophyll content, enzyme activity, and ion leakage were significantly influenced by the acid type, acids, and sodium selenate concentration (Table 1).

Control treatment with 47.3 cm, the lowest shoot length (20.8 cm), and fulvic acids 50 mg/l + 12 mg/l sodium selenate with 38.88 cm has the highest length of the shoot (Fig. 5). Data showed that plant height increased significantly with the enhancement of acids and sodium selenate concentration. Researcher reported that humic materials, especially humic acid, increase growth and plant height by increasing nitrogen content [18].
Humic acid and 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 manganese to be absorbed 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 [19].

The highest amount of chlorophyll (19.64 mg/g F.W) was obtained from fulvic acids 50 mg/l + 12 mg/l sodium selenate treatment. High concentration of sodium selenium (16 mg/l) significantly decreased chlorophyll content compared with control (Fig. 6).

In greenhouse and field studies, the efficacy of five applications with fulvic acids showed significant increased growth, chlorophyll content.

A greenhouse study by the researcher on the chlorophyll content of leaves in wheat showed that fulvic acid significantly increased leaf chlorophyll content [20].

Results indicated acids decreased lipid peroxidation in dill leaves and maximum MDA concentration (2.5 µmol/g FW) obtained from fulvic acids 50 mg/l + 12 mg/l sodium selenate treatment (Fig. 7). Highest catalase activity (CAT) belongs to plants treated with (fulvic acids 50 mg/l + 12 mg/l sodium selenate (2.49 µmol/g FW).
Figure 3: Changes in root fresh weight to selenium trace supplemented with fulvic acids and humic acids. F1: fulvic acids (0 mmol/l), F2: fulvic acids (15 mmol/l), F3: fulvic acids (50 mmol/l), H1: humic acids (0 mmol/l), H2: humic acids (15 mmol/l), H3: humic acids (50 mmol/l); (error bar indicates standard deviation).

Figure 4: Changes in root dry weight to selenium trace supplemented with fulvic acids and humic acids. F1: fulvic acids (0 mmol/l), F2: fulvic acids (15 mmol/l), F3: fulvic acids (50 mmol/l), H1: humic acids (0 mmol/l), H2: humic acids (15 mmol/l), H3: humic acids (50 mmol/l); (error bar indicates standard deviation).

Figure 5: Changes in shoot length to selenium trace supplemented with fulvic acids and humic acids. F1: fulvic acids (0 mmol/l), F2: fulvic acids (15 mmol/l), F3: fulvic acids (50 mmol/l), H1: humic acids (0 mmol/l), H2: humic acids (15 mmol/l), H3: humic acids (50 mmol/l); (error bar indicates standard deviation).

Figure 6: Changes in chlorophyll to selenium trace supplemented with fulvic acids and humic acids. F1: fulvic acids (0 mmol/l), F2: fulvic acids (15 mmol/l), F3: fulvic acids (50 mmol/l), H1: humic acids (0 mmol/l), H2: humic acids (15 mmol/l), H3: humic acids (50 mmol/l); (error bar indicates standard deviation).

Figure 7: Changes in MDA to selenium trace supplemented with fulvic acids and humic acids. F1: fulvic acids (0 mmol/l), F2: fulvic acids (15 mmol/l), F3: fulvic acids (50 mmol/l), H1: humic acids (0 mmol/l), H2: humic acids (15 mmol/l), H3: humic acids (50 mmol/l); (error bar indicates standard deviation).

Figure 8: Changes in Catalase to selenium trace supplemented with fulvic acids and humic acids. F1: fulvic acids (0 mmol/l), F2: fulvic acids (15 mmol/l), F3: fulvic acids (50 mmol/l), H1: humic acids (0 mmol/l), H2: humic acids (15 mmol/l), H3: humic acids (50 mmol/l); (error bar indicates standard deviation).
and lowest in control) (1 μmol/g FW) (Fig. 8). 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 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 H₂O₂ and MDA contents, these increases were 3.2- and 2.8-fold at 10 ppm Se in comparison to control, respectively.

Similarly, selenium level in shoot treated with (fulvic acids 50 mg/l + 12 mg/l sodium selenate (8.8 mg/kg DW) was significantly higher than other treatment; this was significantly different from the control, which averaged (0.3 mg/kg DW). 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 (Figs 9 and 10). 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 in order 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 selenium.

Using GC and GC–MS, 33 constituents and 16 compounds were found as shown in Table 2. Results indicated that selenium along with acids increased some major oil components including α-Pinene, β-Myrcene, α-Phellandrene, 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. Comparison of average showed that the application of mild treatment Fulvic acids 50 mg/l + 12 mg/l sodium selenate (F4S3) significantly increased the β-Myrcene 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%). Mean comparison showed that the highest percentage of α-Phellandrene (48.94%) was observed in treatment with acids
and selenium. These responses are similar to those obtained by [23] with spinach (Spinacia oleracea L.) plants [24] with ryegrass [25] with tobacco, and [26] with lettuce as well as with potato [27]. 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 [28].

This study indicated that used mixtures of selenium and humic substance (humic and fulvic acids) in the soil led to increases morphological, photochemical, and enzymatic traits. Only low concentrations of applied selenium led to improve growth. 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 a combination of humic substance and selenium but high concentration of these elements significantly decreased Dill growth, photosynthesis rate, and essential oil yield.

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