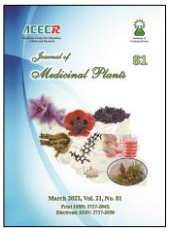
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Research Article

The effect of nano selenium foliar application on some secondary metabolites of *Hypericum perforatum* L.

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ABSTRACT

Background: *Hypericum perforatum* L. belongs to the Hypericaceae family has been considered due to its medicinal properties. The use of nanofertilizers can improve the yield and medicinal value of plants. Selenium has a protective role and a positive effect on the quantitative and qualitative characteristics of plants. **Objective:** Due to the importance of secondary metabolites of this plant and its economic value as well as the use of environmentally friendly fertilizers, this study was performed in the greenhouse of Shariati University of Tehran in 2019 in the form of a randomized complete block design with 3 replications. **Methods:** Nano selenium and selenate selenium were applied at concentrations of 6, 8, 10 and 12 mg/L in the rosette stage. Essential oil components were identified using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). **Results:** The results showed that the highest amount of *α*-pinene with 22 % was obtained in the control treatment and also in the treatments of 6 and 8 mg/L sodium selenate with 21.6 % and 19.5 %. The maximum content of *n*-octane was in the foliar application of 12 mg/L nano selenium with 16 %. Maximum nonane was obtained with 18 % and 16 % of 12 and 10 mg/L sodium selenate, respectively. **Conclusion:** Sodium selenate treatments are recommended if the purpose is producing normal hydrocarbon compounds.

1. Introduction

Hypericum perforatum L. belongs to the Hypericaceae family and has more than 469 species in the world, of which 17 to 19 species of this genus have been reported in Iran [1]. *Hypericum perforatum* L. is a perennial shrub with yellow flowers. The species is native to Europe, but has spread to all regions in Asia, Australia, and North and South America [2]. It is widely used in depression treatment, wound healing, antioxidants, antimicrobials, antibacterial, anti-anxiety, seizures, antifungal,

Abbreviations: GC, Gas Chromatography; GC-MS, Gas Chromatography-Mass Spectrometry; NH, Normal Hydrocarbon; MH, Monoterpene Hydrocarbon; SH, Sesquiterpene Hydrocarbon; OS, Oxygenated Sesquiterpene

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asthma, tuberculosis, burns and infections [3, 4]. The effect of *n*-octane, nonane and *α*-pinene compounds in controlling gram-positive diseases of the genus Staphylococcus, which causes endocarditis, has been confirmed [5]. The inhibitory effect of *Hypericum perforatum* essential oil has been observed on *Bacillus cereus*, *Micrococcus luteus*, *Salmonella typhimurium* and *Candida albicans* [6]. Collectively, findings demonstrated the potential therapeutic role of SiO_2 NPs against *Rhizoctonia solani* infection via the simultaneous activation of a multilayered defense system to suppress the pathogen, neutralize the destructive effect of ROS, lipid peroxidation, and methylglyoxal, and maintain their homeostasis within *R. solani*-infected plants [7]. In all studied metabolites, the ANOVA showed a significant effect of both time and origin factors, but the occurrence of significant interactions evidenced that the effect of climatic variability was often different according to the genotype [8]. It was stated that the percentage of fresh essential oil of *H. perforatum* was 0.12 %. Also, 45 compounds in the essential oil were identified in a total of 94.9 %, which were *α*-pinene (21.8 %), followed by alkanes (24.7 %), sesquiterpenes (20.9 %) and alcohols with *n*-dodecanol (6.8 %) [9]. One of the causes of diversity in essential oil is environmental factor. In one study on the essential oil composition of *Hypericum perforatum* in the central region of Iran, *α*-pinene was the predominant compound, as well as nonane and octane with 18.9 % of the total essential oil [10]. *β*-Caryophyllene and 2-methyl octane were the predominant components of the essential oil of *H. perforatum* in Greece [11]. Other studies in France and Turkey have found that monoterpenes (*α*-pinene) were the main constituents of the essential oils [12, 13]. In another experiment, analysis of *Hypericum perforatum* essential oils consisted mainly of monoterpenes (52.8 %), along with several oxygen monoterpenes, while sesquiterpenes were found in small amounts [14].

Selenium (Se) is a trace element that is required in relatively small amounts in the nutrition of plants, animals and humans, but in high concentrations, it is toxic to humans and wildlife. Research shows that only 5-20 % of selenium fertilizers are consumed by the plant and the rest is insoluble in soil that will not be available to the plant. This can raise concerns about contamination due to the soil application of selenium fertilizers. Selenium cannot be added directly to food, so the selenium content in plants can be increased by various methods, including adding selenium to the soil, soaking the seeds in selenium solution before planting, using in hydroponic and aeroponic crops, and foliar spraying [15]. The shape and form of selenium are important factors. In advanced agriculture, a variety of factors affect the qualitative and quantitative performance of crops, which are considered to be among the most effective aspects of improvement and success. Therefore, recently, the use of environmentally friendly materials for growing and propagating plants has become inevitable. After the development of technology, the use of new nanoscience has expanded in various fields, such as nanotechnology has gained an important position in agricultural systems. The use of nanoparticles in biological systems is not well known. There is

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little research on the effect of nanoparticles on plants [16]. Despite these significant effects, some nanoparticles have been studied on plants. The results of foliar application of nano selenium on clustered chrysanthemums improved the evaluated traits [17]. Previous Study showed that the effect of selenium foliar application was significant on marigold traits [18]. Due to the purpose review about the role of nano-selenium and sodium selenate in improving and increasing the secondary metabolites of some medicinal plants and since little research has been done in this field, the present study investigates the effect of different levels of selenium and nano-selenium on essential oils of valuable species *Hypericum perforatum* under greenhouse conditions.

2. Materials and Methods

To investigate the effect of selenium and nano selenium on essential oil compounds of St. John's wort, this study was conducted in the greenhouse of Shariati University of Tehran in 2019. In this experiment, selenium nanoparticles solution in red color were purchased from Jahan Sani Toos Company in Mashhad with 99 % purity. The initial concentration of nanoparticles was 3000 ppm and their size were 10-15 nm. The seedlings Topaz cultivar were prepared and planted in pots in March 2018. The specimen was presented in central herbarium of Tehran University (Herbarium Code: TUH). (6398 No). The research was carried out in a randomized complete block design with 3 replications in 30 pots. For this purpose, different levels of sodium selenate and nano selenium at concentrations of 6, 8, 10 and 12 mg/L were applied simultaneously as foliar application after seedling establishment. Foliar spraying of distilled water was used as a control. The information recording and harvesting of plants were done at 50 % flowering stage.

The essential oil was extracted from areal branches in the laboratory of the National Forest and Rangeland Research Institute. For this purpose, 20 g of sample from each treatment was weighed and ground and then 1:10 (dry matter: distilled water) was boiled in clevenger for 2 hours. The essential oils were dehydrated and dried by sodium sulfate, and stored in a completely dark glass jar until refrigerated at 4 °C [17].

Essential oil components were identified using gas chromatography (GC) and gas chromatography-mass spectrometry devices (GC/MS). Essential oil components were identified using gas chromatography GC-7890A dual-channel gas chromatograph (Agilent Technologies, USA) and gas chromatography-mass spectrometry devices (GC/MS) (Varian, Inc., Palo Alto, CA, USA).

All data were subjected to ANOVA using SPSS 18 software after normalization and synchronization tests. Also, significant differences between means were compared by LSD method at $P \leq 0.05$. Pearson correlation coefficient (PCC) between traits was calculated.

3. Results

The results of analysis of variance showed that in 29 extracted substances, which was significantly different between treatments in terms of essential oil yield, *n*-octane , nonane, α-pinene, 5-methyl-3-heptanone, limonene,

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n-undecane, *β*-elemene, *E*-*β*-farnesene, *γ*-Himachalene, *β*-eudesmol, *n*-tetradecanol, *α*-bisabolol, total normal hydrocarbons, total monoterpene hydrocarbons, total sesquiterpene hydrocarbons, total oxygenated sesquiterpene, alcohol ($P \leq 0.01$). Also, there is a statistical difference in terpinolene, *γ*-elemene, *α*-selinene, E-*α*-farnesene, E-nerolidol at the level of 5 % (Table 1).

The obtained results demonstrated that the highest essential oil yield (EOY) was related to the control treatment with 0.14 % and with increasing the concentration of sodium selenate and nano selenium, the percentage of essential oil decreased (Table 2). The highest n-octane content was seen at 12 mg/L nano selenium with 16 % foliar application. Maximum nonane was obtained with 18 % and 16 % of the consumption of 12 and mg/L sodium selenate, respectively. The highest *α*-pinene was with 22 % in the control treatment and also in 6 and 8 mg/L sodium selenate with 21.6 % and 19.5 %, respectively. 5-Methyl-3-heptanone was highest in 10 mg/L sodium selenate and 6 mg/L nano-selenium treatments. *n*-dodecanol in 10 mg/L sodium selenate spraying was 9.3 % higher than other treatments. *γ*-Himachalene had the highest percentage in treatments of 12 mg/L sodium selenate (7.8 %) and 8 mg/L nano selenium (6.9 %).

As can be seen in Table 2, the highest amounts of normal hydrocarbons in the treatments of 10 and 12 mg/L sodium selenate were 33.45 % and 37.4 %, respectively. The highest total of monoterpene hydrocarbons was observed in control treatments and levels of 6, 8 and 10 mg/L sodium selenate. The total hydrocarbons of sesquiterpene were higher in the treatments of 12 mg/L sodium selenate and 6 mg/L nano selenium in the other treatments. Total oxygenated monoterpene was highest in 12 mg/L sodium selenate treatments and nano selenium in 12 mg/L treatments with 3.55 % and 3.6 %, respectively. Finally, the highest amount of the identified compounds was obtained with 8, 10 and 12 mg/L sodium selenate and 6 mg/L nano-selenium.

The results of correlation between traits (Table 3) showed that octane, one of the important constituents of essential oil, had a significant negative correlation with *α*-pinene and limonene. N-octane also showed a significant positive correlation with *E*-*α*-farnesene. It was observed that there is a significant positive correlation between nonane and *β*-pinene, *β*-elemene, *β*-eudesmol and *α*-bisabolol. It was also observed that there was a significant negative correlation between *α*-pinene and terpinolene, *β*-selinene and E-*α*-farnesene at the level of 1 %. 5-methul-3-heptanone showed a significant positive relationship with *β*-pinene, *γ*-elemene at 1 %. *β*-Pinene had a significant positive correlation with *γ*-elemene and *β*-eudesmol and a significant negative correlation with E-nerolidol. Myrcene showed a significant negative correlation with terpinolene, *α*-bisabolol and phytol and a significant positive correlation with *β*-farnesene. The rest of the relationships can be seen in Table 3.

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Table 1. Analysis variance of foliar application of sodium selenate and nano selenium on essential oil compound percentage of *Hypericum perforatum* L.

SOV	df	*n*-octane	nonane	*α*-pinene	5-methul-3-heptanone	*β*-pinene	limonene	terpinolene	nepetalactone	*β*-elemene	*γ*-elemene	*E*-*β*-farnesene	*n*-dodecanol
Block	2	25.33	3.59	1.28	1.86	0.11	0.22	0.07	0.11	0.07	0.1	0.02	1.06
Treat	8	14.84**	17.12**	69.8*	9.65**	0.21^{ns}	0.08**	0.08*	0.11^{ns}	2.36**	0.03*	0.03**	3.69**
error	16	2.2	1.64	2.64	1.54	0.108	0.004	0.03	0.106	0.17	0.01	0.04	0.75
CV.	-	11.35	9.5	10.43	19.67	18.77	8.57	27.36	20.79	14.65	19.6	10.7	11.6

ns, * and ** indicates non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 1. Analysis variance of foliar application of sodium selenate and nano selenium on essential oil compound of *Hypericum perforatum* L. (Continued)

SOV	df	*γ*-Himachalene	*α*-selinene	*E, E*-*α*-farnesene	*E*-nerolidol	*β*-eudesmol	NH: Normal Hydrocarbane	MH: Monoterpene Hydrocarbone	SH: Sesquiterpene Hydrocarbone	OS: Oxygenated Sesquiterpene	alcohol	Total
Block	2	0.21	0.18	0.05	0.25	0.38	7.3	4.42	3.49	2.16	4.69	52.1
Treat	8	3.99**	0.34*	0.17*	0.16*	0.27**	4.71**	6.78**	5.08**	0.47**	4.08**	82.2**
Error	16	0.28	0.13	0.054	0.04	0.04	5.46	2.48	0.72	0.04	0.72	18.7
%CV.	-	9.1	17.6	18.7	18.8	17.8	7.6	7.8	5.03	6.92	9.43	4.87

ns, * and ** indicates non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

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Table 2. Mean comparison of foliar application of sodium selenate and nano selenium on essential oil compound of *Hypericum perforatum* L**.**

	n-octane	nonane	*α*-pinene	5-methul-3-heptanone	*β*-pinene	limonene	terpinolene	*n*-undecane	nepetalactone	*β*-elemene	*γ*-elemene	*E*-*β*-farnesene	*n*-dodecanol
Treat/RI	800	900	936	943	985	1033	1090	1100	1365	1392	1440	1460	1473
Co (0)	9^{e}	10^{d}	22^{a}	4.8^{de}	1.5^{c}	0.7^{cd}	0.5^{bc}	4.5^{abc}	1.5^{ab}	2.5^{cd}	0.5^{cd}	0.4^{d}	6.8^{bcd}
si6	11.5^{cd}	12.4^{bc}	21.6a	3.9^{e}	1.7^{ab}	0.6^{de}	0.4^{c}	3.7^{de}	1.2^{b}	3^{bc}	0.6^{abc}	0.8^{a}	8.3^{ab}
8	11^{de}	13.3^{bc}	19.5^{ab}	8^{ab}	1.9^{ab}	0.9^{ab}	0.6^{abc}	3.3^{e}	1.8^{a}	3.5^{ab}	0.7^{ab}	0.6^{bc}	7.9^{abc}
10	13.4^{bc}	16^{a}	18.4^{b}	7.8^{ab}	2.1^{ab}	1^{a}	0.5^{bc}	4^{bcd}	1.7^{ab}	4^{a}	0.5^{cd}	0.4^{d}	9.3^{a}
12	14.8^{ab}	18^{a}	12.4^{c}	6.5^{bcd}	1.7^{abc}	0.5^{e}	0.8^{ab}	4.6^{ab}	1.4^{ab}	4.2^{a}	0.4^{d}	0.5^{cd}	6.5^{cd}
nanosi6	15^{ab}	12.4^{bc}	13.4^{c}	9.1^{a}	2.2^{a}	0.6^{de}	0.6^{abc}	5^{a}	1.5^{ab}	2.1^{d}	0.7^{a}	0.6^{b}	5.8^{d}
8	13^{bcd}	13.5^{bc}	12.6^{c}	7^{abc}	1.6^{bc}	0.8^{bc}	0.7^{abc}	4.2^{bcd}	1.8^{a}	2^{d}	0.6^{abc}	0.5^{bcd}	6.6^{cd}
10	14^{abc}	13.6^{b}	11.3^{cd}	5.3^{cde}	1.4^{c}	0.9^{ab}	0.9^{a}	4^{bcd}	1.6^{ab}	1.9^{d}	0.5^{bcd}	0.5^{cd}	8.3^{ab}
12	16^{a}	11.4^{cd}	9^{d}	4.5^{de}	1.7^{abc}	0.7^{cd}	0.8^{ab}	3.9^{cde}	1.6^{ab}	2.2^{d}	0.4^{cd}	0.6^{b}	7.6^{bc}

Means within each column followed by the same letter are not different according to the LSD test at 5 and 1 %
Co: (control); si: (sodium selenate); nanosi (nano selenium)

Table 2. Mean comparison of foliar application of sodium selenate and nano selenium on essential oil compound of *Hypericum perforatum* L. (Continued)

	γ-Himachalene	*β*-selinene	*α*-selinene	E, E-*α*-farnesene	E-nerolidol	*β*-eudesmol	n-tetradecanol	NH: Normal Hydrocarbane	MH: Monoterpene Hydrocarbone	SH: Sesquiterpene Hydrocarbone	OS: Oxygenated Sesquiterpene	alcohol	Total
Treat/RI	1458	1492	1504	1509	1570	1655	1670						
Co (0)	6^{bc}	2^{a}	1.9^{bc}	0.6^{c}	1.1^{bcde}	1.2^{bcd}	1.3^{ef}	23.5^{d}	26.2^{a}	15.5^{d}	2.8^{de}	8.1^{ed}	83.6^{d}
Si6	5.2^{cd}	2.3^{a}	2.2^{abc}	1^{bc}	1^{cde}	1^{cde}	1.5^{cde}	27.6^{c}	25.7^{a}	16.5^{cd}	2.6^{e}	9.8^{abc}	88.6^{bcd}
Si8	4.9^{de}	2.2^{a}	2.4^{ab}	1^{bc}	0.9^{de}	1.4^{ab}	1.4^{def}	27.6^{c}	24.5^{a}	16.5^{cd}	3^{cd}	9.3^{bcd}	91.6^{abc}
Si10	4^{e}	2.6^{a}	1.8^{bc}	1.3^{abc}	0.8^{e}	1.6^{a}	1.7^{abc}	33.4^{ab}	23.6^{a}	15.9^{d}	3.2^{bc}	11^{a}	97.8^{a}
Si12	7.8^{a}	2.4^{a}	1.6^{c}	1.2^{abc}	1^{cde}	1.6^{a}	1.2^{f}	37.4^{a}	16.7^{b}	19.4^{a}	3.55^{ab}	7.7^{e}	93.3^{ab}
nano6	6.7^{b}	2.7^{a}	2.4^{ab}	1.4^{ab}	1.2^{abcd}	1^{cde}	1.7^{abc}	32.4^{b}	18.5^{b}	18.2^{ab}	2.6^{e}	7.5^{e}	91.1^{abc}
nano8	6.9^{ab}	2.3^{a}	2.6^{a}	1.3^{abc}	1.5^{a}	0.9^{de}	1.8^{ab}	30.7^{bc}	16.9^{b}	17.6^{bc}	3.2^{bc}	8.4^{cde}	86.9^{bcd}
nano10	5.4^{cd}	2.5^{a}	$2a^{bc}$	1.5^{a}	1.3^{abc}	0.8^{e}	1.6^{bcd}	31.6^{b}	16.1^{bc}	17.7^{d}	2.6^{e}	9.9^{ab}	84.3^{cd}
nano12	5.6^{cd}	2.6^{a}	1.8^{bc}	1.6^{a}	1.4^{ab}	1.3^{abc}	1.9^{a}	31.3^{bc}	13.7^{c}	16.4^{cd}	3.6^{a}	9.5^{bcd}	82^{d}

Means within each column followed by the same letter are not different according to the LSD test at 5 and 1 %

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Table 3. Correlation of essential oil compound of *Hypericum perforatum* L. by foliar application of sodium selenate and nano selenium

	***n*-octane**	**nonane**	***α*-pinene**	**5-methul-3-heptanone**	***β*-pinene**	**myrcene**	**limonene**	**terpinolene**	***n*-undecane**	**nepetalactone**	***β*-elemene**
	y1	y2	y3	y4	y5	y6	y7	y8	y9	y10	y11
y_1	1.00										
y_2	0.21^{ns}	1.00									
y_3	-0.6^{**}	-0.21^{ns}	1.00								
y_4	0.01^{ns}	0.3^{ns}	-0.1^{ns}	1.00							
y_5	0.09^{ns}	0.38^{*}	-0.01^{ns}	0.58^{**}	1.00						
y_6	0.3^{ns}	-0.11^{ns}	0.11^{ns}	-0.07^{ns}	-0.02^{ns}	1.00					
y_7	-0.42^{*}	0.14^{ns}	0.11^{ns}	0.24^{ns}	0.23^{ns}	-0.05^{ns}	1.00				
y_8	0.13^{ns}	0.24^{ns}	-0.7^{**}	0.16^{ns}	0.07^{ns}	-0.38^{*}	0.25^{ns}	1.00			
y_9	0.14^{ns}	0.11^{ns}	-0.2^{ns}	0.26^{ns}	0.28^{ns}	0.03^{ns}	-0.15^{ns}	0.11^{ns}	1.00		
y_{10}	-0.04^{ns}	-0.11^{ns}	0.06^{ns}	0.25^{ns}	-0.28^{ns}	0.22^{ns}	0.45^{*}	-0.09^{ns}	-0.01^{ns}	1.00	
y_{11}	-0.08^{ns}	0.65^{**}	0.35^{ns}	0.24^{ns}	0.3^{ns}	0.06^{ns}	0.01^{ns}	-0.2^{ns}	-0.18^{ns}	-0.05^{*}	1.00

Table 3. Correlation of essential oil compound of *Hypericum perforatum* L. by foliar application of sodium selenate and nano selenium (Continued)

	***n*-octane**	**nonane**	***α*-pinene**	**5-methul-3-heptanone**	***β*-pinene**	**myrcene**	**limonene**	**terpinolene**	***n*-undecane**	**nepetalactone**	***β*-elemene**
	y1	y2	y3	y4	y5	y6	y7	y8	y9	y10	y11
y_{12}	-0.06^{ns}	-0.11^{ns}	-0.07^{ns}	0.1^{ns}	-0.08^{ns}	0.47^{*}	0.14^{ns}	-0.01^{ns}	0.32^{ns}	0.48^{*}	-0.07^{ns}
y_{13}	-0.3^{ns}	-0.02^{ns}	0.1^{ns}	0.48^{*}	0.65^{**}	-0.15^{ns}	0.46^{*}	0.21^{ns}	0.2^{ns}	0.05^{ns}	-0.13^{ns}
y_{14}	0.01^{ns}	-0.06^{ns}	0.07^{ns}	-0.07^{ns}	0.3^{ns}	0.01^{ns}	-0.08^{ns}	-0.15^{ns}	-0.08^{ns}	-0.14^{ns}	-0.08^{ns}
y_{15}	-0.03^{ns}	0.22^{ns}	0.26^{ns}	-0.08^{ns}	0.26^{ns}	0.26^{ns}	0.57^{**}	-0.06^{ns}	-0.36^{ns}	0.11^{ns}	0.38^{*}
y_{16}	0.24^{ns}	0.2^{ns}	-0.36^{ns}	0.09^{ns}	0.02^{ns}	-0.07^{ns}	-0.43^{*}	0.3^{ns}	0.57^{**}	-0.1^{ns}	-0.08^{ns}
y_{17}	0.08^{ns}	0.12^{ns}	-0.4^{*}	0.1^{ns}	0.17^{ns}	-0.02^{ns}	0.23^{ns}	0.31^{ns}	0.16^{ns}	-0.05^{*}	-0.08^{ns}
y_{18}	0.03^{ns}	-0.32^{ns}	0.15^{ns}	-0.01^{ns}	-0.23^{ns}	0.06^{ns}	-0.07^{ns}	-0.3^{ns}	-0.14^{ns}	0.31^{ns}	-0.39^{*}
y_{19}	0.4^{*}	0.3^{ns}	-0.66^{**}	-0.02^{ns}	0.20^{ns}	0.2^{ns}	0.19^{ns}	0.29^{ns}	0.12^{ns}	-0.02^{ns}	-0.26^{ns}
y_{20}	0.21^{ns}	0.53^{**}	-0.28^{ns}	0.3^{ns}	0.3^{ns}	0.1^{ns}	-0.18^{ns}	0.19^{ns}	0.45*	0.05^{ns}	0.32^{ns}
y_{21}	-0.16^{ns}	0.41*	-0.22^{ns}	0.12^{ns}	0.21^{ns}	-0.09^{ns}	0.42^{*}	0.42^{*}	0.02^{ns}	0.20^{ns}	0.34^{ns}
y_{22}	-0.08^{ns}	0.19^{ns}	0.18^{ns}	-0.04^{ns}	0.28^{ns}	0.25^{ns}	0.68^{**}	0.01^{ns}	-0.3^{ns}	0.22^{ns}	0.27^{ns}
Y_{23}	0.09^{ns}	0.67^{**}	0.28^{ns}	0.52^{**}	0.6^{**}	0.25^{ns}	0.26^{ns}	-0.18^{ns}	0.19^{ns}	0.21^{ns}	0.72^{**}

ns, * and ** indicates non-significant, significant correlation at $P \leq 0.05$ and $P \leq 0.01$, respectively.

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Table 3. Correlation of essential oil compound of *Hypericum perforatum* Lby foliar application of sodium selenate and nano selenium (Continued)

	γ-Himachalene	**β-selinene**	**α-selinene**	**E, E-α-farnesene**	**E-nerolidol**	**β-eudesmol**	**n-tetradecanol**	**α-bisabolol**	**phytol**	**NH:**	**alcohol**	**Total**
	Y12	y13	y14	Y15	Y16	Y17	Y18	Y19	y20	Y21	Y22	Y23
Y_{12}	1.00											
Y_{13}	-0.1^{ns}	1.00										
y_{14}	0.09^{ns}	0.01^{ns}	1.00									
y_{15}	0.05^{*}	0.3^{ns}	-0.2^{ns}	1.00								
Y_{16}	0.3^{ns}	0.07^{ns}	0.22^{ns}	0.31^{ns}	1.00							
Y_{17}	0.06^{ns}	0.13^{ns}	-0.62^{**}	0.12^{ns}	-0.3^{ns}	1.00						
Y_{18}	-0.5^{ns}	0.35^{ns}	-0.07^{ns}	0.5^{*}	0.64^{**}	0.16^{ns}	1.00					
Y_{19}	0.16^{ns}	0.19^{ns}	-0.42^{*}	0.26^{ns}	0.16^{ns}	0.71^{**}	0.51^{*}	1.00				
y_{20}	0.02^{ns}	0.11^{ns}	-0.27^{ns}	0.07^{ns}	0.37^{ns}	0.02^{ns}	0.58^{*}	0.41^{*}	1.00			
y_{21}	0.35^{ns}	0.15^{ns}	-0.19^{ns}	0.46^{*}	-0.19^{ns}	0.24^{ns}	-0.11^{ns}	0.24^{ns}	-0.17^{ns}	1.00		
y_{22}	-0.46^{*}	0.09^{ns}	-0.32^{ns}	0.17^{ns}	-0.05^{ns}	0.34^{ns}	0.47^{*}	0.4^{*}	0.39^{*}	0.02^{ns}	1.00	
Y_{23}	0.08^{ns}	-0.04^{ns}	-0.15^{ns}	-0.05^{*}	-0.3^{ns}	0.57^{**}	0.11^{ns}	0.31^{ns}	-0.02^{ns}	0.47^{*}	0.44^{*}	1.00

ns, * and ** indicates non-significant, significant correlation at $P \leq 0.05$ and $P \leq 0.01$, respectively.
NH: Normal Hydrocarbane, MH: Monoterpene Hydrocarbone, SH: Sesquiterpene Hydrocarbone, OS: Oxygenated Sesquiterpene

4. Discussion

The chemical composition and biological activity of essential oils can be affected by a number of factors, including harvest time and part of the plant. Regarding the importance of the herb, it can be said that so far, a wide range of biologically active compounds such as hypericin and hyperforin, naphthodiantrons, phloroglucinols, xanthones and flavonoids have also been reported from the essential oil of *Hypericum perforatum* L. [4]. Therefore, the above content indicates the importance of selected plants for this study.

The highest *α*-pinene was with 22 % in the control treatment and also in 6 and 8 mg/L sodium selenate with 21.6 % and 19.5 %, respectively. *n*-octane, nonane, *α*-pinene were the most abundant compounds identified in this study, which is consistent with the findings of [20]. On the other hand, the effects of *n*-octane, nonane, *α*-pinene compounds in controlling gram-positive diseases of the genus Staphylococcus (the cause of endocarditis infection) and the impact of *α* and *β*-pinene in inhibiting the growth of Staphlococcus epidermidis and S. Aureus has been observed by *Hypericum perforatum* essential oil, which indicated the value of research on the essential oil of this plant [6]. On the other hand, the increase in *n*-octane with the use of nano selenium,

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nonane with the application of sodium selenate and *α*-pinene with selenium selenate treatments relieved a value obtained from this survey.

Decreased essential oil yields in foliar spraying treatments can be due to reduced environmental stresses or increased stress intensity, so that if the amount of cell-regulating osmolality increases, it indicates the stressful application of sodium selenate and nano-selenium to the plant. The stress-relieving effect of high selenium intake has also been confirmed in research [12-21], so in the present study the decrease in essential oil yield may be due to the severity of stress. It has been reported that in plants under severe stress, instead of increasing the essential oil, the percentage of other specific compounds in the essential oil and regulating osmolytes such as proline increases [22].

Comparison of means showed that the percentage of normal compounds of hydrocarbons (octane, nonane) increased with 10 and 12 mg/L sodium selenate and 6, 10 and 12 mg/L sodium selenate compared to other treatments. Normal hydrocarbon was higher in 10 and 12 mg/L sodium selenate than the other treatments. Therefore, if the purpose of production is to use all normal compounds of hydrocarbons or nonane, treatments of 10 and 12 mg / l sodium selenate are recommended, and if the goal is to apply octane, 6 and 12 mg/L nano selenium or 12 mg/L sodium selenate can be used. One of the notable points in these compounds is that the precursors of these multiplex seem to be different because the increase of one does not cause a decrease of the other, which does not have a significant negative relationship according to Table 3.

Monoterpene hydrocarbon compounds, after normal hydrocarbons, formed most of the total essential oil compounds, among which *α*-pinene was the main constituent of essential oil, and this compound was the highest in the conditions without selenium. It is produced without more stress, and if the goal is to produce plants with a high percentage of *α*-pinene, using selenium compounds were not recommended. It was also observed in Table 3 that this compound has a negative correlation with normal hydrocarbon compounds, so it is not possible to increase the three main compounds (*n*-octane, nonane, *α*-pinene) simultaneously, but this allows the targeted production of essential oil compounds.

As shown in Table 2, the different hydrocarbon compositions of sesquiterpene increased with the consumption of different amounts of selenium and nano-selenium. In general, due to the positive or negative correlation between the components of essential oil (Table 3), it is possible to change the composition of essential oil by using nutritional treatments such as selenium and nano-selenium and can manage the production of essential oil in this plant. Therefore, considering the positive response of *Hypericum perforatum* to the use of non-chemical fertilizers, it seems that the use of these fertilizers does not have adverse environmental consequences and therefore is a suitable method for healthy and sustainable production of such products. The finding is consistent with Sadegh et al. [23]. The future prospects of selenium nanoparticles include the development of new fast and environment-friendly methodology for their synthesis to obtain nanomaterial with the corresponding size, shape, and properties for the desired [24]. The results of [25] demonstrated that mostly selenium and CS-Se NPs treatments could lessen negative effects of stress conditions through enhancing agronomic traits, photosynthetic pigments, proline, phenols, antioxidant enzymes activities and some dominant constituents of essential oils. Generally, the tested treatments including

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selenium, nano selenium, and glycine betaine varied in their significant effects on the enhancing chemical composition and secondary metabolites of essential oils characters in *Coriandrum sativum* plants [26]. Treated chives varieties with Selenium doses improved the essential oil and major constituents of essential oil were changed. Under Se treatments the M2K variety produced higher values of essential oil content than M2P while no differences were found in PHP. Different variations were obtained in the major constituents of essential oil of both varieties under Se applications [27].

5. Conclusion

The aim of production herbal medicines is in high quality plants free of toxins and chemical fertilizers. Therefore, replacing a part of chemical fertilizer with biomaterials, in addition to improving its quantitative and qualitative properties, reduces the use of chemical fertilizer. According to the results, the maximum amount of α-pinene with 22 % was observed in the control treatment and also in the treatments of 6 and 8 mg/L sodium selenate with 21.6 and 19.5 %. The highest amount of octane was achieved with 12 mg/L nano selenium with 16 %. Maximum nonane was obtained with 18 % and 16 % of 12 and 10 mg/L sodium selenate, respectively. However, the highest essential oil yield, the highest α-pinene, the highest total of monoterpene hydrocarbons was observed in control treatments. Observing the differences in the secondary compounds in response to different treatments of different selenium compounds can be very important and help to increase their production efficiency.

Author contributions

M.R.N. and V.A.; Conceptualization, Methodology, Formal analysis and investigation, M.R.N. Writing-original draft preparation, M.R.N., V.A., F.Z.H. and K.L.; Writing - review and editing, V.A., F.Z.H. and K.L.; Resources, Supervision; All authors have read and approved the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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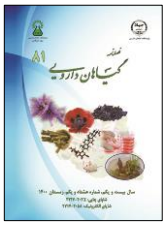
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مقاله تحقیقاتی

بررسی اثر محلول‌پاشی نانو سلنیوم بر برخی متابولیت‌های ثانویه گل راعی

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اطلاعات مقاله

گل‌واژگان:

گل راعی

اسانس

آلفا پینن

اکتان نرمال

سلنیوم

چکیده

مقدمه: گل‌راعی (*Hypericum perforatum* L.) به خانواده گل راعی (Hypericaceae) تعلق دارد و به دلیل خواص دارویی آن مورد توجه قرار گرفته است. استفاده از نانوکودها می‌تواند سبب بهبود عملکرد و ارزش گیاهان دارویی گردد. عنصر سلنیوم نقش حفاظتی و تاثیر مثبتی روی خصوصیات کمی و کیفی گیاهان دارد. **هدف:** با توجه به اهمیت متابولیتهای ثانویه این گیاه و ارزش اقتصادی آن و همچنین استفاده از کودهای سازگار با طبیعت، این تحقیق در گلخانه دانشگاه شریعتی تهران در سال ۱۳۹۸ در قالب طرح بلوک‌های کامل تصادفی با ۳ تکرار انجام شد. **روش بررسی:** تیمارها شامل محلول‌پاشی برگی با سطوح مختلف سلنیوم از منبع سلنات سدیم و نانوسلنیوم در غلظت‌های ۶، ۸، ۱۰ و ۱۲ میلی‌گرم در لیتر به در مرحله رزت بود. شناسایی اجزای اسانس با استفاده از دستگاه‌های کروماتوگرافی گازی و کروماتوگرافی گازی متصل به طیف‌سنج جرمی صورت گرفت. **نتایج:** نتایج نشان داد که بالاترین مقدار آلفا پینن با ۲۲ درصد در تیمار شاهد و نیز در تیمارهای ۶ و ۸ میلی‌گرم بر لیتر سلنات سدیم با ۲۱/۶ درصد و ۱۹/۵ درصد حاصل شد. بیشترین میزان اکتان نرمال در تیمار محلول‌پاشی ۱۲ میلی‌گرم بر لیتر نانوسلنیوم با ۱۶ درصد بود. حداکثر نونان به ترتیب با ۱۸ درصد و ۱۶ درصد از مصرف ۱۲ و ۱۰ میلی‌گرم بر لیتر سلنات سدیم بدست آمد. **نتیجه‌گیری:** در صورتی که هدف از تولید، استفاده از ترکیبات هیدروکربنی نرمال باشد، تیمارهای سلنات سدیم قابل توصیه می‌باشد.

مخففها: GC، کروماتوگرافی گازی؛ GC/MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی ؛ NH، هیدروکربن نرمال؛ MH، مونوترپن هیدروکربنه؛ SH، سزکوئی‌ترپن هیدروکربنه؛ OS، سزکوئی‌ترپن اکسیژنه

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