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Research Article

Effects of nitric oxide on composition of the isolated essential oil from *Satureja hortensis* L. (Lamiaceae), under the cadmium stress

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Abstract: Cadmium (Cd) is one of the heavy metals that cause environmental pollution and biochemical changes in plants grown in contaminated soils. In plants, sodium nitroprusside is used as a nitric oxide (NO) release agent. In this research, a glasshouse pot experiment was conducted to examine the effect of exogenous NO on the essential oil composition of the savory plant, *Satureja hortensis*, under the Cd stress. For this, the plants were treated by different levels of Cd concentration including 0 (control), 75, 100, and 150 µM in the contaminated soil. Plants were also foliar sprayed with concentrations of 0 (control), 50, 100, and 200 µM NO. The results indicated that carvacrol was the main compound in all examined essential oils. Also, there were significant differences among the essential compounds under treatments of Cd and NO. Moreover, the differences among minor constituents were not significant in most of treatments. In apposite, carvacrol (approximately 60% of total volume) showed a significant difference than the others. The results indicated the role of exogenous agents on the changes of essential oil constituents in *S. hortensis*.

1. INTRODUCTION

One of the major environmental concerns is the contamination of soils by heavy metals, which have severe negative effects on production and safety of plants. Cd is one of the common pollutants which have toxic effects on all living organisms (Benavides *et al.*, 2005). Cd interferes with functionally active ions on enzyme sites when inhibits many key enzymes involved in various metabolic pathways including secondary metabolism (Andresen & Küpper, 2013). In the presence of polyphenolic compounds, *Satureja hortensis* L. essential oil has antioxidant, antimicrobial, antiparasitic, pesticidal, anti-inflammatory, antinociceptive, hepatoprotective, and anticancer effects (Fierascu *et al.*, 2018). Plant secondary metabolism can seriously affect the quality and efficacy of valuable natural products such as essential oils derived from medicinal plants (Azizollahi *et al.*, 2019). Cd toxicity induces production of reactive oxygen species (ROS) at the cellular level and impairs redox homeostasis (Asopa *et al.*, 2017). During normal plant growth, ROS molecules are produced and detoxified in balance due to metabolic processes. Abiotic stresses such as heavy metal toxicity disturb the balance

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between ROS production and removal which causes membrane peroxidation, enzyme inhibition, as well as DNA, RNA, and protein damage (Anjitha *et al*., 2021). In response to stressful metal concentrations, plants employ various defense strategies to detoxify ROS (Haider *et al*., 2021). Plants can accumulate secondary metabolites when metal-induced biosynthesis pathways are activated. Plants synthesize them when they undergo physiological changes that require primary metabolism (Bali *et al*., 2020). Although plants are sessile organisms and have no immune system, they have adopted various defense strategies including a wide range of secondary metabolites to overcome environmental stresses (Anjitha *et al*., 2021). In plants, the secondary metabolites fall into three categories based on their biosynthesis pathways. These include terpenes, phenolic compounds, and nitrogen-containing compounds (Ashraf *et al*., 2018).

NO (Nitric oxide) is a gaseous molecule. It had variable effects on different organisms' strata and animals' neurotransmitters. NO also has significant importance in plant signaling which regulates the growth of plants and development processes such as germination, formation of roots, movement of stomata, maturation, and plant defense (Azizi *et al*., 2021; Kumar & Ohri, 2023). Essential oils are natural products with economic potential that are formed mostly of terpenes (Asadi *et al*., 2018, 2021). These compounds play a key role in human activities including medical treatment and industrial manufacturing. There are extensive studies on essential oils as natural products; but the effects of environment on their production and composition are poorly understood (Ribeiro *et al*., 2019).

The summer savory, *Satureja hortensis,* (Figure 1) is one of more than 30 species of plants in the family Lamiaceae that grow in the Eastern Mediterranean (Şahin *et al*., 2003). It is an old plant that has been used for vegetable, medicinal, and aromatic purposes (Mohtashami *et al*., 2018). Essential oil of summer savory is used in the food (conservation and beverage) and pharmaceutical industries (Mihajilov-Krstev *et al*., 2009). It is applied in Türkiye for curing high blood pressure and as an antispasmodic agent against upper respiratory tract problems and inflammations of reproductive system (Selvi *et al*., 2022). Its essential oil contains higher carvacrol and thymol contents that display strong antimicrobial impacts (Mohtashami *et al*., 2021; Azizi & Asadi, 2024). Carvacrol has many biological effects: it is antiseptic, anti-inflammatory, deworming, antioxidant, analgesic, antifungal, and antibacterial as well as yeast inhibitor (Fierascu *et al*., 2018).

Figure 1. Above-ground parts of *S. hortensis.*

The other compounds also have similar importance. Changes in the secondary metabolites of medicinal plants treated with chemical compounds are very important. Accordingly, the present research was designed. We have investigated the constituents of *S. hortensis* under Cd and NO treatments, which are important from different aspects.

2. MATERIAL and METHODS

2.1. Plant Material and Experimental Designs

A factorial plot experiment was conducted in the form of a completely randomized design (CRD) with three replications in the greenhouse of the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran. The main aim was to investigate the response of NO foliar spraying on the content of essential oil in *S. hortensis* under Cd stress. $CdCl_2$ and sodium nitroprusside (NO) were obtained from Merck (Darmstadt, Germany). Soil pollution with different concentrations of $CdCl_2$ solution (0, 75, 100, and 150 mM) is considered as Cd stress under field capacity moisture. The soils were exposed to wet/dry cycles for 4 months to prove close to natural conditions and long-term contamination.

Savory seeds were purchased from Tehran Agricultural Research Station and sterilized with sodium hypochlorite; then, rinsed with the deionized water. The seeds were planted in the contaminated soil. After emergence of the seedling stage, foliar spraying of NO solution was done in four concentrations (0, 50, 100, and 200 µM), under three replications. Spraying was performed in three stages (final amount of 200 ccs for each pot) and every two weeks. The first spraying was done after appearance of two true leaves in the seedling stage (Azizi *et al*., 2021).

2.2. Essential Oil Isolation

The aerial parts of the savory plant, which contained the most essential oil, were separated from each sample and dried at room temperature (about 25°C) under shade. Then, were powdered by an electric mill and 50 grams of each sample were added to 500 ml of the distilled water. Their essential oils were isolated by a Clevenger apparatus at the temperature of 100 °C. The water of essential oils was deleted by Na_2So_4 and the pure essential oils stored at 4 °C in sealed brown vials until the chemical analysis (Asadi *et al*., 2018, 2019).

2.3. Chemical Analysis of The Isolated Essential Oils

To determine the quantitative and qualitative components of the essential oils, we used the Agilent Technologies 7890B (manufactured in the USA) gas chromatography with an HP-5MS column, length 30 m, diameter 0.25 mm, and film thickness 0.25 mm. Temperature program held at 350 °C. The ranges were determined by using mass data, Kovats index, and retention time. Each essential oil constituent was detected based on patterns of range refraction compared to two standard libraries (Adams, 2001).

2.4. Statistical Analysis

In the present research, three replicates were considered for all treatments. Then, the data were examined for normality status. Finally, the normal data were analyzed by one-way ANOVA and their means were compared by Tukeys' test at probability level $p<0.05$ by using SPSS (version 22) software.

3. RESULTS

3.1. Cd Treatments with 0 µM of NO

The effects of Cd concentrations (0, 75, 100, and 150 µM) with 0 µM of NO on *S. hortensis* essential oil are shown in Table 1. In all of them, carvacrol was the main constituent. Under 0 µM×0 µM (Cd×NO) treatment, the differences among most of the compounds were not significant ($F_{17, 36}= 8.93$). About 75 µM×0 µM (Cd×NO) treatment, carene and limonene were not available in the essential oil structure ($F_{17, 36}= 14.55$). About 100 µM×0 µM (Cd×NO) treatment, carene showed the lowest percentage, and limonene with thymyl acetate were not available ($F_{17, 36}= 17.06$). Finally, under 150 µM×0 µM (Cd×NO) treatment, majority of differences were significant when carene had the lowest percentage in the essential oil ($F_{17, 36}= 27.93$).

Table 1. Effects of different Cd concentrations with 0 µM of NO on *S. hortensis* essential oil.

Compound	Cadmium × Nitric Oxide			
	0 µM × 0 µM	75 µM × 0 µM	100 µM × 0 µM	150 µM × 0 µM
α-Pinene	1.02 ± 0.11 ^{e}	1.06 ± 0.12 ^{efg}	1.36 ± 0.05 ^{ef}	1.27 ± 0.04 ^{de}
α-Terpinene	4.51 ± 0.10 ^{d}	4.45 ± 0.11 ^{d}	5.19 ± 0.15 ^{d}	5.57 ± 0.07 ^{d}
α-Thujene	1.40 ± 0.15 ^{e}	1.26 ± 0.08 ^{e}	1.67 ± 0.23 ^{e}	1.46 ± 0.03 ^{e}
ß-Bisabolene	0.11 ± 0.11 ^{e}	0.23 ± 0.11 ^{gh}	0.31 ± 0.19 ^{f}	0.30 ± 0.05 ^{g}
ß-Myrcene	1.42 ± 0.06 ^{e}	1.20 ± 0.06 ^{ef}	1.72 ± 0.14 ^{e}	1.31 ± 0.14 ^{de}
ß-Pinene	0.40 ± 0.03 ^{e}	0.41 ± 0.03 ^{fgh}	0.59 ± 0.02 ^{ef}	0.43 ± 0.07 ^{efg}
γ-Terpinene	20.22 ± 0.75 ^{b}	19.20 ± 0.23 ^{b}	22.28 ± 0.37 ^{b}	23.42 ± 0.29 ^{b}
Benzene	7.53 ± 0.46 ^{c}	6.07 ± 0.58 ^{c}	7.38 ± 0.83 ^{c}	8.37 ± 0.32 ^{c}
Camphene	0.22 ± 0.07 ^{e}	0.04 ± 0.02 ^{h}	0.07 ± 0.03 ^{f}	0.07 ± 0.02 ^{g}
Carvacrol	59.34 ± 0.57 ^{a}	63.92 ± 0.36 ^{a}	57.02 ± 0.49 ^{a}	55.04 ± 0.65 ^{a}
Carene	0.06 ± 0.06 ^{e}	0.00 ± 0.00 ^{h}	0.03 ± 0.03 ^{f}	0.03 ± 0.03 ^{g}
Caryophyllene	0.39 ± 0.03 ^{e}	0.23 ± 0.11 ^{gh}	0.30 ± 0.07 ^{f}	0.14 ± 0.07 ^{g}
Cyclohexen	0.89 ± 0.39 ^{e}	0.14 ± 0.03 ^{h}	0.04 ± 0.04 ^{f}	0.22 ± 0.11 ^{g}
Limonene	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{h}	0.00 ± 0.00 ^{f}	0.56 ± 0.40 ^{efg}
Octatriene	0.03 ± 0.03 ^{e}	0.06 ± 0.03 ^{h}	0.08 ± 0.04 ^{f}	0.09 ± 0.04 ^{g}
Phellandrene	0.52 ± 0.12 ^{e}	0.36 ± 0.02 ^{fgh}	0.39 ± 0.02 ^{f}	0.43 ± 0.02 ^{efg}
Sabinene	0.09 ± 0.05 ^{e}	0.11 ± 0.05 ^{h}	0.08 ± 0.04 ^{f}	0.17 ± 0.08 ^{g}
Thymyl acetate	0.42 ± 0.21 ^{e}	0.03 ± 0.03 ^{h}	0.00 ± 0.00 ^{f}	0.13 ± 0.08 ^{g}

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

3.2. Cd Treatments with 50 µM of NO

Cd treatments (0, 75, 100, and 150 µM) with 50 µM of NO on the essential oil of *S. hortensis* were investigated (Table 2). Among all of them, carvacrol was the main constituent. Under 0 µM×50 µM (Cd×NO), carvacrol and limonene had the highest and lowest percentages on total (F $_{17, 36}$= 22.58). Under 75 µM×50 µM (Cd×NO) treatment, carvacrol had the highest percentage while camphene and carene showed the lowest percentages (F $_{17, 36}$= 23.58). Moreover, about 100 µM×50 µM (Cd×NO) treatment, most of the differences among compounds were significant (F $_{17, 36}$= 4.12). Finally, by treatment of 150 µM×50 µM (Cd×NO), carvacrol and γ-terpinene showed higher percentages compared to the others, respectively (F $_{17, 36}$= 18.79).

Table 2. Effects of different Cd concentrations with 50 µM of NO on *S. hortensis* essential oil.

Compound	Cadmium × Nitric Oxide			
	0 µM × 50 µM	75 µM × 50 µM	100 µM × 50 µM	150 µM × 50 µM
α-Pinene	0.85 ± 0.16 ^{df}	1.30 ± 0.03 ^{d}	1.20 ± 0.14 ^{def}	1.23 ± 0.04 ^{e}
α-Terpinene	4.42 ± 0.10 ^{c}	4.67 ± 0.09 ^{c}	5.23 ± 0.23 ^{d}	5.59 ± 0.32 ^{c}
α-Thujene	1.29 ± 0.11 ^{d}	1.60 ± 0.10 ^{d}	1.88 ± 0.19 ^{e}	1.66 ± 0.04 ^{de}
ß-Bisabolene	0.24 ± 0.08 ^{e}	0.20 ± 0.08 ^{d}	0.19 ± 0.11 ^{g}	0.30 ± 0.08 ^{fg}
ß-Myrcene	1.36 ± 0.01 ^{d}	1.27 ± 0.06 ^{d}	1.79 ± 0.34 ^{de}	1.75 ± 0.02 ^{d}
ß-Pinene	0.43 ± 0.02 ^{df}	0.40 ± 0.05 ^{d}	0.64 ± 0.06 ^{fg}	0.58 ± 0.05 ^{f}
γ-Terpinene	0.43 ± 0.03 ^{b}	20.51 ± 0.65 ^{b}	25.17 ± 0.22 ^{b}	23.21 ± 0.18 ^{b}
Benzene	4.89 ± 0.15 ^{c}	4.58 ± 0.78 ^{c}	6.50 ± 0.75 ^{c}	5.64 ± 0.15 ^{c}
Camphene	0.10 ± 0.01 ^{e}	0.03 ± 0.01^{d}	0.07 ± 0.05 ^{g}	0.12 ± 0.02 ^{fg}
Carvacrol	62.52 ± 0.35 ^{a}	63.64 ± 0.83 ^{a}	56.73 ± 0.41 ^{a}	57.02 ± 0.14 ^{a}
Carene	0.02 ± 0.02 ^{e}	0.03 ± 0.03 ^{d}	0.03 ± 0.03 ^{g}	0.05 ± 0.05 ^{fg}
Caryophyllene	0.12 ± 0.13 ^{e}	0.37 ± 0.03 ^{d}	0.42 ± 0.08 ^{g}	0.25 ± 0.06 ^{fg}
Cyclohexen	1.26 ± 0.14 ^{d}	0.19 ± 0.01 ^{d}	0.63 ± 0.24 ^{fg}	0.21 ± 0.07 ^{fg}
Limonene	0.00 ± 0.00 ^{e}	0.07 ± 0.07 ^{d}	0.37 ± 0.22 ^{g}	0.18 ± 0.12 ^{fg}
Octatriene	0.03 ± 0.03 ^{e}	0.13 ± 0.06 ^{d}	0.14 ± 0.08 ^{g}	0.00 ± 0.00 g
Phellandrene	0.43 ± 0.06 ^{e}	0.36 ± 0.04 ^{d}	0.44 ± 0.06 ^{g}	0.43 ± 0.04 ^{fg}
Sabinene	0.09 ± 0.06 ^{e}	0.20 ± 0.10 ^{d}	0.40 ± 0.27 ^{g}	0.16 ± 0.02 ^{fg}
Thymyl acetate	0.11 ± 0.11 ^{df}	0.07 ± 0.07 ^{d}	0.05 ± 0.05 ^{g}	0.03 ± 0.03 ^{fg}

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

3.3. Cd Treatments with 100 µM of NO

The effects of Cd concentrations (0, 75, 100, and 150 µM) with 100 µM of NO on *S. hortensis* essential oil are shown in Table 3. Among all of the investigated essential oils, carvacrol was a major constituent. About the treatment of 0 µM×100 µM (Cd×NO), carvacrol and octatriene showed the highest and lowest percentages compared to the others (F $_{17, 36}$= 5.47). Moreover, under 75 µM×100 µM (Cd×NO) treatment, most of differences among constituents were not significant (F $_{17, 36}$= 5.95). On 100 µM×100 µM (Cd×NO) treatment, carvacrol and γ-terpinene showed higher percentages than the others (F $_{17, 36}$= 5.12). Finally, by treatment of 150 µM×100 µM (Cd×NO), carvacrol had the highest while octatriene showed the lowest percentages compared to the other compounds (F $_{17, 36}$= 10.22).

Table 3. Effects of different Cd concentrations with 100 µM of NO on *S. hortensis* essential oil.

Compound	Cadmium × Nitric Oxide			
	0 µM × 100 µM	75 µM × 100 µM	100 µM × 100 µM	150 µM × 100 µM
α-Pinene	0.79 ± 0.06 ^{e}	1.32 ± 0.12 ^{e}	1.27 ± 0.13 ^{ef}	0.90 ± 0.11 ^{fg}
α-Terpinene	4.60 ± 0.11 ^{c}	4.43 ± 0.06 ^{c}	5.84 ± 0.23 ^{c}	6.53 ± 0.14 ^{c}
α-Thujene	1.43 ± 0.14 ^{e}	1.35 ± 0.09 ^{e}	1.37 ± 0.08 ^{ef}	1.74 ± 0.09 ^{e}
ß-Bisabolene	0.20 ± 0.10 ^{e}	0.38 ± 0.07 ^{e}	0.56 ± 0.24 ^{e}	0.35 ± 0.05 ^{fg}
ß-Myrcene	1.31 ± 0.14 ^{e}	1.34 ± 0.06 ^{e}	2.29 ± 0.22 ^{e}	1.85 ± 0.19 ^{e}
ß-Pinene	0.52 ± 0.03 ^{e}	0.47 ± 0.05 ^{e}	0.46 ± 0.03 ^{e}	0.72 ± 0.02 ^{fg}
γ-Terpinene	21.42 ± 0.51 ^{b}	21.50 ± 0.73 ^{b}	22.49 ± 0.44 ^{b}	24.08 ± 0.12 ^{b}
Benzene	3.30 ± 0.32 ^{d}	3.27 ± 0.31 ^{d}	4.12 ± 0.45 ^{d}	2.85 ± 0.29 ^{d}
Camphene	0.05 ± 0.03 ^{e}	0.10 ± 0.01 ^{e}	0.12 ± 0.00 ^{e}	0.18 ± 0.06 ^{fg}
Carvacrol	63.94 ± 0.77 ^{a}	62.69 ± 0.83 ^{a}	58.58 ± 0.93 ^{a}	57.28 ± 0.28 a
Carene	0.27 ± 0.04 ^{e}	0.07 ± 0.04 ^{e}	0.10 ± 0.05 ^{e}	0.18 ± 0.12 ^{fg}
Caryophyllene	0.15 ± 0.07 ^{e}	0.31 ± 0.08 ^{e}	0.37 ± 0.00 ^{e}	0.31 ± 0.02 ^{fg}
Cyclohexen	0.26 ± 0.13 ^{e}	0.15 ± 0.05 ^{e}	0.05 ± 0.05 ^{e}	0.15 ± 0.00 ^{fg}
Limonene	0.34 ± 0.20 ^{e}	0.83 ± 0.16 ^{e}	0.66 ± 0.35 ^{e}	0.93 ± 0.46 ^{f}
Octatriene	0.07 ± 0.04 ^{e}	0.16 ± 0.04 ^{e}	0.10 ± 0.05 ^{e}	0.04 ± 0.04 ^{g}
Phellandrene	0.46 ± 0.05 ^{e}	0.50 ± 0.09 ^{e}	0.43 ± 0.03 ^{e}	0.45 ± 0.06 ^{fg}
Sabinene	0.21 ± 0.07 ^{e}	0.29 ± 0.08 ^{e}	0.31 ± 0.13 ^{e}	0.26 ± 0.04 ^{fg}
Thymyl acetate	0.20 ± 0.12 ^{e}	0.00 ± 0.00 ^{e}	0.15 ± 0.10 ^{e}	0.24 ± 0.12 ^{fg}

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

3.4. Cd Treatments with 200 µM of NO

Cd treatments (0, 75, 100, and 150 µM) with 200 µM of NO on the essential oil of *S. hortensis* were investigated (Table 4). Among all of them, carvacrol was the main constituent. On 0 µM×200 µM (Cd×NO) treatment, carvacrol and carene showed the highest and lowest percentages (F $_{17, 36}$= 16.74). Under treatment of 75 µM×200 µM (Cd×NO), both compounds (carvacrol and carene) showed similar positions (F $_{17, 36}$= 6.06). Furthermore, on the treatment of 100 µM×200 µM (Cd×NO), differences among most of compounds were not significant (F $_{17, 36}$= 16.44). Finally, in the treatment of 150 µM×200 µM (Cd×NO), carvacrol had the highest while limonene showed the lowest percentage than the others (F $_{17, 36}$= 10.40).

Table 4. Effects of different Cd concentrations with 200 µM of NO on *S. hortensis* essential oil.

Compound	Cadmium × Nitric Oxide			
	0 µM × 200 µM	75 µM × 200 µM	100 µM × 200 µM	150 µM × 200 µM
α-Pinene	0.86 ± 0.05 ^{ef}	1.44 ± 0.72 ^{d}	1.46 ± 0.06 ^{ef}	1.25 ± 0.11 ^{ef}
α-Terpinene	4.49± 0.10 ^{c}	5.23 ± 0.27 ^{c}	5.48 ± 0.29 ^{c}	5.99 ± 0.20 ^{c}
α-Thujene	1.53 ± 0.27 ^{fg}	1.57 ± 0.07 ^{d}	2.11 ± 0.31 ^{de}	1.72 ± 0.07 ^{de}
ß-Bisabolene	0.35 ± 0.04 ^{fg}	0.47 ± 0.08 ^{d}	0.47 ± 0.12 ^{fg}	0.35 ± 0.12 ^{g}
ß-Myrcene	1.29 ± 0.08 ^{de}	1.52 ± 0.22 ^{d}	2.68 ± 0.32 ^{g}	1.96 ± 0.06 ^{e}
ß-Pinene	0.46 ± 0.10 ^{fg}	0.45 ± 0.08 ^{d}	0.71 ± 0.03 ^{fg}	0.78 ± 0.08 ^{fg}
γ-Terpinene	19.32 ± 0.15 ^{b}	22.03 ± 0.53 ^{b}	22.97 ± 0.51 ^{b}	24.41 ± 0.44 ^{b}
Benzene	2.92 ± 0.36 ^{d}	4.60 ± 0.89 ^{c}	4.00 ± 0.15 ^{d}	4.26 ± 0.24 ^{d}
Camphene	0.11 ± 0.02 ^{g}	0.10 ± 0.02 ^{d}	0.16 ± 0.01 ^{g}	0.16 ± 0.05 ^{g}
Carvacrol	65.96 ± 0.08 ^{a}	59.32 ± 0.42 ^{a}	55.31 ± 0.36 ^{a}	57.20 ± 0.26 ^{a}
Carene	0.09 ± 0.09 ^{g}	0.06 ± 0.06 ^{d}	0.22 ± 0.06 ^{g}	0.08 ± 0.08 ^{g}
Caryophyllene	0.32 ± 0.02 ^{fg}	0.32 ± 0.05 ^{d}	0.39 ± 0.02 ^{fg}	0.34 ± 0.04 ^{g}
Cyclohexen	0.17 ± 0.04 ^{fg}	0.17 ± 0.02 ^{d}	0.21 ± 0.12 ^{g}	0.19 ± 0.10 ^{g}
Limonene	0.71 ± 0.20 ^{fgh}	0.26 ± 0.26 ^{d}	1.12 ± 0.33 ^{fg}	0.00 ± 0.00 ^{g}
Octatriene	0.10 ± 0.01 ^{g}	0.17 ± 0.04 ^{d}	0.19 ± 0.04 ^{g}	0.07 ± 0.03 ^{g}
Phellandrene	0.44 ± 0.07 ^{fg}	0.60 ± 0.10 ^{d}	0.59 ± 0.05 ^{fg}	0.50 ± 0.01 ^{g}
Sabinene	0.21 ± 0.07 ^{fg}	0.40 ± 0.15 ^{d}	0.44 ± 0.16 ^{fg}	0.09 ± 0.04 ^{g}
Thymyl acetate	0.19 ± 0.10 ^{fg}	0.14 ± 0.07 ^{d}	0.10 ± 0.10 ^{g}	0.19 ± 0.09 ^{g}

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

4. DISCUSSION

Regarding the effects of NO and heavy metals on secondary metabolites, limited studies have been conducted. NO in plants is absorbed endogenously, by the surrounding atmosphere and soil. The quality of this gaseous composition differs depending on fluctuations in plant and environment. Proper plant growth, vegetative development, and reproduction require hormonal activity with an antioxidant network, as well as maintaining the concentration of active oxygen and nitrogen species on certain ranges. Plants often face abiotic stress conditions such as nutrient deficiency, salinity, drought, high UV radiation, extreme temperatures, and heavy metal stress which can affect growth processes and lead to their growth limitation. The ability of plants to respond and survive under environmental stress involves sensing and signaling events which NO becomes a critical component when mediates hormonal actions, interacts with reactive oxygen species, modulates gene expression, and activity of proteins (Simontacchi *et al*., 2015).

Tripathi *et al*., (2017) evaluated the effect of NO (100 µM) on the stress of nano ZnO in wheat plants. In their study, NO declined accumulation of zinc in vascular tissues and led to a decrease in oxidative stress in wheat plants. Also, the supply of NO led to regulation of antioxidants (ascorbate-glutathione) and non-antioxidant enzymes (ascorbate and glutathione). In this way, the stress of nano ZnO in wheat plants was reduced. Akladious and Mohamed (2017) studied the effects of NO (20 µM) on the mitigation of Zn toxicity in sunflower plants. Their study showed that treatment of NO increased the content of ascorbic acid glutathione and antioxidant enzymes. NO supply increased sunflower oil quality due to the enhancement of unsaturated fatty acid contents. Prieto *et al*., (2007) studied the essential oil of *Origanum vulgare* L. and *Satureja montana* L. under peroxynitrite and concluded that there was a significant difference with decreasing 3-nitrotyrosine. Also, production of compounds was inhibited by peroxynitrite induced with malondialdehyde. Furthermore, thymol and carvacrol inhibited the formation of nitrotyrosine and reduced malondialdehyde. Additionally, p-cymene and γ-terpinene were inactive in both assessments. Their results indicated that thymol and carvacrol had main roles in the prevention of toxic product formation in *O. vulgare*. The type of treatment applied and plant species in the present study were different from our study. These parameters are among the main factors affecting plant secondary metabolites, which should be given special attention.

The effects of salt stress on the biochemical parameters of *S. hortensis* were evaluated by Najafi and Khavari-Nejad (2010). They found that the main constituents were carvacrol and γ-terpinene in the control plants. Under NaCl treatment, with concentration increasing, carvacrol increased when γ-terpinene reduced. In all treated plants by NaCl, growth parameters were negatively reduced. Finally, with salinity increasing, carvacrol increased which is useful in medicinal applications. Despite differences in the type of treatment applied, the dominant composition in the species (carvacrol) was the same, this constituent has a lot of differences with the others, and its dominance did not lost with the applied changes. Vafa *et al*., (2015) studied the effects of Nano Zinc and Humic acid on *S. hortensis* and found that the highest essential oil content was gained under N_4. Also, its minimum content was observed under treatment by N_4H_4. Said-Al Ah *et al*., (2016) evaluated the effect of N and P on the composition of essential oil from *S. montana* when found that carvacrol was major. The other important compounds were p-cymene, γ-terpinene, linalool, thymol, and β-Caryophyllene. The highest percentage of carvacrol was recorded from plants harvested at 2nd cut and fertilized by 50 kgN+30 kg P/fed. They concluded that essential oil constituents are affected by N and F under the first and second cuts. In the study, we did not examine the effects of fertilizer treatments on the essential oil of this medicinal plant; but, this case can be considered as a new field of study about the secondary metabolites in this plant. The effect of methanolic extracts integration from *Tanacetum parthenium* (L.) and *S. Montana* on antioxidant capacity and NO was evaluated by Bahramnezhad *et al*., (2021). They concluded that the combination led to a significant increase in total antioxidant activity compared to the control. Also, in the grease test, their combination significantly reduced NO production compared to the control group. The results of their studies were in agreement with our results, which showed a clear effect of NO treatment on the changes of various compounds in plants.

Regarding heavy metals, Mumivand *et al*., (2011) studied the essential oil content from *S. hortensis* under calcium carbonate and N applications and found that interaction of N and $CaCO_3$ was significant in the essential oil contents. GC-MS results showed that compositions of *S. hortensis* such as carvacrol, γ-terpinene, and β-bisabolene did not change with the compound application. Karimi *et al*., (2013) evaluated Cd accumulation in *S. hortensis* when reported that arsenic, cadmium, and mercury were observed in roots rather than shoots in artichoke and savory. Artichoke had higher uptake from metalloids and heavy metals, a factor of bioaccumulation, and translocation efficiencies from root to shoot than savory. Accordingly, artichokes had a greater accumulating capacity. Moreover, phytoextraction of metals by artichoke can be applied to clean the soils from contamination by heavy metals. Different heavy metals have variable effects on plant secondary compounds and this has been proven in different studies. Naturally, this case should be considered by researchers in the soils contaminated with these metals. In another study, the effect of organic fertilizers on the essential oil composition of *S. hortensis* was studied by Esmaielpour *et al*., (2018). They reported that the highest and lowest contents from its essential oil were in plants under vermicompost 30% and unwashed spent mushroom compost, respectively. Also, its main constituents were carvacrol and γ-terpinene. The highest level of those was observed in plants grown on substrates containing 40% and 20% washed spent mushrooms, respectively. In our studies, the role of organic fertilizers was not investigated; however, this is a new aspect that should be considered. Although, despite the application of different treatments, the main composition of the essential oil from this medicinal plant did not change, this confirms our results.

Azizollahi *et al*., (2019) evaluated Cd accumulation in *S. hortensis* and reported that its main constituent was carvacrol, which showed quality under treatment by this heavy metal. *S. hortensis* can also be considered an invaluable alternative crop for mildly Cd-contaminated soils. Besides, because of the high potential from Cd accumulation in the roots, *S. hortensis* can be a suitable tool for phytostabilization purposes. Finally, Memari-Tabrizi *et al*., (2021) investigated foliar application of silicon nanoparticles to mitigate Cd stress on essential oil constituents from *S. hortensis* and reported the main of them were carvacrol, γ-terpinene, p-cymene, and thymol with changes in their concentrations under Cd and Si-NPs. Azizi & Asadi (2024) studied the effect of selenium on essential oil of *S. hortensis* under the cadmium stress and concluded that carvacrol being major constituent in most of analyses. Furthermore differences among minor constituents in most of the treatments were not significant which is in direction with this study.

5. CONCLUSION

This study indicated that NO treatment has obvious effects on *S. hortensis* essential oil compounds under Cd stress conditions, although the major constituent was not changed due to its high difference with the others. In this way, carvacrol (approximately 60% of total volume) showed a significant difference than the others. These studies recommend improving the biological position of *S. hortensis* under Cd stress. For this, the authors of this article encourage other researchers to examine the essential oils under the treatment of various agents. They hope that it will be possible to determine the conditions in which the highest amount of these compounds with the highest percentage can be isolated.

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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