Journal of Medicinal Plants and By-products (2021) 1: 67-73

Original Article

Essential Oil Profile Changes of Three *Thymus* Species under Nanoparticle Treatments

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Article History: Received: 17 April 2020/Accepted in revised form: 26 September 2020

Abstract

Nanoparticles (NPs) have received much attention recently in various areas of industry, biomedical, and agricultural sectors worldwide. It is important to recognize the consequences of the use and application of NPs and their interaction with ecosystems components including plants, whether in the environmental area or in physiology and crop production. The present study aimed to investigate the changes in essential oil content and composition of *Thymus daenensis* Celak., *Thymus fedtschenkoi* Ronniger and *Thymus vulgaris* L. under silver nanoparticles (AgNPs) and silicon nanoparticles (SiNPs) in four levels (0, 30, 60, 100 ppm). The essential oil content increased at all stress levels and in all three species. The amount of monoterpene hydrocarbons in *T. fedtschenkoi* increased, while it decreased in other two species. The amount of sesquiterpentes, except for oxygenated sesquiterpenes in *T. daenensis*, increased slightly. Compared to other factors, the type of plant species was more determinative in response to treatments. Overall, both AgNPs and SiNps treatments had a distinct effect. However, no interpretable results were observed between the different levels of both treatments.

Keywords: AgNPs, SiNPs, *Thymus daenensis*, *Thymus fedtschenkoi*, *Thymus vulgaris*

Introduction

With the rapid development of nanotechnology in recent years, nanoparticles (NPs) research has become an area of intense scientific interest, due to a wide variety of potential applications in the areas of physical, chemical, biological, health and other interdisciplinary fields of science and engineering [1,2]. Although nanoparticles (NPs) naturally exist in the environment, the concentrations of NPs have increased in the environment due to increasing demand of NPs based products [3–5]. Due to the static nature of NPs and their interaction with soil and air, plants as an important component of the ecological system are most exposed to these materials [1,6]. The impact of various types of NPs on higher plants differently at the physiological, biochemical, nutritional, and genetic levels has also been examined [6–9]. However, there are still many unresolved issues and challenges concerning the biological effects of NPs [10]. The effects vary depending on the physicochemical properties of NPs, as well as plant species, the extent of their sizes, and the exposure duration [10,11]. Currently, NPs researches with the goal to promote their use for improvements of crop agronomic traits engenders considerable interests. Besides the exclusive properties of NPs on plants, certain reports have confirmed that NPs can induce phytotoxicity and have some negative effects as they enhance the generation of reactive oxygen species (ROS) [4,12].

Among the different NPs, silver nanoparticles (AgNPs) have been applied in many industries, such as personal care products, clothing, food, building materials and medical equipment. Exposure to AgNPs seems to have many physiological and biochemical consequences in plants such as transpiration, oxidative stress, and genotoxicity, among others, which in turn have influence

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on key life events of the plants including seed germination, seedling vigor, root initiation, growth and photosynthesis to flowering [4,13].

Silicon (Si), as the second most abundant macro-element, is known to improve plant fitness by mitigation and remediating the toxic effects of biotic and abiotic stresses and toxic substances [14,15]. Silica nanoparticles (SiNPs) have received special interest due to their prominence in cosmetic and biomedical applications and on the other hand as a nano-fertilizer as well as in industry [16,17]. Use of nano-fertilizers in plant nutrition is one of the major roles of nanotechnology in agriculture, soil and water sciences [18,19].

In such a conflicting and ambiguous nature of NPs in plants, research related to the use, application and elucidation of the action mechanism of cellular, biochemical and molecular protection render by the NPs in plants is essential. Given that the essential oil content and composition of medicinal and aromatic plants is highly integrated with the physiology of the whole plant, this study aimed consideration the changes of essential oil composition of three *Thymus* species under different concentrations of AgNPs and SiNPs, whether as a stressor (AgNPs) or a fertilizer (SiNPs).

Experimental

Plant Material and Growth Conditions

The field experiment was conducted from early April to early July 2018, in the research farm of the University of Malayer (Latitude: 34°15′N, Longitude: 48°51′E, Altitude: 1814 m, Malayer, Hamedan Province, Iran). The plants of *T. daenensis* and *T. fedtschenkoi* were collected from their natural habitats in Malayer and Soobashi Mountains in early March 2016, respectively, and propagated by plant separation. *T. vulgaris* transplants were purchased from the Hamedan Botanical Garden. In order to achieve steady and fresh growth, the plants were trimmed after planting. The AgNPs was purchased from Pars Nano Nasb Company with an average particle diameter of 25 nm. Powdered SiO_2 nanoparticles were purchased from TECNAN (Tecnología Navarra de Nanoproductos S.L., Spain) with a range of 10 to 30 nm.

The experiment was arranged in a randomized block design with two NPs (AgNPs and SiNPs) treatments in four levels (0, 30, 60, 100 ppm) and three replications.

Each block had six plants. After deployment and earl May, each of the plant was thoroughly sprayed and soaked with 200 ml of each treatment. NPs treatments were applied weekly for four weeks. The plants were harvest at the flowering stage in mid-June.

Characterization and Application of NPs

X-ray diffraction (XRD) measurement confirmed the structure and particle size distribution of the NPs. The NPs were dispersed in deionized water and sonicated for more than 15 min. All experimental concentrations were prepared by diluting the AgNPs stock solution (1 g L^{-1}) in deionized water. All dilutions were freshly prepared before use. Four levels of AgNPs and SiPNs (0, 30, 60, and 100 ppm) were sprayed with 0.1% Tween 20 on plants as a surfactant. These solutions were sonicated for 15 min.

Isolation of Essential Oils

The essential oils of each treatment was isolated by hydro-distillation of 100 gr air-dried aerial parts of the plants using a Clevenger type apparatus for two hours with three replications. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4 °C until analysis.

GC-FID and GC/MS Conditions

Due to the high number of samples, all three replicates were mixed before injection to the device. Gas chromatography (GC) analysis was performed using a Thermo-UFM ultra-fast gas chromatograph equipped with a ph-5 fused silica column (10 m × 0.1 mm i.d., film thickness 0.40 µm). Oven temperature was held at 60 °C for three minutes and then programmed to 280 °C at a rate of 80 °C/min. Detector (FID) temperature was 285 °C and injector temperature was 285 °C. Helium was used as carrier gas with a linear velocity of 32 cm/s. The oils were manually injected to GC without dilution. The percentages of compounds were calculated by the area normalization method, without considering response factors. Gas chromatography mass spectrometry (GC-MS) analysis was carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm); oven temperature was 50-240 °C at a rate of 4 °C/min, transfer line temperature 260 °C, carrier gas: He, with a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40-300 amu. The oils diluted in dichloromethane (2 µl of the oil in 2 ml solvent), then 2 µl of each was injected to GC/MS manually.

Identification of Volatile Components

The components of oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [20, 21]. Mass spectra from the literature were also compared [22, 23].

Data Analysis

Scatter biplot was performed on the principal components analysis (PCA) discrimination using variance-covariance

matrix by Past software (version 3.04) to obtain a simultaneous representation of both treatments and general characteristic in one oil sample.

Results and Discussion

The main ingredient of *T. daenensis* oil was thymol (ranged from 72.03% to 86.33%), that its highest amount was obtained from Ag60 and Si60 treatments (86.33% and 86.07%, respectively), as well as the highest oxygenated monoterpenes (Table 1). Other main constituents were p-cymene, γ-terpinene, carvacrol methyl ether and *trans*-caryophyllene in all treatments. The most amounts of sesquiterpenes, with the abundance of the hydrocarbon forms, achieved form Ag30 and Ag60 treatments (4.03% and 4.72% respectively). In the case of essential oil content, in comparison with the control, all applied treatments increased essential oil percentage, especially Si30 treatment (1.2%). Scattering of the treatments based on the first two principal components of chemical-composition data of *T. daenensis* is demonstrated in Fig. 1, above. Accordingly, the length and orientation of the vector of the compounds are determinative in scattering of the treatments. Herein, the vectors of thymol, *trans*-caryophyllene, p-cymene, γ-terpinene and carvacrol methyl ether had the longest length, respectively. And with different directions from each other, thymol; *trans*-caryophyllene and γ-terpinene; p-cymene and carvacrol methyl ether had a nearly same orientation. Hence, this layout showed proximity of Ag30 and Si30 treatments in the second quarter, proximity of control and Ag100 in third quarter, and distance of Ag60. Alcoholic compound of linalool was major ingredient of *T. fedtschenkoi* in all treatments, that the highest and the lowest observed in treatments of Ag30 (78.30%) and Ag100 (39.41%) (Table 2).

Table 1 The profile of the essential oil of *Thymus daenensis* Celak. under various levels of AgNPs and SiNPs.

No	RT[a]	Compounds	Control	Ag30	Ag60	Ag100	Si30	Si60	Si100
	Monoterpene hydrocarbons								
1	6.59	α-Thujene	0.74	1.09	0.31	0.74	0.55	0.75	0.51
2	6.80	α-Pinene	0.28	0.42	0.14	0.27	0.20		0.20
3	7.23	Camphene	0.16	0.22		0.12	0.09		0.10
4	8.07	β-Pinene	0.12	0.15	0.05	0.11	0.08	0.15	0.10
5	8.47	β-Myrcene	0.69	1.23	0.36	0.65	0.65	0.72	0.61
6	8.97	α-Phellandrene		0.20	0.06	0.09	0.11		0.15
7	9.38	α-Terpinene	0.80	1.18	0.36	0.50	0.77	0.35	0.67
8	9.74	p-Cymene	4.95	5.19	0.82	3.90	3.41	3.98	4.29
	Oxygenated monoterpenes								
9	10.96	1,8-Cineole		0.43	0.11	0.44	0.37		
10	11.05	γ-Terpinene	3.13	6.40	2.22	1.71	4.55	1.35	3.47
11	11.41	*trans*-Sabinene hydrate		0.24		0.16	0.12	0.26	0.32
12	12.69	Linalool	0.33	0.31		0.33	1.87	0.33	
13	15.41	Borneol	0.21	0.38		0.17	0.28		0.76
14	18.59	Carvacrol methyl ether	5.37	5.95	2.66	4.59	4.16	3.51	4.59
15	21.9	Thymol	79.46	72.03	86.33	77.75	77.58	86.07	80.86
	Sesquiterpene hydrocarbons								
16	25.83	*Trans*-Caryophyllene	1.96	3.92	4.42	1.97	3.25	2.11	3.03
17	27.18	α-Humulene		0.11	0.14	0.06	0.09		0.10
18	29.33	β-Bisabolene			0.16		0.17		
	Oxygenated sesquiterpenes								
19	32.31	Caryophyllene oxide	0.53	0.18	0.25	0.26	0.42	0.42	0.24
	Monoterpene hydrocarbons		7.74	9.68	2.10	6.38	5.86	5.95	6.63
	Oxygenated monoterpenes		88.50	85.74	91.32	85.15	88.93	91.52	90.00
	Sesquiterpene hydrocarbons		1.96	4.03	4.72	2.03	3.51	2.11	3.13
	Oxygenated sesquiterpenes		0.53	0.18	0.25	0.26	0.42	0.42	0.24
	Detected compounds		98.73	99.63	98.39	93.82	98.72	100	100
	Essential oil content (%)		0.25	1	0.5	1	1.2	1.05	1

[a] Retention Time

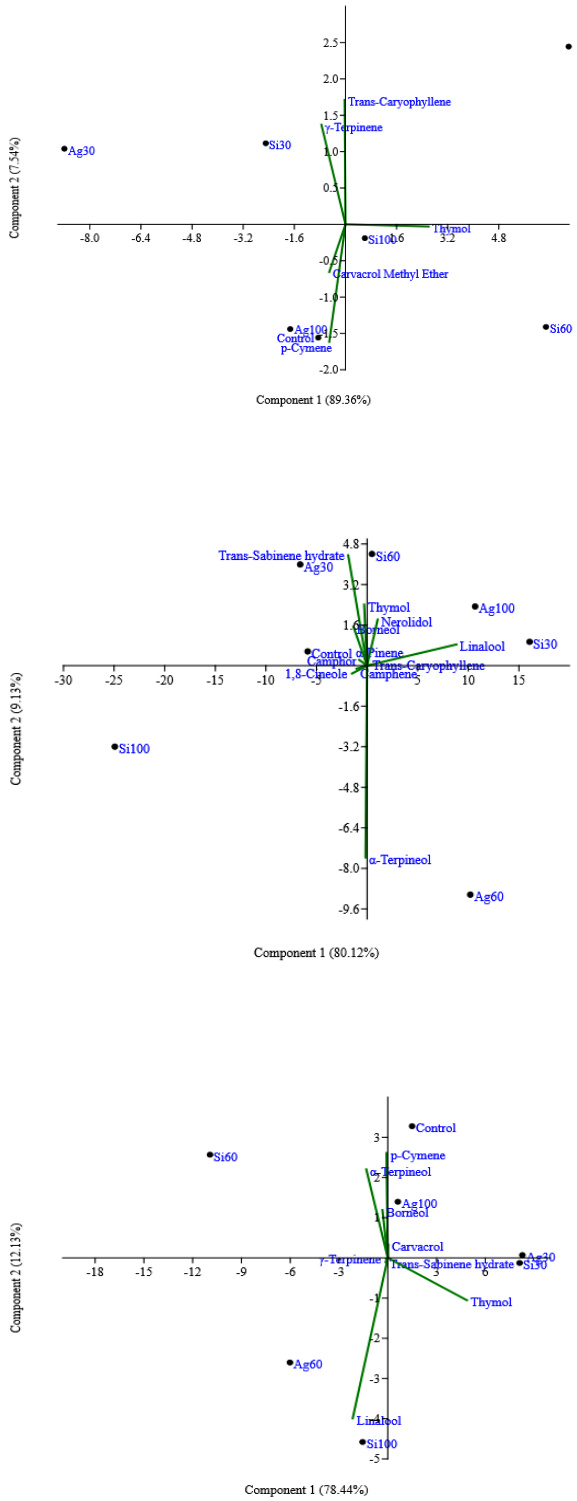

Fig. 1 Scatter plot based on the first two principal components of chemical-profile data, demonstrating the relationships among some important characteristics in the essential oil of *Thymus daenensis* Celak. (above), *Thymus fedtschenkoi* Ronniger (middle) and *Thymus vulgaris* L. (below).

Other main constituents included 1,8-cineole, borneol and *trans*-caryophyllene. The high amount of *trans*-sabinene hydrate (13.22%) and α-terpineol (11.59%) found in treatments of Ag30 and Ag60, respectively. Furthermore, the amount of 1,8-cineole in Si100 (10.12%), *trans*-sabinene hydrate in control (8.67%) and Si100 (7.21%), thymol in Si60 (7.90%) and nerolidol in Ag100 (7.38%) and Ag30 (5.67%) was high. In comparison with two other species, the number of major compounds was more and various. The high amount of sesquiterpene hydrocarbons were observed in Si treatments. Regardless of the high levels of nerolidol in Ag30 and Ag100, which, of course, should be investigated, the total amount of sesquiterpenes was increased in Si treatments. Like the *T. daenensis*, the essential oil content increased in all applied treatments, especially in Ag60 (1.1%). Scatter plot of this species showed remoteness of Ag60, similar to *T. daenensis*, and Si100 (Fig. 1, middle).

In the case of *T. vulgaris*, the main compound was thymol, and it's following γ-terpinene and p-cymene (Table 3). The highest amount of thymol obtained from Ag30 (61.85%) and Si30 (61.53%), and it's lowest found in Ag60 (48.70%) and Si60 (44.63%). In comparison with the control, the amount of γ-terpinene increased in all treatments, especially in treatments of Ag60, Ag100 and Si30. On the contrary, the amount of p-cymene decreased, especially in Ag60, Si30 and Si100. It can also be said that the amount of sesquiterpene hydrocarbons have increased. Like the two previous species, all applied treatments increased essential oil content, especially in Ag60 (1.05%). Scattering biplot set Ag60 and Si100 apart from other treatments, similar to the two species before (Fig. 1, below).

Altogether and despite the different effects, the treatments of silver and silicon nanoparticle decreased the amount of monoterpene hydrocarbons in *T. daenensis* and *T. vulgaris* compared to control, however in *T. fedtschenkoi* vice versa (Table 4). In the case of oxygenated monoterpenes, the same changes were observed in both *T. daenensis* and *T. vulgaris* so that a slight decrease and increase was seen under AgNPs and SiNPs, respectively. However, these compounds decreased in *T. fedtschenkoi* under both AgNPs and SiNPs. Likewise, the similar trend of changes was observed in sesquiterpene hydrocarbons in both *T. daenensis* and *T. vulgaris*. That is, it increased in both. In *T. fedtschenkoi*, the increase was only seen in SiNPs. The amount of oxygenated sesquiterpenes decreased in *T. daenensis* while that increased in *T. fedtschenkoi* and *T. vulgaris*. In general, both AgNPs and SiNPs treatments at all levels increased the essential oil content in all three species. As secondary and latent results, it is worth noting that regardless of the treatments effects, the amount of monoterpene hydrocarbons in *T. vulgaris* was higher than that of *T. daenensis* and in *T. daenensis* more than *T. fedtschenkoi*. Instead, the amount of oxygenated monoterpenes and sesquiterpenes in *T. fedtschenkoi* was generally higher than in *T. daenensis* and *T. vulgaris* (Table 4).

Table 2 The profile of the essential oil of *Thymus fedtschenkoi* Ronniger under various levels of AgNPs and SiNPs.

No	RT[a]	Compounds	Control	Ag30	Ag60	Ag100	Si30	Si60	Si100
Monoterpene hydrocarbons									
1	6.6	α-Thujene	0.04	-	0.12	-	0.09	-	0.11
2	6.8	α-Pinene	0.39	3.11	0.30	0.51	0.29	0.70	3.52
3	7.24	Camphene	0.45	1.14	0.37	0.28	0.49	1.39	3.02
4	7.96	Sabinene	0.20	-	0.19	-	0.19	-	0.53
5	8.08	β-Pinene	0.16	0.63	0.21	0.30	0.22	0.34	0.64
6	8.51	β-Myrcene		0.30	0.29	-	-	-	0.25
7	9.38	α-Terpinene	0.12	0.12	0.22	-	-	-	0.11
8	9.74	p-Cymene	0.51	-	0.98	-	0.22	-	-
Oxygenated monoterpenes									
9	10.02	1,8-Cineole	4.01	4.77	2.64	2.87	3.82	4.58	10.12
10	10.49	*Cis*-β-Ocimene	0.06	0.41	0.46	0.68	0.43	0.29	0.22
11	10.91	γ-Terpinene	0.57	0.29	2.45	0.16	0.24	-	0.34
12	11.54	*Trans*-Sabinene hydrate	8.67	13.22	1.37	5.25	0.74	-	7.21
13	11.64	linalool oxide	0.97	0.47	-	-	-	1.60	0.35
14	13.45	Linalool	57.31	57.37	71.92	72.92	78.3	64.18	39.41
15	14.55	Camphor	2.39	2.57	0.38	0.77	0.63	0.76	4.88
16	15.55	Borneol	3.64	3.02	1.27	1.92	2.28	8.71	8.07
17	15.92	Terpinen-4-ol	1.26	1.13	0.28	0.46		0.29	0.90
18	16.66	α-Terpineol	4.96	1.23	11.59	0.78	0.47	0.46	4.67
19	21.21	Thymol	7.96	0.70	0.66	1.11	3.14	7.90	2.31
Sesquiterpene hydrocarbons									
20	25.86	*Trans*-Caryophyllene	1.68	1.07	2.35	2.24	3.26	3.71	2.64
21	28.27	Germacrene D	0.49	0.16	0.47	0.41	0.61	0.14	0.31
22	28.88	Bicyclogermacrene	0.38	0.17	0.64	0.48	1.10	0.53	1.70
23	29.33	β-Bisabolene	0.26	0.22	-	-	-	-	-
Oxygenated sesquiterpenes									
24	31.78	Nerolidol	-	5.67	-	7.38	-	-	0.10
25	32.21	Spathulenol	-	-	-	-	0.32	-	1.22
26	32.37	Caryophyllene oxide	1.85	1.61	0.17	1.47	1.09	2.89	2.01
Monoterpene hydrocarbons			1.87	5.30	2.68	1.09	1.50	2.43	8.18
Oxygenated monoterpenes			91.80	85.18	93.02	86.92	90.05	88.77	78.48
Sesquiterpene hydrocarbons			2.81	1.62	3.46	3.13	4.97	4.38	4.65
Oxygenated sesquiterpenes			1.85	7.28	0.17	8.85	1.41	2.89	3.33
Detected compounds			98.33	99.38	99.33	99.99	97.93	98.47	94.64
Essential oil content			0.4	1	1.1	0.65	0.6	0.7	0.65

[a] Retention Time

Per the reported literature, the effect produced by NPs is depend on the type and concentration of NPs, plant species, and the exposure media and these effects are inconsistent among the different studies [11]. In plants, NPs are adsorbed to plant surfaces and taken up through natural nano or micrometer scale plant openings. Several pathways exist or are predicted for NP association and uptake in plants was explained [24].

There were no interpretable results between the different levels of both treatments. In other words, the effects of different levels of treatments were ambiguous. However, AgNPs and SiNPs treatments had a distinct effect. These effects were similar in most cases, in that the plant exhibited similar responses to these compounds; perhaps these compounds induced the same stress in the plant. In some cases, the responds were somewhat different, such as the different effects of AgNPs and SiNPs on the oxygenated monoterpenes in *T. daenensis* and *T. vulgaris* (Table 4). However, the plant species showed different responses to the treatments. In other words, the species of plants was more determinant in response to treatments than other factors.

All the stress levels increased the essential oil content in all three species. In the most cases, stresses (biotic and/or abiotic) caused an enhancement in essential oil content [25–27]. However, the essential oil constituents have different variations in exposure to various stresses in different plants. The amount of sesquiterpentes, except for oxygenated sesquiterpenes in *T. daenensis*, increased slightly. Sesquiterpene compounds constitutes a small portion of the essential oil of *Thymus*. These compounds are mostly synthesized confronting of biotic stresses [28–30]. It seems that applied treatments actives a general mechanism than one specific pathway.

In the case of monoterpenes, the amount of monoterpene hydrocarbons in *T. fedtschenkoi*, which was the lowest, increased. However, in the other two species it decreased. Tangible changes in the monoterpene compounds are due to the face that most constitutes of the *Thymus* essential oil are these compounds.

Table 3 The profile of the essential oil of *Thymus vulgaris* L. under various levels of AgNPs and SiNPs.

No	RT[a]	Compounds	Control	Ag30	Ag60	Ag100	Si30	Si60	Si100
Monoterpene hydrocarbons									
1	6.60	α-Thujene	0.96	1.23	1.85	0.76	1.10	2.03	0.88
2	6.81	α-Pinene	0.62	-	-	0.37	0.45	-	0.45
3	7.25	Camphene	0.67	0.21	0.31	0.23	0.24	0.55	0.35
4	8.08	β-Pinene	0.22	-	0.28	-	0.23	0.42	0.14
5	8.30	1-Octen-3-ol	0.53	-	-	-	-	-	0.58
6	8.49	β-Myrcene	1.18	1.97	2.24	2.03	2.36	2.5	1.25
7	8.97	α-Phellandrene	0.21	0.33	0.37	-	0.36	0.41	0.19
8	9.41	α-Terpinene	1.65	1.64	1.93	1.57	1.80	1.99	1.53
9	9.85	p-Cymene	12.06	10.3	9.12	11.67	9.24	11.15	8.28
Oxygenated monoterpenes									
10	10.01	1,8-Cineole	1.75	-	-	-	-	-	0.76
11	11.12	γ-Terpinene	13.07	14.51	16.39	16.94	16.74	15.75	13.82
12	11.46	*Trans*-Sabinene hydrate	1.91	1.65	3.00	2.42	1.39	1.50	1.42
13	12.67	Linalool	1.44	1.72	8.23	3.53	1.37	7.49	9.07
14	15.48	Borneol	3.59	0.64	1.07	1.32	0.63	2.34	1.44
15	15.89	Terpinen-4-ol	0.54	0.46	0.79	0.56	0.53	-	0.23
16	17.22	α-Terpineol	0.36	0.21	0.29	-	0.20	6.74	0.16
17	18.52	Carvacrol methyl ether	-	0.62	-	-	-	-	-
18	19.87	Z-Citral	-	-	0.16	-	0.16	0.18	0.15
18	21.62	Thymol	54.3	61.85	48.70	54.45	61.53	44.63	53.99
19	21.82	Carvacrol	3.62	-	-	-	-	-	2.90
20	23.38	Thymyl acetate	0.11	0.29	0.25	-	0.35	0.29	0.15
Sesquiterpene hydrocarbons									
21	25.81	*Trans*-Caryophyllene	0.50	0.85	1.47	0.62	0.83	1.09	1.16
22	28.28	Germacrene D	-	0.41	0.19	-	0.24	0.25	0.29
23	29.54	Bicyclogermacrene	-	-	-	-	-	0.14	0.17
24	29.88	δ-Cadinene	-	0.11	-	-	-	-	-
25	30.64	*Cis*-α-Bisabolene	0.13	-	-	-	-	-	-
Oxygenated sesquiterpenes									
26	30.71	Nerolidol	-	-	2.40	-	-	-	-
27	32.33	Caryophyllene Oxide	0.24	0.36	0.64	0.25	0.17	0.49	0.24
Monoterpene hydrocarbons			18.10	15.68	16.10	16.63	15.78	19.05	13.65
Oxygenated monoterpenes			80.69	81.33	78.88	79.22	82.90	78.92	84.09
Sesquiterpene hydrocarbons			0.63	1.37	1.66	0.62	1.07	1.48	1.62
Oxygenated sesquiterpenes			0.24	0.36	3.04	0.25	0.17	0.49	0.24
Detected compounds			99.66	98.74	99.68	96.72	99.92	99.94	99.60
Essential oil content (%)			0.20	0.65	1.05	0.85	0.95	0.80	0.85

[a] Retention Time

Table 4 Average effects of the various levels of silver and silicon nanoparticles on profile of the essential oil of *Thymus daenensis* Celak., *Thymus fedtschenkoi* Ronniger and *Thymus vulgaris* L.

Compounds	*T. daenensis*			*T. fedtschenkovi*			*T. vulgaris*		
	Control	AgNPs	SiNPs	Control	AgNPs	SiNPs	Control	AgNPs	SiNPs
Monoterpene hydrocarbons	7.74	6.05	6.15	1.87	3.02	4.04	18.10	16.14	16.16
Oxygenated monoterpenes	88.50	87.40	90.15	91.80	88.37	85.77	80.69	79.81	81.97
Sesquiterpene hydrocarbons	1.96	3.59	2.92	2.81	2.74	4.67	0.63	1.22	1.39
Oxygenated sesquiterpenes	0.53	0.23	0.36	1.85	5.43	2.54	0.24	1.22	0.30
Essential oil content	0.25	0.83	1.08	0.40	0.92	0.65	0.20	0.85	0.87

Conclusion

As per the reported literature, the effects depend on the concentration of the NPs, exposure media, and plant species. Therefore, many subjects, studies and challenges involving the biological effects of NPs remain unresolved. In this study, it was found that species have a definite role in determining the response compared to

other factors. Stimulation of increased essential oil content and lack of a specific pattern of changes in the constituents indicate induction of a general response by the applied treatments, not a specific response. However, more studies are needed concerning about the plant growth and physiological responses in different species when exposed to various NPs.

References

1. Capaldi Arruda SC, Diniz Silva AL, Moretto Galazzi R, Antunes Azevedo R, Zezzi Arruda MA. Nanoparticles applied to plant science: A review, Talanta. 2015;131:693-705.
2. Taylor R, Coulombe S, Otanicar T, Phelan P, Gunawan A, Lv W, Rosengarten G, Prasher R, Tyagi H. Small particles, big impacts: A review of the diverse applications of nanofluids, J Appl Phys. 2013;113:11301-11319.
3. Corral-Diaz B, Peralta-Videa JR, Alvarez-Parrilla E, Rodrigo-García J, Morales MI, Osuna-Avila P, Niu G, Hernandez-Viezcas JA, Gardea-Torresdey JL. Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L). Plant Physiol Biochem. 2014;84:277-285.
4. Khan MN, Mobin M, Abbas ZK, AlMutairi KA, Siddiqui Z.H. Role of nanomaterials in plants under challenging environments. Plant Physiol Biochem. 2017;110:194-209.
5. Ma X, Geiser-Lee J, Deng Y, Kolmakov A. Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation. Sci Total Environ. 2010;408:3053-3061.
6. Zhu H, Han J, Xiao JQ, Jin Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J Environ Monit. 2008;10:713-717.
7. Navarro E, Baun A, Behra R, Hartmann N.B, Filser J, Miao A.J, Quigg A, Santschi P.H, Sigg L. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. Ecotoxicology. 2008;17:372-386.
8. Seeger EM, Baun A, Kästner M, Trapp S. Insignificant acute toxicity of TiO2 nanoparticles to willow trees. J Soils Sediments. 2009;9:46-53.
9. Thuesombat P, Hannongbua S, Akasit S, Chadchawan S. Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth, Ecotoxicol. Environ Saf. 2014;104:302-309.
10. Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, Zhao Y, Chai Z. Effects of rare earth oxide nanoparticles on root elongation of plants, Chemosphere. 2010;78:273-279.
11. Rizwan M, Ali S, Qayyum M.F, Adrees M, Ibrahim M, Zia-ur-Rehman M, Farid M, Abbas F. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review, J Hazard Mater. 322;2017:2-16.
12. Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW. Applications of nanomaterials in agricultural production and crop protection: A review, Crop Prot. 2012;35:64-70.
13. Zuverza-Mena N, Martínez-Fernández D, Du W, Hernandez-Viezcas J.A, Bonilla-Bird N, López-Moreno M.L, Komárek M, Peralta-Videa J.R, Gardea-Torresdey J.L. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review, Plant Physiol Biochem. 2017;110:236-264.
14. Mateos-Naranjo E, Andrades-Moreno L, Davy AJ. Silicon alleviates deleterious effects of high salinity on the halophytic grass Spartina densiflora. Plant Physiol Biochem. 2013;63:115-121.
15. Zhang W, Yu X, Li M, Lang D, Zhang X, Xie Z. Silicon promotes growth and root yield of Glycyrrhiza uralensis under salt and drought stresses through enhancing osmotic adjustment and regulating antioxidant metabolism. Crop Prot. 2018;107:1-11.
16. Anselmann R, Nanoparticles and Nanolayers In Commercial Applications, J. Nanoparticle Res. 2001;3:329-336.
17. Meyer DE, Curran MA, Gonzalez MA. An Examination of Existing Data for the Industrial Manufacture and Use of Nanocomponents and Their Role in the Life Cycle Impact of Nanoproducts. Environ Sci Technol. 2009;43:1256-1263.
18. Kalteh M, Alipour ZT, Ashraf S, Marashi Aliabadi M, Falah Nosratabadi A. Effect of silica Nanoparticles on Basil (*Ocimum basilicum*) Under Salinity Stress. J Chem Heal Risks. 2018;4.
19. Rezaei R, Hoseini SM, Shabanali Fami H, Safa L, Identification and analysis of barriers in the development of nanotechnology in Iran agriculture sector from the researchers point of view. Sci Technol Policy. 2010;2:17-26.
20. Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J Chromatogr A. 1990;503:1-24.
21. Shibamoto T, Retention indices in essential oil analysis. In: Sandra P, Bicchi C (eds.) Capill. Gas Chromatogr. Essent. Oil Anal., Alfred Heuthig-Verlag, New York, 1987, pp. 259-275.
22. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed., Allured publishing corporation Carol Stream, Illinois, USA. 2007.
23. Stenhagen E, Abrahamsson S, McLafferty F.W, Registry of mass spectral data. 1974.
24. Dietz KJ, Herth S. Plant nanotoxicology. Trends Plant Sci. 2011;16:582-589.
25. Gondim Filho H, Petterson CCS, Gomes da Silva M, Mourato Pereira M, Tales M.S, André D. de A.N, Rafael S.V, Raj Gheyi H. Growth, Production and Essential Oil Content of Basil Genotypes in Hydroponic Conditions under Salt Stress. J Exp Agric Int. 2018;25:1-10.
26. Lung I, Soran ML, Opriş O, Truşcă MRC, Niinemets Ü, Copolovici L, Induction of stress volatiles and changes in essential oil content and composition upon microwave exposure in the aromatic plant *Ocimum basilicum*. Sci. Total Environ. 2016;569-570:489-495.
27. Shayganfar A, Azizi M, Rasouli M. Various strategies elicited and modulated by elevated UV-B radiation and protectant compounds in *Thymus* species: Differences in response over treatments, acclimation and interaction. Ind. Crops Prod. 2018;113:298-307.
28. Palmer-Young EC, Veit, D. Gershenzon J, Schuman MC. The sesquiterpenes(E)-β-farnesene and (E)-α-bergamotene quench ozone but fail to protect the wild tobacco Nicotiana attenuata from ozone, UVB, and drought stresses, PLoS One. 2015;10.
29. Copolovici L, Kännaste A, Pazouki L, Niinemets Ü. Emissions of green leaf volatiles and terpenoids from Solanum lycopersicum are quantitatively related to the severity of cold and heat shock treatments. J. Plant Physiol. 2012;169:664-672.
30. Copolovici L, Niinemets Ü. Temperature dependencies of Henry's law constants for different plant sesquiterpenes. Chemosphere. 2015;138:751-757.