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Research Article

Effects of selenium on structure of the essential oil isolated from *Satureja hortensis* L. (Lamiaceae) under the cadmium stress

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Abstract: One of the heavy metals which cause severe environmental pollution and biochemical changes in plants is Cadmium (Cd) while Selenium (Se) acts as an important anti-stress agent in plants. In the present research, the glasshouse experiment was conducted to examine the effect of Cd and Se on structure of the essential oil isolated from the savory plant, *Satureja hortensis* L. To do so, the plants were polluted with different Cd levels including 0 (the control), 75, 100, and 150 µM. Moreover, Se was used in the form of sodium selenite salt in concentrations of 0 (control), 10, 20, and 40 µM. The results show that carvacrol was the main constituent in most of the essential oil analyses, except for one of them (0 µM of Cd×10 µM of Se). Furthermore, differences among minor constituents in most of treatments were not significant. Therefore, these results indicate the role of Cd and Se in the compositional changes of *S. hortensis* essential oil.

1. INTRODUCTION

Trace pollutants such as cadmium (Cd) are toxic to humans, animals, and plants. They enter the environment through human activities. This chemical compound is easily absorbed by root and transported inside the plant, where it is toxic for living cell even at very low concentrations (Gallego *et al*., 2012). Plants affected by cadmium are disturbed in photosynthesis, nutrition, and water balance. Several cellular interactions indicate the toxicity by Cd. Metals bind to enzymes ligands, so their toxic effects are mainly determined by interaction with those when enzyme inhibition is probably due to the masking of catalytically active groups (Saidi *et al*., 2014). Several studies have indicated that Cd generates oxidative stress either by causing oxygen free radical production and reactive oxygen species (ROS) or by declining enzymatic and non-enzymatic antioxidants activity due to its high affinity towards sulfur-containing peptides and protein (Wang *et al*., 2014).

Selenium (Se) as an essential element for humans and animals is also useful for plants (Pezzarossa *et al*., 2012). Se has not been confirmed as an essential micronutrient in higher

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plants, but several studies have shown that relatively low concentrations of it can have beneficial effects on plant growth under normal and stressed conditions. Se acts as an antioxidant agent and can increase plant tolerance (Hawrylak-Nowak *et al*., 2014). Several studies have suggested that Se can limit production of ROS and participate in their quenching through both direct and indirect mechanisms. Three possible mechanisms have been proposed for Se to scavenge ROS (Feng *et al*., 2013). In the first mechanism, superoxide anions are dismutated into H_2O_2 without involvement of superoxide dismutase. Secondly, its presence in seleno compounds, quenching superoxide anions, and hydroxyl radicals has been proven. The third mechanism involves increasing antioxidative enzyme activity (Handa *et al*., 2019).

Secondary metabolites are derivatives of primary metabolites that are produced by plants when their physiological processes change (Ashraf *et al*., 2018). Plant growth and survival are significantly improved by the secondary metabolites under different environmental stresses, making them important metabolic molecules (Zandalinas *et al*., 2018). Under natural conditions, plants are susceptible to biotic and abiotic stresses. Secondary metabolites are synthesized by plants in response to these environmental stresses, so they can counteract the adverse effects of both biotic and abiotic stresses. Thus, stresses in the environment strongly influence the production of plant secondary metabolites (Raduisene *et al*., 2012).

Essential oils make up a significant part in aromatic plants. When a plant is under stress, essential oil is generally considered remnants of its main metabolic processes (Mohtashami *et al*., 2018). Essential oils are made in medicinal and aromatic plants and known as the secondary metabolites. They are used in pharmaceutical, food, and cosmetic industries, due to their antimicrobial, anti-inflammation, anti-spasmodic, sedative, carminative, and appetizer properties (Hajhashemi *et al*., 2003; Mohtashami *et al*., 2021). The genus *Satureja* (the family Lamiaceae) comprises 200 species of herbs and shrubs which are widely distributed in the Mediterranean regions. Savory plant is used as an antiseptic for the digestive system as it improves blood pressure and reduces cough (Mohtashami *et al*., 2021). The summer savory, *Satureja hortensis* L. (Figure 1) has medicinal and food values due to its prominent essential oil compounds such as carvacrol and thymol (Alizadeh *et al*., 2020). It is very important to investigate the changes of secondary metabolites in medicinal plants treated with different chemical compounds. Based on this, the present research was designed. We investigated the changes in structure of the essential oil from this medicinal plant under Cd and Se treatments, which is important from different aspects.

Figure 1. The medicinal plant, *S. hortensis.*

2. MATERIAL and METHODS

2.1. Research Design and Treatments

The present research was conducted during 2017 in the greenhouse of Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran under a factorial experiment in the form of a completely randomized design (CRD) under three replications. The main goal was to investigate the effect of Cadmium (stress agent) and Selenium (anti-stress factor) on the essential oil structure of *S. hortensis*. Cadmium chloride ($CdCl_2$) and Sodium Selenite salt were obtained from Merck and Sigma Companies, Germany, respectively. Soil contamination was performed with different doses of $CdCl_2$ (0, 75, 100, and 150 mM) and Sodium Selenite salt (0, 10, 20, and 40 µM) under the Field Capacity (FC) moisture. The soils were exposed to wet/dry cycles for 4 months as approximate natural conditions for a long-term contamination. Finally, the seeds of *S. hortensis* were planted in the contaminated soil and monitored until seedling emergence. In the control, no chemical compounds were applied (Azizi *et al*., 2021).

2.2. Essential Oil Isolation

The aerial parts of savory plant which contained the most essential oil content were collected from each treated sample and the control plants. Then, they completely dried under the room temperature (about 25°C) in shadow for about a week. Then, the dried samples were powdered by an electric mill and 50 g of each was added to 500 ml of the distilled water. Finally, their essential oils were separated by a Clevenger apparatus in a temperature of 100 °C for 4 h (Figure 2). The water in each essential oil was removed by Na_2So_4 and the pure essential oils were stored at 4°C in special sealed vials (dark glass bottles) until the chemical analysis by GC-MS (Asadi *et al*., 2018, 2019).

Figure 2. The essential oil isolation from *S. hortensis*.

2.3. Chemical Analysis of The Isolated Essential Oils

Quantitative and qualitative analyses of the essential oils were done by the Agilent Technology 7890B gas chromatography-mas spectroscopy (made in USA) with the following specifications:

- Column: HP-5MS
- Long: 30 m
- Diameter: 0.25 mm
- Film thickness: 0.25 mm.
- Analysis temperature: 350°C

Determining of ranges was done by mass data bank, coates index, and retention time. At the end, each compound from the essential oil was identified by extent pattern compared to the valid libraries (Adams *et al*., 2001).

2.4. Statistical Analysis

The present study was repeated three times for all treatments and uninfected plants. Then the data obtained were checked for normality. Finally, the normalized data (if needed) were analyzed by using one-way ANOVA and their means compared by Tukey's test (p<0.05) with SPSS software version 20.

3. RESULTS

3.1. Treatment of Cd Concentrations with 0 µM of Se

Treatments of Cd (0, 75, 100, and 150 µM) with 0 µM of Se were investigated on the structure of *S. hortensis* essential oil (Table 1). About all treatments, Carvacrol was determined as the main constituent. By treatment of 0 µM×0 µM (Cd×Se), differences among most of the constituents were significant ($F_{17, 36}$= 10.16). Under treatment of 75 µM×0 µM (Cd×Se), carvacrol, γ-terpinene, and benzene had high percentages in the essential oils structure, respectively ($F_{17, 36}$= 4.90). Also, with 100 µM×0 µM (Cd×Se) treatment, limonene showed the lowest percentage compared to the others ($F_{17, 36}$= 14.16). Finally, about 150 µM×0 µM (Cd×Se) treatment, most of differences were significant when octatriene being absent in the essential oil structure ($F_{17, 36}$= 7.93).

Table 1. The effects of different Cd concentrations with 0 µM of Se on the structure of *S. hortensis* essential oil.

Compound	Cadmium × Selenium			
	0 µM × 0 µM	75 µM × 0 µM	100 µM × 0 µM	150 µM × 0 µM
α-Pinene	1.03 ± 0.15 efg	1.04 ± 0.07 e	1.15 ± 0.04 ef	1.21 ± 0.02 e
α-Terpinene	4.14 ± 0.37 d	4.37 ± 0.05 d	4.94 ± 0.12 d	4.99 ± 0.35 d
α-Thujene	1.32 ± 0.11 ef	1.31 ± 0.08 e	1.52 ± 0.09 e	1.51 ± 0.04 e
ß-Bisabolene	0.07 ± 0.03 g	0.22 ± 0.07 e	0.36 ± 0.16 f	0.32 ± 0.05 ef
ß-Myrcene	1.34 ± 0.14 e	1.26 ± 0.11 e	1.50 ± 0.11 e	1.48 ± 0.17 e
ß-Pinene	0.37 ± 0.02 efg	0.40 ± 0.01 e	0.49 ± 0.02 ef	0.46 ± 0.05 ef
γ-Terpinene	19.92 ± 0.86 b	19.66 ± 0.40 b	21.43 ± 0.42 b	23.42 ± 0.49 b
Benzene	7.98 ± 0.09 c	6.24 ± 0.52 c	7.16 ± 0.65 c	6.70 ± 0.06 c
Camphene	0.18 ± 0.06 efg	0.04 ± 0.02 e	0.07 ± 0.03 f	0.06 ± 0.00 f
Carvacrol	60.00 ± 0.40 a	62.59 ± 0.78 a	58.71 ± 0.48 a	56.84 ± 0.63 a
Carene	0.09 ± 0.05 g	0.07 ± 0.07 e	0.09 ± 0.02 f	0.03 ± 0.03 f
Caryophyllene	0.26 ± 0.02 efg	0.32 ± 0.08 e	0.27 ± 0.03 f	0.28 ± 0.03 ef
Cyclohexen	0.06 ± 0.11 efg	0.20 ± 0.02 e	0.08 ± 0.04 f	0.25 ± 0.09 ef
Limonene	0.06 ± 0.16 g	0.46 ± 0.31 e	0.03 ± 0.03 f	0.53 ± 0.42 ef
Octatriene	0.06 ± 0.03 g	0.08 ± 0.05 e	0.05 ± 0.02 f	0.00 ± 0.00 f
Phellandrene	0.45 ± 0.14 efg	0.34 ± 0.03 e	0.34 ± 0.04 f	0.39 ± 0.04 ef
Sabinene	0.13 ± 0.02 fg	0.08 ± 0.05 e	0.08 ± 0.04 f	0.20 ± 0.04 ef
Thymyl acetate	0.35 ± 0.06 efg	0.03 ± 0.03 e	0.04 ± 0.04 f	0.13 ± 0.08 ef

The values in each column with different letters show significant differences (Tukey's test, p<0.05).

3.2. Treatment by Cd Concentrations with 10 µM of Se

Treatments by Cd (0, 75, 100, and 150 µM) with 10 µM of Se were investigated on *S. hortensis* essential oil (Table 2). About 0 µM×10 µM (Cd× Se), carvacrol and camphene had the highest and lowest percentage in total, respectively ($F_{17, 36}$= 9.32). By treatment of 75 µM×10 µM (Cd×Se), carvacrol had the highest percentage and carene being the lowest ($F_{17, 36}$= 13.28). Moreover, about 100 µM×10 µM (Cd× Se), carvacrol and carene had the highest and lowest percentage in comparison to the others ($F_{17, 36}$= 5.21). Finally, by treatment of 150 µM×10 µM (Cd×Se), carvacrol, γ-terpinene, and benzene showed high percentages in the essential oil structure, respectively ($F_{17, 36}$= 17.18).

Table 2. The effects of different concentrations of Cd with 10 µM of Se on the structure of *S. hortensis* essential oil.

Compound	Cadmium × Selenium			
	0 µM × 10 µM	75 µM × 10 µM	100 µM × 10 µM	150 µM × 10 µM
α-Pinene	1.33 ± 0.12 ef	1.24 ± 0.02 d	1.31 ± 0.09 d	1.23 ± 0.02 d
α-Terpinene	1.08 ± 0.15 efg	4.63 ± 0.06 c	5.22 ± 0.17 c	5.11 ± 0.44 c
α-Thujene	1.43 ± 0.10 e	1.43 ± 0.05 d	1.52 ± 0.08 d	1.55 ± 0.04 d
ß-Bisabolene	4.44 ± 0.13 d	0.22 ± 0.07 d	0.50 ± 0.12d	0.29 ± 0.08 d
ß-Myrcene	6.09 ± 0.58 c	1.28 ± 0.05 d	1.54 ± 0.14 d	1.59 ± 0.03 d
ß-Pinene	19.82 ± 0.15 b	0.42 ± 0.02 d	0.48 ± 0.05 d	0.54 ± 0.07 d
γ-Terpinene	62.18 ± 0.49 a	20.57 ± 0.40 b	22.04 ± 0.23 b	23.43 ± 0.17 b
Benzene	0.44 ± 0.03 fg	4.87 ± 0.69 c	6.04 ± 1.03 c	5.62 ± 0.68 c
Camphene	0.05 ± 0.03 g	0.03 ± 0.01 d	0.09 ± 0.02 d	0.14 ± 0.00 d
Carvacrol	0.23 ± 0.12 g	62.97 ± 0.91 a	58.65 ± 0.90 a	58.15 ± 1.59 a
Carene	0.06 ± 0.03 g	0.10 ± 0.06 d	0.03 ± 0.03 d	0.08 ± 0.04 d
Caryophyllene	0.14 ± 0.06 g	0.32 ± 0.00 d	0.32 ± 0.00 d	0.30 ± 0.02 d
Cyclohexen	0.35 ± 0.02 g	0.20 ± 0.03 d	0.28 ± 0.06 d	0.20 ± 0.09 d
Limonene	0.17 ± 0.08 g	0.16 ± 0.08 d	0.33 ± 0.23 d	0.21 ± 0.12 d
Octatriene	0.22 ± 0.04 g	0.16 ± 0.01 d	0.13 ± 0.00 d	0.03 ± 0.03 d
Phellandrene	0.09 ± 0.06 g	0.34 ± 0.03 d	0.41 ± 0.01 d	0.40 ± 0.04 d
Sabinene	0.10 ± 0.10 g	0.16 ± 0.09 d	0.19 ± 0.02 d	0.18 ± 0.04 d
Thymyl acetate	0.54 ± 0.08 efg	0.12 ± 0.06 d	0.05 ± 0.05 d	0.10 ± 0.00 d

The values in each column with different letters show significant difference (Tukey's test, p<0.05).

3.3. Treatment by Cd Concentrations with 20 µM of Se

Treatments by Cd (0, 75, 100, and 150 µM) with 20 µM of Se were investigated on the constituents of *S. hortensis* essential oils (Table 3). About all essential oils, carvacrol was tha major constituent. On treatment of 0 µM×20 µM (Cd× Se), carvacrol and octatriene showed the highest and lowest percentages than the others ($F_{17, 36}$= 8.27). Moreover, the treatment of 75 µM×20 µM (Cd× Se), carvacrol, γ-terpinene, and α-terpinene showed high percentages in the essential oil structure ($F_{17, 36}$= 5.64).

Table 3. The effects of different Cd concentrations with 20 µM of Se on the structure of *S. hortensis* essential oil.

Compound	Cadmium × Selenium			
	0 µM × 20 µM	75 µM × 20 µM	100 µM × 20 µM	150 µM × 20 µM
α-Pinene	1.03 ± 0.15 d	1.34 ± 0.07 e	1.29 ± 0.01 e	1.40 ± 0.03 de
α-Terpinene	4.84 ± 0.19 c	4.51 ± 0.06 c	5.35 ± 0.37 c	4.96 ± 1.08 c
α-Thujene	1.44 ± 0.09 d	1.46 ± 0.08 e	1.55 ± 0.07 de	1.75 ± 0.07 de
ß-Bisabolene	0.38 ± 0.12 d	0.30 ± 0.03 e	0.49 ± 0.08 e	0.38 ± 0.07 e
ß-Myrcene	1.45 ± 0.13 d	1.35 ± 0.07 e	1.77 ± 0.03 de	1.84 ± 0.13 de
ß-Pinene	0.45 ± 0.03 d	0.47 ± 0.02 e	0.56 ± 0.00 e	0.67 ± 0.03 e
γ-Terpinene	20.87 ± 0.28 b	21.16 ± 0.74 b	22.51 ± 0.32 b	23.27 ± 0.10 b
Benzene	4.31 ± 0.70 c	3.41 ± 0.40 d	3.78 ± 0.81 cd	2.92 ± 0.40 d
Camphene	0.18 ± 0.07 d	0.14 ± 0.02 e	0.11 ± 0.01 e	0.14 ± 0.03 e
Carvacrol	62.59 ± 0.89 a	62.85 ± 0.62 a	59.59 ± 1.93 a	59.08 ± 1.44 a
Carene	0.18 ± 0.03 d	0.12 ± 0.02 e	0.10 ± 0.01 e	0.22 ± 0.10 e
Caryophyllene	0.33 ± 0.07 d	0.37 ± 0.02 e	0.38 ± 0.02 e	0.42 ± 0.06 e
Cyclohexen	0.38 ± 0.03 d	0.17± 0.04 e	0.18± 0.04 e	0.18± 0.0.0 e
Limonene	0.25 ± 0.15 d	0.70 ± 0.22 e	0.40 ± 0.23 e	0.81 ± 0.09 e
Octatriene	0.12 ± 0.00 d	0.21 ± 0.02 e	0.17 ± 0.00 e	0.24 ± 0.03 e
Phellandrene	0.41 ± 0.03 d	0.44 ± 0.06 e	0.42 ± 0.02 e	0.50 ± 0.06 e
Sabinene	0.19 ± 0.08 d	0.25± 0.08 e	0.28± 0.11 e	0.24± 0.05 e
Thymyl acetate	0.21 ± 0.12 d	0.22 ± 0.05 e	0.22 ± 0.01 e	0.29 ± 0.05 e

The values in each column with different letters show significant differences (Tukey's test, p<0.05).

By treatment of 100 µM×20 µM (Cd× Se), carvacrol and carene showed the highest and lowest percentage in comparison to the other constituents, respectively ($F_{17, 36}$= 7.87). Finally, by treatment of 150 µM×20 µM (Cd× Se), carvacrol had the highest when camphene showed the lowest percentage in the essential oil ($F_{17, 36}$= 6.73).

3.4. Treatment of Cd Concentrations with 40 µM of Se

Treatments of Cd (0, 75, 100, and 150 µM) with 40 µM of Se were investigated on the constituents of *S. hortensis* essential oil (Table 4). About all, carvacrol was the major constituent. By treatment of 0 µM×40 µM (Cd× Se), carvacrol showed the highest percentage when camphene being the lowest compared to the others ($F_{17, 36}$= 20.21). Moreover, under the treatment of 75 µM×40 µM (Cd× Se), carvacrol and carene showed the highest and lowest percentages ($F_{17, 36}$= 8.06). About 100 µM×40 µM (Cd× Se) treatment, carvacrol, γ-terpinene, and α-terpinene showed high percentages, respectively ($F_{17, 36}$= 8.15). Finally, by treatment of 150 µM×40 µM (Cd×Se), carvacrol had the highest percentage, while sabinene showed the lowest percentage compared to the others ($F_{17, 36}$= 10.52).

Table 4. The effects of different treatments of Cd with 40 µM of Se on composition of *S. hortensis* essential oil.

Compound	Cadmium × Selenium			
	0 µM × 40 µM	75 µM × 40 µM	100 µM × 40 µM	150 µM × 40 µM
α-Pinene	1.16 ± 0.11 ^{ef}	1.41 ± 0.07 ^{d}	1.42 ± 0.08 ^{efg}	1.35 ± 0.03 ^{ef}
α-Terpinene	4.54 ± 0.13 ^{c}	4.84 ± 0.06 ^{c}	5.57 ± 0.15 ^{c}	5.37 ± 0.27 ^{c}
α-Thujene	1.51 ± 0.11 ^{e}	1.52 ± 0.08 ^{d}	2.04 ± 0.34 ^{def}	1.76 ± 0.07 ^{ef}
ß-Bisabolene	0.32 ± 0.03 ^{g}	0.32 ± 0.02 ^{d}	0.41 ± 0.10 ^{gh}	0.37 ± 0.02 ^{ef}
ß-Myrcene	1.48 ± 0.11 ^{e}	1.52 ± 0.13 ^{d}	2.11 ± 0.11 ^{de}	1.91 ± 0.04 ^{e}
ß-Pinene	0.53 ± 0.01 ^{fg}	0.56 ± 0.02 ^{d}	0.72 ± 0.03 ^{gh}	0.66 ± 0.05 ^{ef}
γ-Terpinene	20.50 ± 0.41 ^{b}	21.40 ± 1.42 ^{b}	22.64 ± 0.26 ^{b}	23.24 ± 0.53 ^{b}
Benzene	3.65 ± 0.26 ^{d}	3.53 ± 0.34 ^{c}	2.96 ± 0.63 ^{d}	3.35 ± 0.12 ^{d}
Camphene	0.18 ± 0.04 ^{g}	0.17 ± 0.04 ^{d}	0.22 ± 0.06 ^{h}	0.12 ± 0.08 ^{f}
Carvacrol	63.27 ± 0.26 ^{a}	61.11 ± 1.08 ^{a}	58.65 ± 0.42 ^{a}	59.38 ± 1.19 ^{a}
Carene	0.25 ± 0.08 ^{g}	0.18 ± 0.00 ^{d}	0.17 ± 0.02 ^{g}	0.25 ± 0.05 ^{f}
Caryophyllene	0.43 ± 0.03 ^{fg}	0.40 ± 0.02 ^{d}	0.39 ± 0.01 ^{gh}	0.39 ± 0.03 ^{ef}
Cyclohexen	0.23± 0.08 ^{g}	0.28± 0.01 ^{d}	0.21± 0.10 ^{h}	0.18± 0.06 ^{f}
Limonene	0.57 ± 0.31 ^{fg}	0.71 ± 0.20 ^{d}	1.01 ± 0.39 ^{gh}	0.32 ± 0.17 ^{f}
Octatriene	0.19 ± 0.03 ^{g}	0.22 ± 0.02 ^{d}	0.16 ± 0.02 ^{g}	0.17 ± 0.02 ^{f}
Phellandrene	0.44 ± 0.01 ^{fg}	0.52 ± 0.09 ^{d}	0.57 ± 0.07 ^{gh}	0.46 ± 0.05 ^{ef}
Sabinene	0.19± 0.08 ^{g}	0.38± 0.14 ^{d}	0.38± 0.14 ^{h}	0.11± 0.03 ^{f}
Thymyl acetate	0.30± 0.07 ^{g}	0.20 ± 0.01 ^{d}	0.19 ± 0.09 ^{h}	0.25 ± 0.04 ^{f}

The values in each column with different letters show significant differences (Tukey's test, p<0.05).

4. DISCUSSION and CONCLUSION

Very limited studies have been carried out on the effects of Se and the other heavy metals on the secondary metabolites of *S. hortensis*. Mumivand *et al*., (2011) studied the changes in *S. hortensis* essential oil under calcium carbonate and nitrogen treatments and found their effect on the difference in essential oil composition as significant. The GC-MS results showed that *S. hortensis* constituents changed by relative percentage of carvacrol, γ-terpinene, and β-bisabolene. The above results were different from ours despite different treatment, because we observed certain changes in the secondary metabolites of this medicinal plant. Karimi *et al*., (2013) studied the effects of Cd on *S. hortensis* and reported that arsenic, Cd, and mercury were observed in artichoke and savory root in comparison to aerial parts. Futhermore, artichoke showed higher heavy metals absorption as bioaccumulation factor and transfer efficiency from root to stem than savory plant. Accordingly, artichoke showed higher accumulation capacity than savory. Based on this, extraction of metals by artichoke can be applied to clean the soils from heavy metals pollution. Heavy metals have different effects on secondary metabolites and this subject has been proven in different studies. This issue should be considered by researchers in soils contaminated with these metals.

Ashraf *et al*., (2018) reported that plants are exposed to abiotic stresses such as fertilizers, soil type and its composition, high temperatures, light intensity, lack of access to water, and salinity that can affect their life. Plants need specific amounts of abiotic components while lack or excess of them causes changes in biosynthesis of secondary metabolites and determine plants growth and development. Also, concentration of them in plants increases in response to environmental stresses such as temperature, lack of nutrients, wounds, and UV rays. Plants also show changes in concentration of phenols due to the lack of effective substances in their nutrition. In the present study, significant changes in the secondary metabolites were observed by application of Se and Cd treatments, which is in line with the results of our study. The effect of organic fertilizers on the essential oil of *S. hortensis* was studied by Esmailpour *et al*., (2018) while the highest and lowest essential oil content were obtained in plants treated with vermicompost 30% and unwashed mushroom compost, respectively. Also, the main compounds were determined as carvacrol and γ-terpinene. The highest level of them was observed in plants grown on 40% and 20% substrates including washed spent mushroom, respectively. In our study, the role of organic fertilizers was not investigated, but this is a new aspect that should be considered in future. Despite the application of different treatments, the main constituents of the essential oil from this medicinal plant has not changed, which confirms our results.

Azizollahi *et al*., (2019) studied Cd accumulation in *S. hortensis* and reported that its main constituent was carvacrol, which showed suitable values under treatment by this heavy metal. *S. hortensis* can also be considered as an invaluable alternative crop for contaminated soils by Cd. Besides, due to suitable potential of Cd accumulation in the root, it can be a suitable tool for phytostabilization purposes. Finally, Memari-Tabrizi *et al*., (2021) investigated foliar spraying of silicon nanoparticles in reducing cadmium stress on *S. hortensis* essential oil and reported the dominant compounds were carvacrol, γ-terpinene, p-cymene, and thymol. Their results are somewhat consistent with ours. The type of treatment was different, which affected the results to some extent.

About the other plants, Manquián-Cerda *et al*., (2016) studied the effect of Cd in concentrations of 50 and 100 μM on antioxidant potential and phenolic compounds accumulation in *Vaccinium corymbosum* L. (Ericaceae) in the form of DPPH activity in which free-radical of DPPH interacts with an odd electron to yield a strong absorbance at 517 nm. Furthermore, the production of phenolic compounds was significantly affected under Cd stress. The highest amount of chlorogenic acid was produced in blueberry plants at higher levels of Cd. It has been suggested that antioxidant activity of the plant had a positive correlation with phenolic compounds. The results of their research are consistent with ours regarding the effect of Cd stress on the amount of phenolic compounds. In another study, Handa *et al*., (2019) evaluated the effect of Se on biochemical factors and secondary metabolites in *Brassica juncea* L. (Brassicaceae) under chromium stress conditions. The co-application of Se and Cr led to increasing of total phenolic, flavonoid, and anthocyanin content in plants grown inside Cr-amended soils. According to their findings, Se has a similar effect on secondary metabolites under heavy metal stress as we have found this in our study as well.

In another study conducted by Elguera *et al*., (2013), the effect of Se and Cd on the phenolic compounds of *Lepidium sativum* L. (Brassicaceae) was investigated when quantity of phenolic compounds increased under the treatment of these two elements. Azizi *et al*., (2021) investigated the effects of foliar application by Se on morphological and physiological indices of *S. hortensis* under Cd stress and found that Se spray reduced the toxic effects of Cd stress on it by increasing proline, stimulating enzymes, and limiting leakage in cell membrane. Also, Se foliar application under Cd stress conditions improved chlorophyll and reduced Cd accumulation in the root. Overall, their study showed that Se foliar application can reduce Cd toxicity and improve growth under different levels of Cd and another heavy metals. Although, changes in secondary metabolites were not investigated in their study, the role of Se has been effective in reducing negative effects of Cd and it was consistent with the present research. Karimi *et al*., (2022) investigated Se- and Silicon-mediated recovery of *Satureja mutica* Fisch. chemotypes subjected to drought stress followed by rewatering and concluded that no separation was observed in savory chemotypes in response to foliar Se and silicon applications. However, plants treated with Se showed a decrease in proline accumulation under drought stress conditions. Based on their results, *S. mutica* was a valuable medicinal plant resistant to drought especially in areas with low rainfall and can be introduced into the agricultural systems. In our studies, we observed certain changes in the essential oil of this medicinal plant under Se and Cd treatments. However, plant species was different in two studies and this important issue is very effective in changing the results.

In conclusion, this research indicated that treatment by Se and Cd made obvious effects on *S. hortensis* essential oil although major constituents were not changed due to their high differences with the others. These studied recommend improving the biological position of *S. hortensis* plants under Cd stress and Se as its anti-stress agent. For this, the authors of this article encourage other researchers to examine the essential oils structure from the other medicinal plants under treatment of various agents. They hope that it will be possible to determine the conditions in which the highest number of useful compounds with the highest percentage can be produced.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Iraj Azizi: Investigation, Methodology, Supervision, and Validation. **Mohammad Asadi**: Resources, Visualization, Software, Formal Analysis, and Writing Original draft.

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