EJTAS
ISSN 2786-7447
EUROPEAN JOURNAL OF THEORETICAL AND APPLIED SCIENCES
OPEN ACCESS
PEER REVIEWED

Studying the Response of Greek Basil *Ocimum basilicum var. minimum* to Treatment with High Doses of Selenium

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Suggested Citation

Neamah, W.H., Hasan, F.A., & Hachim, A.J. (2024). Studying the Response of Greek Basil *Ocimum basilicum var. minimum* to Treatment with High Doses of Selenium. *European Journal of Theoretical and Applied Sciences, 2*(4), 1-5.
DOI: 10.59324/ejtas.2024.2(4).xx

Abstract:

Previous published literatures have been established the impact of selenium application in a trace amount on yield and active constituents of basil plants. In the current study, the effect of high doses of selenium application on growth of Greek basil *Ocimum basilicum var. minimum* growth and its essential oil compounds was investigated. Obtained result exhibited that the foliar application with (0, 40, 80, 120, 160) mg/L of selenium reduced the plants growth parameters and plant content of phytopigments and primary metabolites such as carbohydrates, protein, and proline. Refractive index of essential oil diminished post selenium application, while, specific gravity increased at 160 mg/L. Selenium application also caused alteration in the secondary metabolites profile. A reduction was obtained in the volatile compounds of essential oil such as Linalool, Eugenol and Methyleugenol with selenium treatment. On the other hand, unsaturated fatty acids such as Linolenic acid and phytosterols such as Campesterol, Stigmasterol and beta-Sitosterol increased with applicated selenium concentration.

Keywords: *Ocimum basilicum var. minimum, Selenium, Primer metabolites, Secondary metabolites*

Introduction

The Lamiaceae family is one of the most recognized sources of aromatic herbs worldwide and an excessive source of extracts with powerful antipathogens and antioxidant properties (Kaya, Yigit, & Benli, 2008). Genus Ocimum, known as basil, provide 150 species that belong to Lamiaceae family. Ocimum basilicum L is a one of species that show large morphological differences relying to species including size and colour of leaf or flower, and height and shape of plant. The chemical composition such as volatile organic and phenolic compounds also affected according to species (Mkaddem Mounira et al., 2022). The major aroma constituents of *Ocimum basilicum* L according to Lee and his colleagues are linalool, estragole, methyl cinnamate, eugenol, and 1,8-cineole (Lee, Umano, Shibamoto, & Lee, 2005). However, the existence and formation of these components are effected by numerous agents such as environment (Tursun & Telci, 2020), growing and harvesting states (Bowes & Zheljazkov, 2004) as well as drying and

extraction methods (Calín-Sánchez, Lech, Szumny, Figiel, & Carbonell-Barrachina, 2012; Chenni, El Abed, Rakotomanomana, Fernandez, & Chemat, 2016).

Ocimum basilicum var. minimum or Greek basil is the most common cultivated basil in Greece , it could be distinguished by its small leaves and shape of a loose or compact ball or reverse cone (Koutsos, Chatzopoulou, & Katsiotis, 2009). Greek basil represent a remarkable source of bioactive compounds including Cineole, Estragole and especially linalool with a high potency of biological activity (Šovljanski et al., 2022). Existing stress conditions around the plant is one of effects that cause alteration with increase or decrease in phytocomponents according to stress type and plant species (Yeshi, Crayn, Ritmejerytė, & Wangchuk, 2022).

Selenium (Se) was reported as an essential trace element for human health in regarding to its important role as anti-inflammatory, antioxidant and in immunity activates (Rayman, 2012). The application of Se in trace amounts can promote the plant growth under stress and normal conditions (da Silva et al., 2020; Hussein, Darwesh, & Mekki, 2019). However, Selenium in high concentration caused toxicity for human, animal, and plant due to replacing sulfur with Se in amino acids which influence negatively proteins biosynthesis and functions (Salhani, Boulyga, & Stengel, 2003). Se toxicity diminishing plant growth, development, and impeding plant ecophysiology, causing chlorosis and necrosis, restricted growth, and declined protein biosynthesis (Molnár et al., 2018). Wang and his team found an increase in the photosynthesis rate in rice seedlings that received low doses of selenium, this increase turn into dwindling when selenium dose increased gradually (Wang, Wang, & Wong, 2012). The toxicity of selenium occurs in the plant by two mechanisms, malformed selenoproteins and inducing oxidative stress, both of two mechanisms cause harms to plant and influence passively its growth and development (Gupta & Gupta, 2016). Malformed selenoproteins are formed initially due to misincorporation of SeCys/SeMet instead of Cys/Met in protein chain, SeCys is larger, more reactive and easily deprotonated than cysteine which effect prejudicially in proteins structure and function (Hondal, Marino, & Gladyshev, 2013) Moreover, under excessive does of selenium, Se act as pro-oxidant, produce reactive oxygen species and cause consequently oxidative stress in plants, for instance, under Se stress there was increase in the lipid peroxidation that observed obviously in wheat seedlings (Łabanowska et al., 2012).

Basil is not among plants that can accumulate high concentrations of selenium, thus, it is more sensitive to high doses of selenium, which can lead to reduce quantitively and qualitatively the yield (Skrypnik, Novikova, & Tokupova, 2019). In the current study, a novel cultivation of *Ocimum basilicum var. minimum* Greek basil in Basrah under stress of high excessive application of selenium was executed. Furthermore, physiological and biochemical characteristics of plants as well as physical traits and chromograph profile of the essential oil investigated under Se stress.

Materials and Methods

O. basilicum var. minimum Cultivation and Treatment

Seeds of Greek basil were fetched up from Turkey and sown in trays with peatmoss substrate. Post seedlings reached to 5 cm, they transplanted in 35 cm diameter pots with 3:1 ratio of soil to peatmoss substrate. Selenium foliar were applied on plants in 5 concentration (0, 40, 80, 120, 160) mg\L and with 3 times during the growth season.

Growth Criteria

Plant height, fresh weight and biomass were recorded before flowering stage

Biochemical Criteria

Plants were dried at room temperature, ground to small particles and utilized to value the carbohydrates, proline, protein, and phytopigments content. Essential oil extracted also from dry material and its physical traits and chromograph profile was estimated.

Photosynthesis Pigments

The content of leaves from chlorophyll A, chlorophyll B, total chlorophyll, and carotenoids was estimated as described earlier by (Makeen, Babu, Lavanya, & Grard, 2007). 0.5 g of plant leaf was incubated with 10 ml of 80% acetone for 48 hours in 4 ^{0}C. Then, suspend filtrated and the absorbance measured at 663, 645, and 452 nm against the blank and pigments concentration calculated using follow equations:

Chlorophyll a (mg/100g FW)

$$= (10.3 \text{ x } A663 - 0.92 \text{ x } A645)/1000 \text{ x } 10/ 0.5 \text{ x} 100 \quad (1)$$

Chlorophyll b (mg/100g FW)

$$= (19.7 \text{ x } A645 - 3.87 \text{ x } A663)/ 1000 \text{ x } 10/0.5 \text{ x} 100 \quad (2)$$

Total chlorophyll (mg/100g FW)

Chl a + Chl b (3)

Carotenoids (mg/100g FW)

$$= (4.2 \text{ x } A452) - ((0.026 \text{ x Chl a}) + (0.426 \text{ x Chl b})) \ /1000 \text{ x } 10/ 0.5 \text{ x } 100 \quad (4)$$

Carbohydrates Content

Plant content of carbohydrates was assessed according to (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). 0.5 g of dried samples were homogenized with70 ml of distilled water in 70 ^{0}C for 1 hour. Post bringing the extract to the room temperature, the extract was filtrated, and 5 ml of filtrate diluted with 25 ml of distilled water in separated tubes. 1 ml of solution was placed in the new tube, 1 ml of 5% of phenol added to the tube and then 5 ml of pure sulfuric acid added rapidly to the tube with cautious vortex. The absorption was recorded spectrometry at 490 nm and carbohydrates concentration was determined from glucose standard curve and calculated in dry weight following the equation:

$$\text{Carbohydrates content mg/} = (\text{mg glugose/ ml x final sample volume ml}) / \text{ sample weight g} \quad (5)$$

Proline Content

Proline content estimate was according to (Bates, Waldren, & Teare, 1973) with little modification. Acid-ninhydrin was prepared by dissolving 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid on heat plate with agitation and kept cool at 4 ^{0}C. 0.20 g of dried plant samples homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate filtered through filter paper. 2 ml of filtrate was reacted with 2 ml of glacial acetic acid and 2 ml of acid-ninhdrin in test tubes at 100 ^{0}C. After 1 hour the reaction terminated with ice bath, toluene added to the tubes and vortexed for 15-20 sec. Separated upper layer was read spectrophotometrically at 520 nm using toluene as a blank and proline content determined from proline standard curve and calculated in dry weight following the equation:

$$\mu\text{moles prolin/g} = [(\mu\text{g proline/ml} \times \text{ml toluene})/115.5\ \mu\text{g/}\mu\text{mole}]/ [(\text{g sample})/5] \quad (6)$$

Protein Content

Kjeldahl method was followed to determine protein content in dried plant samples. 0.20 g of plant sample was digested with 5 ml of concentrated sulfuric acid at 350 ^{0}C for 30 minutes. Then, 3 ml of the mixture of 6 ml perchloric acid and 96 ml sulfuric acid was added to the sample solution and placed on digestion tablet at 350 ^{0}C until the solution be clear. The volume of sample solution was made up to 50 ml by distilled water. 10 ml of sample was mixed with 10 ml of 6 N of sodium hydroxide in Kjeldahl tube and placed in Kjeldahl analyzer. 150 ml of the distillate was collected in a conical flask containing 2 % Boric acid and 2 % of bromocresol green and methyl red indicator (0.099 g + 0.066 g in 100 ml Ethanol). The indictor was titrated with 2 % HCl until the

solution has a slightly violet color, the portion of Nitrogen was calculated following the equation:

$$\% N = (V X N X MW X TV)100/ 1000 X SW X VS) \quad (7)$$

% N= Portion of Nitrogen

V= Volume of titration acid

N= Normality of HCl

MW= Molecular weight of Nitrogen

TV= Total volume of digested solution

SW= Weight of plant sample

VS= Volume of digested solution

The portion of protein was estimated following the question:

$$\% protein = \% N X 6.25 \quad (8)$$

Essential Oil Extraction

The essential oil of Greek basil was obtained by Soxhelt extractor. 10 g of dried sample was placed in a Soxhelt with 150 ml of petroleum ether as a solvent and at 50 ^{0}C, the extraction cycles were ended when the solvent color in the last cycle was semi clear. Then, extracted essential oil was concerted by Rotary evaporator and its specific gravity and refractive index (Abbe-Refractometer, /6500, Germany) estimated as well as GC-MS (Agilent 7890B GC, 5977A MSD, US) runed for the components profile detection.

Experiment Design and Statistical Analysis

The experiment was designed as a randomized complete block design (R.C.B.D.) in three blocks with three replicates. The results were analyzed by Graph prism program version 6.01, and the averages of treatment were compared at the probability level.0.05 by one-way analysis of variance ANOVA.

Results

Growth Traits

Figure 1 illustrates the impact of five concentrations of selenium on vegetative growth traits of *O. basilicum var. minimum* including the plant high (Fig. 1a), fresh weight (Fig. 1b) and biomass (Fig. 1c). Fresh weight and biomass were remarkably reduced due to selenium foliar application compared to the control. On the other hand, Se2 (80 mg/L)-treated plants recorded increasing in the plant high feature compared to the control and other treated plants.

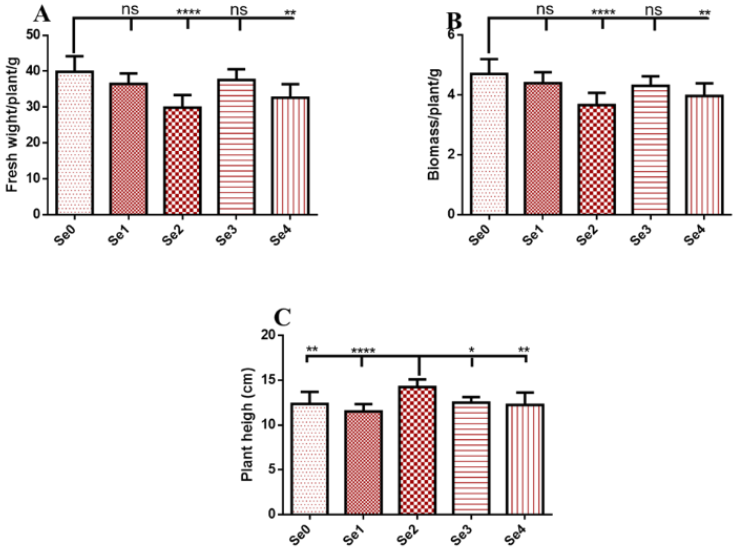

Figure 1. Effect of Treatment with Selenium on Vegetative Growth of *O. basilicum var. minimu*

(A) Representative the effect of treatment with selenium on fresh weight of Greek basil plants. Se0 control or untreated plants. Se1 plants that treated with 40 mg/L of selenium. Se2 plants that treated with 80 mg/L of selenium. Se3 plants that treated with 120 mg/L of selenium. Se4 plants that treated with 160 mg/L of selenium. (B) Representative the effect of treatment with selenium on biomass of Greek basil plants. (C) Representative the effect of treatment with selenium on height of Greek basil plants. A multiple ANOVA was performed using ordinary one-way ANOVA multiple comparisons to compare between the averages of treatments. Significance was designated as follows: *p < 0.05, **P < 0.01, ****p < 0.0001.

Photopigments Content

The foliar spray with selenium at 160 mg/L caused diminishing in Chlorophyll A, B and total Chlorophyll compared to treated or untreated Greek plants (Fig. 2a, b, c). While, Carotenoids content reduced significantly with selenium treatment at all levels (Fig. 2d)

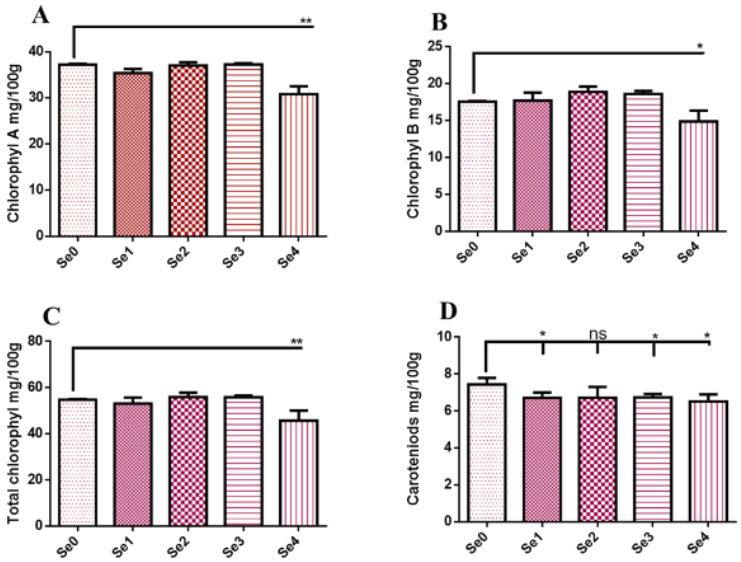

Figure 2. Effect of Treatment with Selenium on Phytopigments Content in Leaves of *O. basilicum var. minimum*

(A) Representative the effect of treatment with selenium on Chlorophyll A content in leaves of Greek basil plants. Se0 control or untreated plants. Se1 plants that treated with 40 mg/L of selenium. Se2 plants that treated with 80 mg/L of selenium. Se3 plants that treated with 120 mg/L of selenium. Se4 plants that treated with 160 mg/L of selenium. (B) Representative the effect of treatment with selenium on Chlorophyll B content in leaves of Greek basil plants. (C) Representative the effect of treatment with selenium on total Chlorophyll content in leaves of Greek basil plants. (D) Representative the effect of treatment with selenium on Carotenoids content in leaves of Greek basil plants. A multiple ANOVA was performed using ordinary one-way ANOVA multiple comparisons to compare between the averages of treatments. Significance was designated as follows: *p < 0.05, **P < 0.01.

Biochemical Traits

Figure 3 represent the impact of selenium application stress on biochemical criteria of Greek plants. Obtained result showed a decrease in the carbohydrates content with all Se concentration (Fig. 3a). Likewise, protein percentage reduced in the plants at selenium

treatment compared to control plants (Fig. 3b). There was no alteration in the proline content obtained under the selenium stress (Fig. 3c).

Physical Traits of Essential Oil

Figure 3 illustrate the physical features of extracted essential oil from O. basilicum var. minimum. Both specific gravity and refractive index reduced markedly due to selenium stress excepting the treatment with 160 mg/L that increased the gravity of essential oil compared to the control (Fig 3d&e).

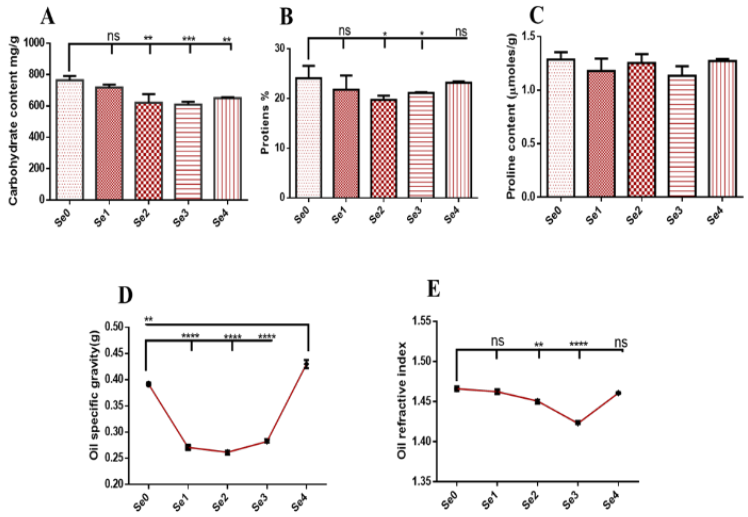

Figure 3. Effect of Treatment with Selenium on Biochemical and Physical Traits of Plant and Essential oil of *O. basilicum var. minimu*

(A) Representative the effect of treatment with selenium on carbohydrates content of Greek basil plants. Se0 control or untreated plants. Se1 plants that treated with 40 mg/L of selenium. Se2 plants that treated with 80 mg/L of selenium. Se3 plants that treated with 120 mg/L of selenium. Se4 plants that treated with 160 mg/L of selenium. (B) Representative the effect of treatment with selenium on protein percentage of Greek basil plants. (C) Representative the effect of treatment with selenium on proline content of Greek basil plants. (D) Representative the effect of treatment with selenium on specific gravity of essential oil of Greek basil plants. (E) Representative the effect of treatment with selenium on refractive index of essential oil of Greek basil plants A multiple ANOVA was performed using ordinary one-way ANOVA multiple comparisons to compare between the averages of treatments. Significance was designated as follows: *p < 0.05, **P < 0.01***P < 0.001, ****p < 0.0001.

GC-MS Profile

Chromogram profile of Greek basil essential oil in figure 4 and table 1 represent the percentage area of volatile compounds that are mainly existent in the Greek basil oil. Linalool is formed the most findable compound among other volatile compounds of Greek basil essential oil including Eugenol, Methyleugenol, tau.-Cadinol and Phytol. Furthermore, other phytocomponents such as Linolenic acid, Vitamin E, Campesterol, Stigmasterol, gamma.-Sitosterol, and beta.-Sitosterol are obtained from the essential oil of Greek basil and influenced widely beside the volatile compounds due to selenium doses (Fig 4a,b,c,d,e) (Table 1).

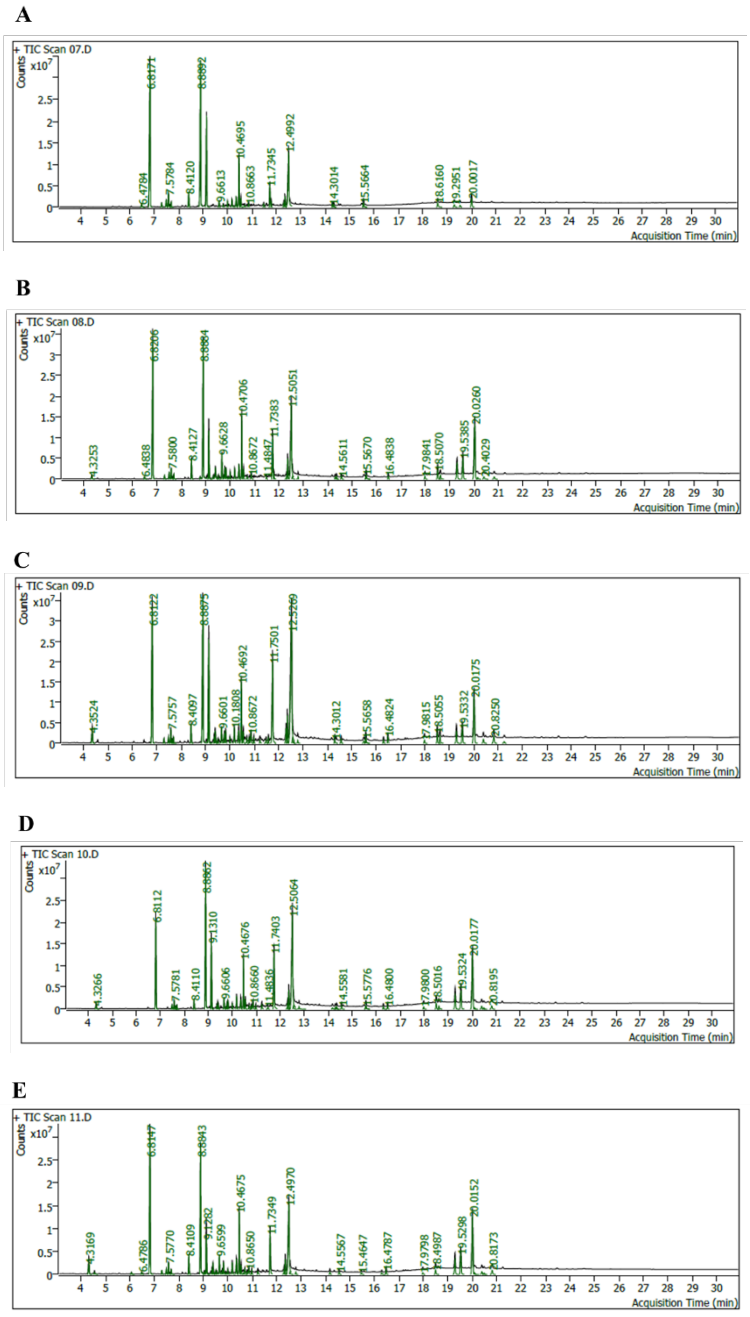

Figure 4. GC-MS chromatograph of essential oil of *O. basilicum var. minimum*

(A) Representative GC-MS profile of essential oil of Se0 control or untreated plants. (B) Representative GC-MS profile of essential oil of Se1 plants that treated with selenium at 40 mg/L. (C) Representative GC-MS profile of essential oil of Se2 plants that treated with selenium at 80 mg/L. (D) Representative GC-MS profile of essential oil of Se3 plants that treated with selenium at 120 mg/L. (E) Representative GC-MS profile essential oil of Se4 plants that treated with selenium at 160 mg/L.

Table 1. GC-MS Profile of Greek Basil *O. basilicum var. minimum* Essential Oil

			Peak Area %				
No	R.T	Compound Name	Se0	Se1	Se2	Se3	Se4
1	6.82	Linalool	23.8642	15.0202	11.0957	9.1867	15.3296
2	7.57	Terpinen-4-ol	1.6206	0.889	0.9242	0.7352	1.0316
3	8.41	Borneol acetate	1.7523	1.6211	1.1553	0.8337	1.6296
4	8.88	Eugenol	19.833	12.8163	11.0392	13.7595	11.9645
5	9.13	Methyleugenol	12.2124	5.7047	9.3317	7.9807	6.2669
6	9.82	.gamma.-Muurolene	0.4003	0.9792	0.898	0.7649	1.1411
7	10.18	Spathulenol	1.1211	1.1621	1.3222	1.5063	1.4995
8	10.47	.tau.-Cadinol	5.9325	5.306	3.9964	4.5393	5.4704
9	12.35	Phytol	1.1933	1.1933	1.7718	1.6702	1.3074
10	12.49	Linolenic acid	12.7774	14.3143	22.693	19.8191	13.2034
11	18.50	Vitamin E	-	1.2223	1.0092	1.0321	0.7658
12	19.29	Campesterol	-	-	-	1.9287	2.0208
13	19.54	Stigmasterol	-	2.7219	1.3325	2.5239	2.8354
14	19.99	gamma.-Sitosterol	1.5583	-	-	-	-
15	20.02	beta.-Sitosterol	-	8.4625	4.8853	8.611	9.0896

Discussion

It is a known that the application of selenium in the high level cause maceration the plant growth, the data of vegetative parameters of *O. basilicum var. minimum* showed decrease in fresh weight and biomass traits compared to the control. Likewise, the high concentration of selenium caused reduce the number of leaves, biomass, and the starch/chloroplast area ratio in strawberry which was related to changes in the activity and/or biosynthesis of enzymes post the selenium application (Valkama, Kivimäenpää, Hartikainen, & Wulff, 2003). Treated plants with 80 mg/L of selenium showed increase in the plant height compered to 40,120 and 160 mg/L Se-treated plant and Se-untreated plants. These plants showed a considerable reduction in the fresh wight and biomass which could be elucidated the increase in the height of plants.

Chlorophyll A, B, and total chlorophyll decreased remarkably with 160 mg/L of Se treatment only. Nawaz and his colleagues reported that excessive Se concentrations reduced chlorophyll and carotenoids content in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) under water deficit conditions (Fahim Nawaz et al., 2016; Nawaz, Ashraf, Ahmad, & Waraich, 2013). Also, Se foliar application at all concentration significantly reduced the carotenoids content in leaves, diminution of carotenoids content in *Pterocypsela laciniata* leaves was reported with gradual increase of exogenous application of selenium (Xu et al., 2018).

Numerous of studies reported increasing in the carbohydrates content with low doses of selenium treatment. In the current study, carbohydrates content reduced progressively in *O. basilicum var. minimum* with increase of selenium concentrations. Carbohydrates reduction could be attributed to the negative effect of Se on vegetative growth of plants and photosynthesis pigments content which effect consequently on the efficiency of photosynthesis production. Likewise, protein proportion reduced in Se-treated plants due to the decrease in the carbohydrates output. Carbohydrates provide carbon skeleton and energy for the synthesis of amino acids that are precursors for proteins and many secondary metabolites, act as nitrogen transporter and reservoir in C and N metabolism (Cao et al., 2008). Although it is not a significant, the reduction in the proline was obtained with selenium application, Aggarwal and his team found that proline content reduced in bean (*Phaseolus vulgaris* L.) seedlings concomitant with the reduced growth after were subjected to high level of selenium (Aggarwal et al., 2011).

Physical criteria of *O. basilicum var. minimum* essential oil exhibited decrease in the specific gravity and refractive index at 40, 80 and 120 mg/L of selenium. However, the treatment at

160 mg/L significantly increased the essential oil gravity compared to the control and other treatments which could be attributed to increase the phytosterols such as Campesterol, gamma-Sitosterol and beta.-Sitosterol in the essential oil of treated plants. Phytosterols are triterpenes compounds have a big molecular weight compared to monoterpene and sesquiterpene compounds that component the Greek basil essential oil (Hosseini & Pereira, 2023; Moreau, Whitaker, & Hicks, 2002). Refractive index usually can be used as indicator to the purity and quality of the oil, refractive index of *O. basilicum var. minimum* essential oil diminished with selenium treatment special at 120 mg/L concentration compared to the control. As is shown in Table 1 and GC-MS profile the terpenes including monoterpenes such as Linalool, sesquiterpene such as Spathulenol and diterpene such as Phytol declined due to Se exogenous application which caused consequently descent in the refractive index value and then quality of essential oil. According to Ospina and his colleagues who found that reflective index value of essential oil of *Lippia origanoides* was direct related with its content of thymol (Ospina, Tovar, Flores, & Orozco, 2016).

Post Se application, it was high alteration in chromogram profile of *O. basilicum var. minimum* essential oil. For example terpenoid compound such as Linalool, Eugenol, and tau.-Cadinol, decreased due to Se treatment. The reduction in the volatile compounds could be related to reduce the primary carbon metabolists including carbohydrates which provide the precursors for terpenoid compounds biosynthesis through mevalonate (MVA) and methylerythritol 4-phosphate (MEP) pathways (Verma & Shukla, 2015). The unsaturated fatty acids (UFAs) such as Linolenic acid increased with Se treatment compared to Se0. Increasing of UFAs including Linolenic acid is one of elaborate strategies have been developed by plant to avoid or abide the adverse effects of stress. UFAs are acting as stocks of extracellular barrier constituents precursors of various bioactive molecules, regulators of stress signaling and ingredients, modulators of cellular membranes in glycerolipids, and reserve of carbon and energy in triacylglycerol (He & Ding, 2020). Vitamin E or Tocopherol was present post Se treatment due to its antioxidant activity which increase the plant tolerance against several abiotic stresses (e.g., salinity, metal toxicity, drought, ozone, UV radiation) (Hasanuzzaman, Nahar, & Fujita, 2014). GC-MS profile of *O. basilicum var. minimum* essential oil exhibited variation in the appearance and percentage area of sterols post Se treatment. Campesterol, Stigmasterol, and beta.-Sitosterol increased with Se treatment. While gamma.-Sitosterol vanished with all Se concentrations treatment. The alteration in plant sterols content due to stress has been reported in the previous studies (Neamah, Hasan, Jasim, & Hachim, 2024; Ozolina, Gurina, Nesterkina, & Nurminsky, 2020; Rogowska & Szakiel, 2020). Increasing sterols under abiotic stress could improve the plants resistance through boosting the cohesion of cell membranes to change the membranes permeability and affecting proton efflux (Dufourc, 2008; Valitova, Sulkarnayeva, & Minibayeva, 2016).

Conclusion

Greek basil *O. basilicum var. minimum* that exposed to selenium stress through high doses application exhibited diminishing in physical and biochemical traits. Also, selenium treatment caused reduction in physical features of essential oil and alteration its active constituents.

Conflict of Interests

No conflict of interest.

https://doi.org/10.1007/s12011-010-8699-9

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