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Original Article

Effects of nanoparticles treatments and salinity stress on the genetic structure and physiological characteristics of *Lavandula angustifolia* Mill.

Efeitos de tratamentos com nanopartículas e estresse salino na estrutura genética e características fisiológicas de *Lavandula angustifolia* Mill.

S. M. Talebi[a], M. Askary[a], R. Amiri[a], M. R. Sangi[b*] and A. Matsyura[c]

[a]Arak University, Faculty of Sciences, Department of Biology, Arak, Iran
[b]Arak University, Faculty of Sciences, Department of Chemistry, Arak, Iran
[c]Altai State University, Barnaul, Russian Federation

Abstract

Lavandula angustifolia Mill. is an aromatic herb of the Lamiaceae family, which has been widely used by humans for many centuries. In the current study, we treated *L. angustifolia* samples with various concentrations of ZnO and Fe_2O_3 nanoparticles in the presence/ absence of NaCl salinity stress to evaluate the composition of essential oils, genetic structure, glandular trichome density and cellular Zn^{2+} and Fe^{2+} contents. We used Inter Simple Sequence Repeat (ISSR) molecular markers to investigate the parameters of genetic diversity among the treated samples. Furthermore, the hydro-distilled essential oil from the aerial parts of the samples was subjected to GC and GC / MS analyses. SPSS ver. 15, PAST, PopGene, and GenAlex software were employed for statistical analyses. Intracellular concentrations of Fe^{2+} and Zn^{2+} differed under various concentrations of nanoparticles and salinity treatments, and a significant negative correlation was observed between these elements, however, nanoparticles treatment significantly increased intracellular concentrations of iron and zinc ions. We found four types of glandular trichomes on the surface of the leaf of the treated plants, and the ANOVA test revealed a significant variation for most of them. Meanwhile, the short-stalked capitate trichomes were the most frequent in most of the evaluated samples. The main and trace essential oil compounds were the same among the treated plants, meanwhile, their percentages varied among the samples. The percentages of 1,8- cineole and camphor decreased in treated plants, which affects the quality of essential oils. Parameters of genetic diversity differed among the treated samples. Furthermore, the AMOVA test demonstrated a significant genetic variation that its substantial part belonged to among treated samples. These findings revealed that the treatment of nanoparticles and salinity stress strongly influenced the genetic diversity, trichomes density, iron and zinc ions content in lavender plants.

Keywords: essential oil, genetic diversity, *Lavandula angustifolia*, nanoparticles, salinity.

Resumo

Lavandula angustifolia Mill. é uma erva aromática da família Lamiaceae, que tem sido amplamente utilizada pelo homem há muitos séculos. No presente estudo, tratamos amostras de *L. angustifolia* com várias concentrações de nanopartículas de ZnO e Fe_2O_3 na presença/ausência de estresse salino de NaCl para avaliar a composição de óleos essenciais, estrutura genética, densidade de tricomas glandulares e teores celulares de Zn^{2+} e Fe^{2+}. Usamos marcadores moleculares Inter Simple Sequence Repeat (ISSR) para investigar os parâmetros de diversidade genética entre as amostras tratadas. Além disso, o óleo essencial hidrodestilado das partes aéreas das amostras foi submetido às análises de GC e GC/MS. SPSS ver. 15, os softwares PAST, PopGene e GenAlex foram empregados para análises estatísticas. As concentrações intracelulares de Fe^{2+} e Zn^{2+} diferiram sob várias concentrações de nanopartículas e tratamentos de salinidade, e uma correlação negativa significativa foi observada entre esses elementos. No entanto, o tratamento com nanopartículas aumentou significativamente as concentrações intracelulares de íons de ferro e zinco. Encontramos quatro tipos de tricomas glandulares na superfície da folha das plantas tratadas, e o teste ANOVA revelou uma variação significativa para a maioria deles. Enquanto isso, os tricomas capitados de haste curta foram os mais frequentes na maioria das amostras avaliadas. Os compostos do óleo essencial principal e traço foram os mesmos entre as plantas tratadas, entretanto, seus percentuais variaram entre as amostras. As porcentagens de 1,8-cineol e cânfora diminuíram nas plantas tratadas, o que afeta a qualidade dos óleos essenciais. Os parâmetros de diversidade genética diferiram entre as amostras tratadas. Além disso, o teste AMOVA demonstrou uma variação genética significativa que sua parte substancial pertencia entre as amostras tratadas. Esses achados revelaram que

*e-mail: rezasangi@yahoo.com
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o tratamento de nanopartículas e o estresse salino influenciaram fortemente a diversidade genética, densidade de tricomas, teor de íons ferro e zinco em plantas de lavanda.

Palavras-chave: óleo essencial, diversidade genética, *Lavandula angustifolia*, nanopartículas, salinidade.

1. Introduction

Several aromatic and medicinal plants are found in the Lamiaceae family, which are widely distributed in various regions of the world (Détár et al., 2020; Shanaida et al., 2021). *Lavandula* is a genus of this family that contains more than 40 taxa, with several cultivars and hybrids. Some of the *Lavandula* species, such as *L. angustifolia* Mill., *L. stoechas* L. and *L. latifolia* Medik., are used in pharmaceutical, perfume and food industries. Therefore, the species mentioned or their cultivars are grown in various regions of the world (Bejar, 2020; Dong et al., 2020).

According to previous studies (Balchin, 2002; Cavanagh and Wilkinson, 2002) *L. angustifolia*, which is also called English lavender, true lavender, *L. vera* or *L. officinalis*, is considered to be a perennial herbaceous species, and is one of the most loved aromatic and medicinal herbs in the world. Its name comes from the Latin word "lavando", which is derived from the verb "lavare", which means "to wash". This species was used by the Romans to have a pleasant smell in their bath, and for its beneficial effects on human health.

The essential oil of *L. angustifolia* is highly valued due to its unique properties, such as its attractive fragrance and low camphor content. Meanwhile, its oil production is less than that of other species of the genus, such as *L. latifolia* or *L. x intermedia*. Ivanišová et al. (2021) suggested that lavender oil is used in food industries as a flavoring in beverages, ice cream, candies, and chewing gums. Recent evaluations (Ogata et al., 2020) revealed that this essential oil has anxiolytic, mood stabilizing, sedative, anti-inflammatory, analgesic, anticonvulsive, and neuroprotective properties. Its oil shows biological activities against species of several microorganisms (Białón et al., 2019): for example, it demonstrated high activity against some bacteria, yeast, and filamentous fungi (Smigielski et al., 2018). Several studies (Bejar, 2020; Ogata et al., 2020) indicated that *L. angustifolia* oil is more expensive and is often adulterated with cheaper oils.

The effects of salinity stress on physiological characteristics have been investigated in some lavender taxa, including *L. dentata* var. *dentate*, *L. dentata* var. *candicans*, *L. multifida*, *L. stoechas,* and *L. angustifolia*. The results of these evaluations showed that specific concentrations of salinity stress can affect growth and development of lavender plants (Plaza et al., 2015; García-Caparrós et al., 2016: Paraskevopoulou et al., 2020).

Zinc is one of the basic elements in plants and is used to improve soil quality in calcium carbonate-rich soils. However, its deficiency in the soil is resolved using zinc oxides or zinc sulfates. Furthermore, the application of zinc oxide nanoparticles significantly leads to zinc residues and availability (Sirelkhatim et al., 2015).

Iron plays a prominent role in metabolic processes of plants, and its deficiency is considered as a type of abiotic stress, which widely occurred in alkaline and calcium rich soils. The solubility of this element significantly declined with an enhancement in soil pH. In recent years, fertilizers contain iron oxide nanoparticles are widely used as the rich sources of Iron, due to its potential to release this ion gradually in a wide range of pH (Askary et al., 2018).

In this evaluation, we examine the effects of zinc oxide and iron oxide nanoparticles in the presence/absence of salinity stress on some physiological characteristics of lavender plants such as intracellular Fe^{2+} and Zn^{2+} content, essential oil composition, glandular trichomes type and density parameters, and genetic diversity.

The objectives of this study were (1) to investigate the effects of treatment and salinity stress on the parameters of genetic diversity, some physiological and anatomical characteristics, (2) are there significant correlations between essential oil variation, trichome density, and genetic diversity in the treated samples, and (3) how the examined nanoparticles can ameliorate the effects of salinity stress? As far as we could search, no similar evaluation is available for this plant in the world.

2. Material and Methods

2.1. Cultivation and treatment

The same plant samples of *L. angustifolia* were used in this investigation. We conducted this study in a growth chamber maintained at an air temperature of 25 to 20 °C (day/ night, respectively). The light period was 14 h during the experiment and its intensity was 420 µmol $m^{-2}s^{-1}$. We planted the samples in plastic flower pots (14 × 12 cm) that were filled with perlite and soil (1: 1).

We treated plant samples with NaCl (Merck-Germany), iron oxide, and zinc oxide nanoparticles (Sigma-Aldrich, United Kingdom), according to Table 1. We prepare salinity by adding sodium chloride solutions to pots containing 6-leafed plants. We applied the nanoparticles weekly after salinity induction by leaf spraying.

Although nanoparticles were purchased from a reputable company, they were tested with X-ray diffraction (XRD) for greater reliability. We determined the crystal phase of these nanoparticles by XRD using CuKα as a radiation source (40 kV, step size 0.05). The reference code for iron oxide nanoparticles was 00-005-0637, and those for ZnO nanoparticles were 01-079-2205. The Williamson-Hall method was used to estimate nanoparticles size (Prabhu et al., 2014).

2.2. Determination of the ionic content of Zn^{2+} and Fe^{2+}

We utilized the Reeves et al. (1999) method to measure the amounts of intracellular zinc and iron ions in the treated samples. We harvested the fresh leaves of the treated samples and washed them three times with distilled

Table 1. The applied concentrations of NaCl, Fe_2O_3 and ZnO- nanoparticles for samples treatments.

Treatment code	NaCl concentration	Fe_2O_3 nanoparticles concentration	ZnO nanoparticles concentration
1	0	0	0
2	0	0	1.5
3	0	0	3
7	0	20	0
8	0	40	0
4	120	0	1.5
5	120	0	3
6	120	0	0
9	120	20	0
10	120	40	0

water. The leaves were then dried in an oven at 75 °C for 45 h, and 1g of treated leaves was incubated in an electric oven for 15 h at 470 °C. After cooling, we dissolved the resulting ashes in 10 ml of 10% nitric and filtered them. The amounts of intracellular Zn^{2+} and Fe^{2+} were detected by the atomic absorption apparatus.

2.3. Micromorphology study

We harvested six mature and intact leaves from six flowering stems from each treatment group. We fixed these samples in a formaldehyde 5%, acetic acid 5% and ethanol 90% solution (F.A.A.) for 48 h. We dehydrated these samples in a series of ethanol (Johansen, 1940). The trichomes investigation was done according to the semi-thin sections of the leaf blade. Sections were double-stained with 1% aqueous methylene blue and carmine solutions. We shot the microphotographs using an Olympus CH_2 light microscope.

For the scanning electron microscopy (SEM) study, tiny parts of the harvested leaves were washed with distilled water to clean the surface of the samples. We then used a glutaraldehyde solution to fix the samples. A graded series of ethanol (70, 80, 95, and 100%) was used to dehydrate the samples. However, we finish off the final dehydration step with 100% or acetone. Before placing the leaves in a vacuum environment, we dried them at critical point drying (CPD) in liquid CO_2. We fixed the leaves parts on sample stubs with a double-coated carbon tape and coated them with gold using a Polaron SC 7640 sputter coater (Robards 1978). Subsequently, the samples were transferred to a SU 3500 scanning electron microscopy (SEM) instrument, and electron micrographs were shot with various magnifications at 15 kV.

2.4. Essential oil isolation, GC, and GC/MS analysis

The treated plants in the flowering stage were harvested for phytochemical analysis. We dried the aerial parts of plants at room temperature, away from sunlight, and then used them for the extraction of essential oils using the hydro-distillation method using a Clevenger-type apparatus. The following equation was employed for oil yield: Rou = (M / Bm) × 100, where M is the extracted oil mass and Bm is considered as the initial plant biomass according to da Costa et al. (2014). GC and GC–MS apparatus are used for detecting the essential oil composition. An Agilent 6890 N GC system was used for GC analysis, which is equipped with a 5975 MSD and an FID, using an HP–5 MS column (30 m × 0.25 mm, 0.25 µm film thicknesses). The injection volume was 2 µl, and the injector temperature was 200 °C with a 10:1 split ratio. Helium was used as a carrier gas and its column flow rate was 1.0 ml min^{-1} in constant flow mode. The column temperature was linearly programmed in the range of 60-280 °C with the step of 3 °C min^{-1} and held at 280 °C for 5 min. The transfer line was heated to 250 °C. The retention indices of essential oil compounds were detected by two series of n-alkanes: C8–C20 and C21–C40 under the same chromatographic conditions. We identified the constituents of oils by comparing the mass spectra and retention indices with those obtained from authentic samples, the NIST MS Search and Automated Mass Spectral Deconvolution and Identification System (AMDIS) software, and available references. The relative amounts of detected compounds were calculated from the area of the GC peak.

2.5. Phytochemical data analysis

Data related to the percentage of essential oil components were standardized. We used the Podani method (Podani, 2000) to perform the Unweighted Paired Group Method with Arithmetic Mean (UPGMA) analysis. The Multivariate Statistical Package for Windows (MVSP), ver. 2.0 was used for statistical analysis.

2.6. Genetic variability and population structure

The cetyltrimethylammonium bromide (CTAB) modified protocol (using high salt concentrations to remove polysaccharides, the use of polyvinylpyrrolidone (PVP) to remove polyphenols, an extended RNase treatment, and a phenol chloroform extraction) was used for extraction of genomic DNA from fresh leaves.

We remove RNA contamination by adding 2 µl of RNase A to 20 µl of DNA dissolved in Tris-EDTA buffer (pH = 8.0) and incubate for 3.5 h at 35 °C. According to previous genetic studies on various Lamiaceae species (Jedrzejczyk and Rewers, 2018; Sunar et al., 2020), we select seven Inter Simple Sequence Repeats (ISSR) primers for PCR reactions (Table 2). We used the following program for the PCR reaction: 5 min for the initial denaturation step at 94 °C, 40 cycles of 1 min at 94 °C, 1 min at 52-57 °C, 2 min at 72 °C and the final extension step of 7-10 min at 72 °C. The PCR results were observed by running on a 1% agarose gel and staining with ethidium bromide. We used a 100 bp molecular size ladder in order to estimate the fragment size.

2.7. Molecular analyzes

We colinked the observed ISSR bands as binary characteristics (absence = 0, presence = 1) and used them to calculate genetic diversity parameters, such as effective allele number, Shannon information index, polymorphism percentage, expected heterozygosity, unbiased expected heterozygosity. Moreover, Nei's genetic distance among the treated samples was employed for UPGMA tree clustering (Huson and Bryant 2006; Freeland et al. 2011). We employed GenAlex 6.4 to analyze molecular variance (AMOVA) test with 1000 permutations (Peakall and Smouse, 2006; Meirmans, 2012).

3. Results

3.l. XRD analysis

We performed an XRD analysis to determine the crystal structure and phase composition of the used samples and compared them with the standard samples (Figures 1, 2). The average crystallite size for ZnO and Fe_2O_3 nanoparticles was calculated at 75 nm and 3-5 nm, respectively. In Fe_2O_3 nanoparticles the smoothing type was polynomial (cubic).

3.2. Effects of salinity stress and treatment on Fe^{2+} content

We detected the lowest amount (134.62 µg/g DW) of intracellular Fe^{2+} ions in samples stressed with 120 mM salinity, while the highest amount (676.15 µm/g DW) belonged to plants treated with 40 µm nanoparticles.

Table 2. Sequences and characteristics of the used ISSR primers.

No.	Sequence (5'→3')	Annealing temperature (°C)
1	(GACA)4	49.0
2	(GAC)6	63.4
3	(GA)8T	54.3
4	(CA)6AC	48.5
5	(AGG)6	67.2
6	(GA)6CC	54.3
7	(CT)8TG	50.0

The application of iron oxide nanoparticles increased intracellular Fe^{2+} amounts in salinity-stressed plants, compared to control samples. The results revealed that the Fe^{2+} amount decreased by almost 71% under 120 mM salinity stress in the absence of both nanoparticles. Meanwhile, the Fe^{2+} amounts in samples treated with 20 and 40 µm Fe_2O_3 nanoparticles (under 120 mM salinity stress) decreased by 45.5% and 55.7%, respectively, compared to controls.

In samples stressed by 120 mM salinity, the application of zinc oxide nanoparticles significantly decreased the intracellular iron ion content. Under treatment with 1.5 µm and 3 µm ZnO nanoparticles (in the absence of

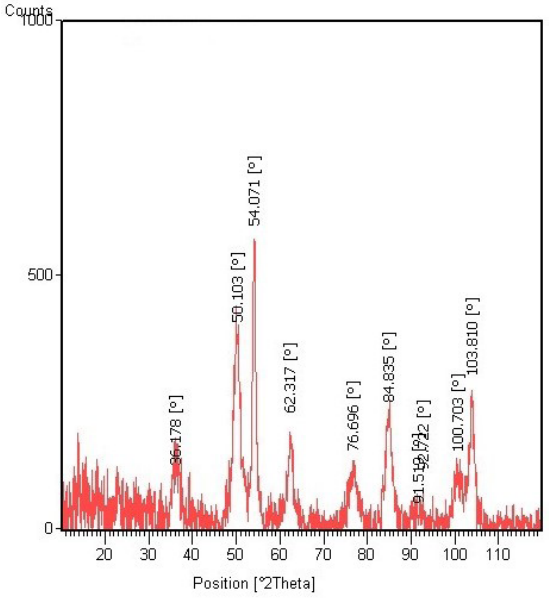

Figure 1. The XRD pattern for the used Fe_2O_3 nanoparticles.

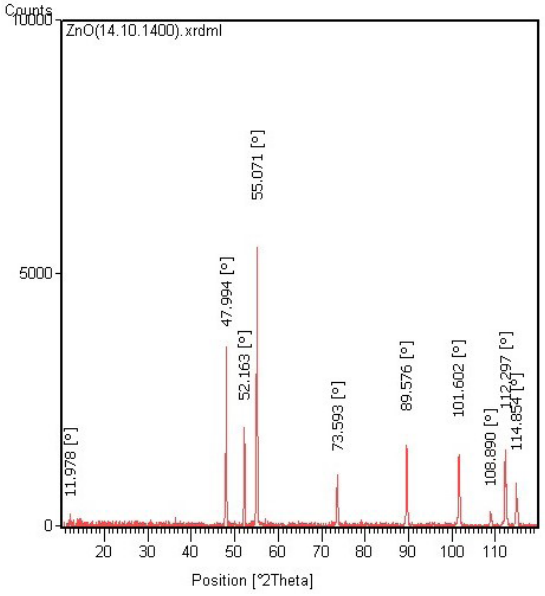

Figure 2. The XRD pattern for the used ZnO nanoparticles.

salinity stress), Fe^{2+} amounts decreased by 52 and 82%, respectively, compared to controls. Furthermore, in plants stressed by salinity, the application of 1.5 µm and 3 µm ZnO nanoparticles decreased intracellular Fe^{2+} contents by 92 and 97%, respectively (Figure 3).

3.3. Effects of salinity stress and nanoparticles treatment on Zn^{2+} concentration

We detected the lowest intracellular concentration of Zn^{2+} under 120 mM salinity stress. However, the highest amount of this ion belonged to samples treated with 3 µm ZnO nanoparticles in the absence of salinity stress, which was 1.89 times higher than controls. The application of ZnO and Fe_2O_3 nanoparticles treatment significantly increased the intracellular Zn^{2+} concentration in plants stressed by 120 mM salinity. Meanwhile, Zn^{2+} ion concentrations in 40 µm Fe_2O_3 and 3 µm ZnO nanoparticles (under 120 mM salinity stress) were reduced by 39.36% and 28.66%, respectively, compared to the control plants (Figure 4).

3.4. Glandular trichomes density

We observed four types of glandular trichomes on the leaves epidermal surface of the evaluated samples: peltate, digitate, short and long-stalked capitate trichomes. The ANOVA test revealed a significant variation for all types, except for short-stalked capitate trichomes (Table 3). The more frequent trichomes type was not the same among the treated samples; meanwhile, in most cases, it was a short-stalked capitate. Furthermore, long-stalked capitate trichomes were rarely observed in the evaluated samples. The highest number of digitate (27.33) and peltate (12.5) trichomes were reported from plants treated with 1.5 µm ZnO nanoparticles and 20 µm Fe_2O_3 nanoparticles treated plants (in the absence of salinity), respectively. Furthermore, the lowest number of these trichomes was found in 40 µm Fe_2O_3 nanoparticle treated plants (0 mM salinity) and 120 mM salinity stressed plants treated with 1.5 µm ZnO nanoparticles, respectively.

We detected the highest number of total trichomes in 1.5 µm ZnO nanoparticles in the absence of NaCl stress and treatment with Fe_2O_3 nanoparticles, while the lowest number was registered in plants treated with 40 µm Fe_2O_3 nanoparticles in the absence of 120 mM NaCl stress and treatment with ZnO nanoparticles treatment.

3.5. Chemical composition of essential oils

We found four classes of compounds in the essential oils of the evaluated samples: oxygenated monoterpenes (78-87.2%)

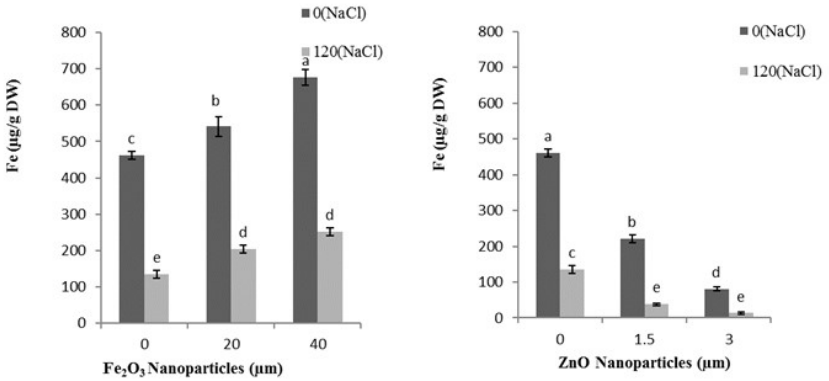

Figure 3. Effects of Fe_2O_3 and ZnO nanoparticles on concentration of the leaves Fe^{2+} amounts. Whiskers indicate the standard deviation, and dissimilar letters showed the significant variation based on Duncan test ($P \leq 0.05$).

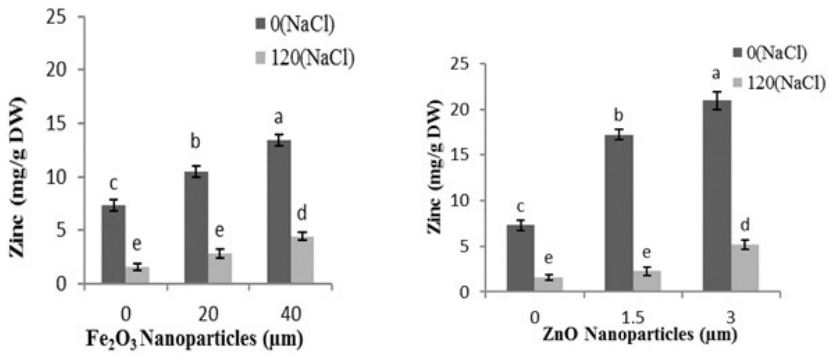

Figure 4. Effects of ZnO and Fe_2O_3 nanoparticles on intracellular Zn^{2+} concentration. Whiskers reveal the standard deviation, and dissimilar letters showed the significant variation according to Duncan test ($P \leq 0.05$).

Table 3. Mean and standard deviation of glandular trichomes in the treated plants (asterisks** show the significant variation $P \leq 0.05$, treatment's code as in Table 1).

Treatment		Digitate**	Peltate**	short-stalked capitate	Long-stalked capitate**
1	Mean	10.33	9.67	17.50	---
	No.	6	6	6	6
	Std. dev.	2.66	2.81	9.65	---
2	Mean	27.33	9.00	18.83	---
	No.	6	6	6	6
	Std. dev.	14.38	2.82	5.19	---
3	Mean	21.83	10.50	16.83	---
	No.	6	6	6	6
	Std. dev.	6.59	2.51	4.45	---
4	Mean	15.17	6.00	25.83	---
	No.	6	6	6	6
	Std. dev.	11.44	3.29	7.63	---
5	Mean	16.00	9.17	12.67	---
	No.	6	6	6	6
	Std. dev.	9.36	2.99	5.32	---
6	Mean	12.83	5.67	16.50	1.00
	No.	6	6	6	6
	Std. dev.	14.22	2.25	4.37	1.27
7	Mean	9.50	12.50	23.50	---
	No.	6	6	6	6
	Std. dev.	0.71	4.95	6.36	---
8	Mean	8.67	9.83	17.00	---
	No.	6	6	6	6
	Std. dev.	5.09	3.49	5.25	---
9	Mean	15.83	11.83	20.33	---
	No.	6	6	6	6
	Std. dev.	2.86	3.13	4.18	---
10	Mean	15.17	12.33	17.33	---
	No.	6	6	6	6
	Std. dev.	5.78	2.16	5.75	---

were the main class of components, followed by monoterpene hydrocarbons (5.1-6.0%). Other compounds (such as aldehydes) were the third class of compounds. We detected oxygenated sesquiterpenes in trace amount; meanwhile, we could not detect any sesquiterpene hydrocarbons compound in the oil of the samples studied (Table 4).

The type of main essential oil compounds was the same among the samples evaluated. In this regard, 1.8-cineole (42.9-57.7%) was the main component. The highest amount (57.7%) of 1.8-cineole was observed in samples treated with 1.5 µm ZnO nanoparticles under 120 mM salinity stress, while its lowest percentage (40.6%) was detected in plants treated with 1.5 µm ZnO nanoparticles in the absence of salinity stress.

The second major oil compound differed among the samples, borneol (11.3-19.8%) was the second oil compound in samples no. 5, 6, 7, 9 and 10, while it was camphor (15.3-18.8%) in the rest of the samples. Additionally, the trace compounds were the same between the populations, while their percentages differed. Furthermore, the highest (19.8%) and lowest (11.3%) percentages of borneol were reported in plants stressed by 120 mM salinity under 40 µm and 1.5 µm of Fe_2O_3 and ZnO nanoparticles treatments, respectively. We found the highest amount (18.8%) of camphor in 1.5 µm ZnO nanoparticles (in the absence of salinity stress), but its

Table 4. Essential oil composition of the nanoparticles and salinity-treated plants (treatment's code as in Table 1).

compounds/ treatment code	1	2	3	4	5	6	7	8	9	10
α-pinene	1.0	0.9	0.8	0.8	1	0.9	1.1	0.9	0.9	0.8
Camphene	1.0	0.9	0.8	0.8	1.0	0.9	1.1	0.9	1.0	0.8
β-pinene	0.7	0.7	0.6	0.6	0.7	0.7	0.8	0.7	0.7	0.6
P-cymene	0.5	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4
O-cymene	1.4	1.3	1.4	1.4	1.4	1.39	1.4	1.35	1.4	1.2
Limonene	1.2	1.4	1.3	1.0	1.4	1.7	1.7	1.4	1.5	1.2
1.8-cineole	52.8	40.6	44.3	57.7	43.8	42.6	51.6	42.9	43.2	44.0
cis-Verbenol	0.4	0.28	0.3	0.5	0.7	0.4	0.6	0.6	0.4	0.7
Camphor	16.7	18.8	15.0	14.2	15.9	16.2	13.0	17.7	16.6	14.3
Borneol	12.9	14.9	14.7	11.3	17.6	17.3	14.8	17.7	17.7	19.8
Terpinen-4-ol	0.7	0.9	0.8	1.0	0.6	1.0	1.1	1.0	1.13	1.01
Cryptone	1.9	2.0	1.6	1.4	2.1	1.9	2.1	2.5	2.17	1.69
α-Terpineol	0.5	0.6	0.5	0.4	0.6	0.6	0.5	0.6	0.6	0.6
Isobornyl formate	1.8	1.5	1.92	1.7	2.1	2.1	1.4	1.8	2.1	1.9
Cumine	1.0	1.76	2.8	0.7	1.3	1.5	1.1	1.3	1.4	1.3
Caryophyllene Oxide	1.1	1.5	1.4	0.9	1.6	1.7	1.5	1.4	1.5	1.5
epi- α -cineol	0.6	1.4	1.09	0.7	1.4	1.77	1.01	1.24	1.47	1.4
Monoterpene hydrocarbons	5.7	5.5	5.4	5.1	5.9	6.1	6.5	5.7	6.0	5.0
Oxygenated monoterpenes	86.5	79.5	78.4	87.2	82.7	81.6	84.7	84.2	83.2	83.6
oxygenated sesquiterpene	1.1	1.49	1.4	0.9	1.58	1.7	1.45	1.4	1.5	1.49
Other compounds	2.9	3.3	4.76	2.4	3.4	3.58	2.5	3.1	3.5	3.19
Total (%)	96.2	89.8	90.0	95.6	93.6	93.0	95.2	94.36	94.2	93.3

lowest amount (13.0%) detected in samples treated with 20 µm Fe_2O_3 (under salinity stress 0 mM).

According to UPGMA tree of essential oil composition, we divided the treated samples into four groups, group I: samples no. 4, 7 and 1, group II: sample no. 3, group III: samples no. 5, 6, 8, 9 and 10 and group IV: sample no. 2 (Figure 5).

3.6. Study of genetic diversity

The AMOVA test demonstrated a significant genetic diversity among the treated samples (PhiPT=0.591, $P \leq 0.01$, Figure 6). The estimated parameters of genetic diversity revealed that expected heterozygosity (He) and unbiased expected heterozygosity (UHe) were 0.115±0.004 and 0.123±0.005, respectively. Furthermore, Shannon's information index (I) was 0.176, and the number of effective alleles was 1.191±0.008. These findings revealed a high level of genetic diversity among the treated samples. According to the UPGMA tree of genetic distance (Figure 7), the treated samples were clustered into three genotype groups, I: samples no. 2, 4 and 6, II: samples no. 1, 8 and III: samples no. 3, 4, 7, 9 and 10.

4. Discussion

In the current study, we evaluated the effects of salinity stress on some physiological characteristics of lavender in the presence/ absence of nanoparticle treatment. Previous investigations (García-Caparrós et al., 2016: Paraskevopoulou et al., 2020) revealed that NaCl concentrations greater than 60 mM affect plant growth and development in lavender species. However, in some *Lavandula* species, salinity concentrations of 100 mM and 200 mM decreased plant biomass (Plaza et al., 2015). Therefore, we treated the samples with salinity concentrations of 0 and 120 mM.

Stein (2010) suggested that 60% and 30% of the world's soil are iron and zinc deficient, affecting crop production. Therefore, the application of these elements as fertilizers in nano shapes decreases the adverse effects. The application of Fe_2O_3 nanoparticles increased the intracellular iron ion concentration of the leaf in both control and nanoparticles-treated plants. A similar condition was found for ZnO nanoparticles and the intracellular concentration of leaf zinc ion concentration. Our findings agree with a previous investigation in which significant correlations were detected between ZnO and the amount of Fe_2O_3 nanoparticles and these elements in the leaves and roots of *Raphanus sativus* (Mahmoud et al., 2019).

We found that intracellular Fe^{2+} amount decreased in plants and those that were treated with ZnO nanoparticles. Zinc disrupts plant metabolism by affecting the rate of uptake as well as the activity rate of some enzymes at their function site. A negative interaction was reported

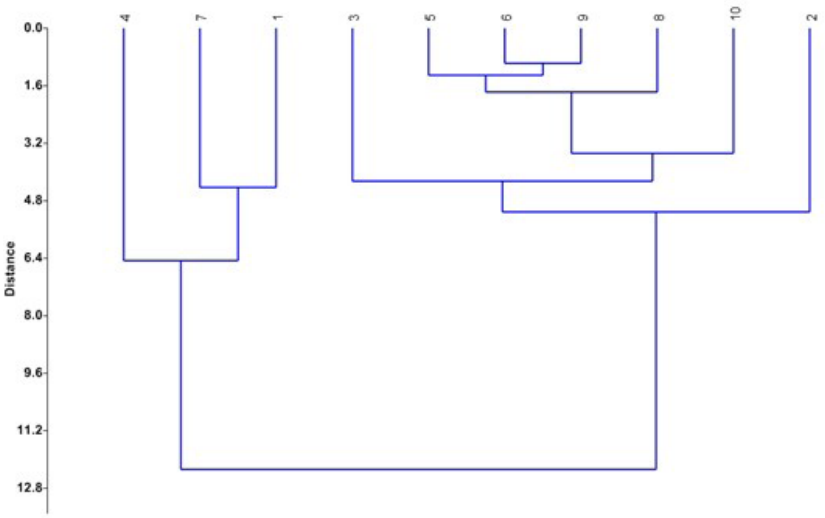

Figure 5. UPGMA tree of the studied samples according to essential oil compositions (treatment's code as in Table 1).

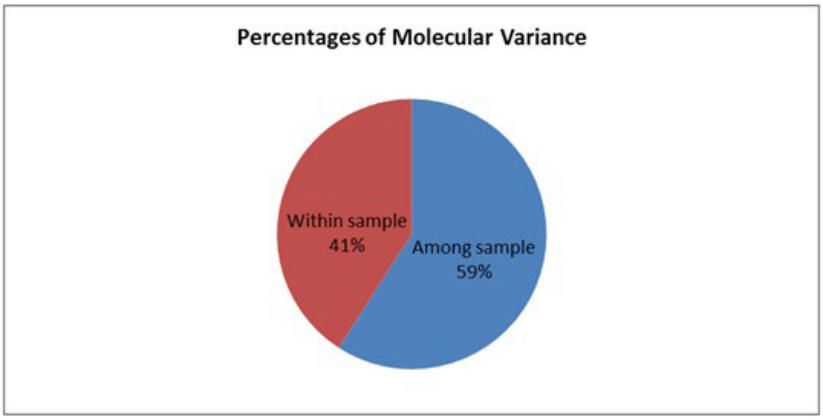

Figure 6. Results of the AMOVA test revealed a significant genetic diversity between the treated samples

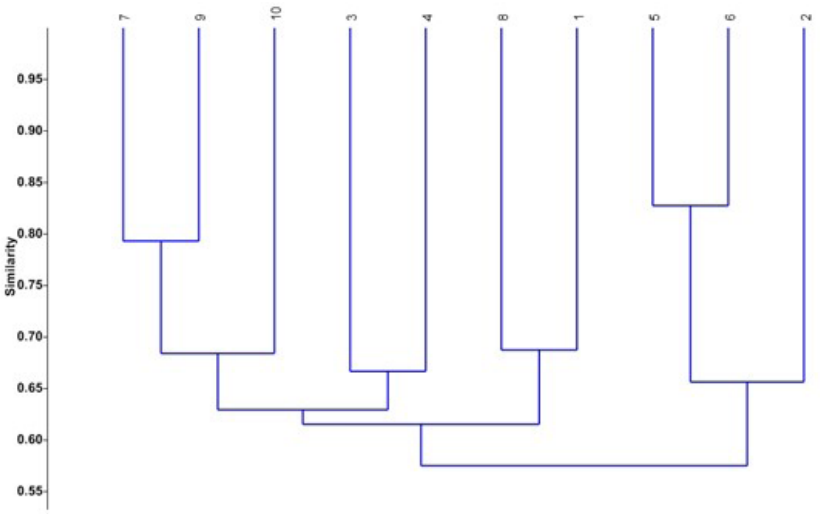

Figure 7. UPGMA tree of the evaluated samples based on the molecular ISSR data (treatment's code as in Table 1).

between zinc and iron ions. The antagonism between these elements is related to the competitive effect at the adsorption site. Furthermore, Tandon (2002) indicated that each increase in zinc uptake reduces intracellular iron ion accumulation. It seems that the zinc ion inhibits chelation processes during the absorption and transfer of iron ions from the roots to the shoots. Moreover, the effect of dilution and increased iron transfer of iron from roots to shoot is another effective factor in the interaction between these elements (Cakmak, 2000).

According to previous studies (Talebi et al., 2012, 2018; Talebi and Shayestehfar, 2014), trichomes play several important roles in plants in the survival and adaptation of their habitats. Two main types of trichomes, glandular and non-glandular, cover aerial surfaces of plant organs. However, in Lamiaceae taxa, the glandular ones are more important than the non-glandular trichomes, due to their role in the biosynthesis, storage, and secretion of essential oil.

We found four types of glandular trichomes in the evaluated samples, whose densities varied between the treated samples. Furthermore, the type of trichome that was more frequent varied between treated samples. Under ZnO nanoparticles treatments (except for 120 mM NaCl stress and 1.5 µm ZnO nanoparticles treatment), the more frequent trichome was digitate, while it was short-stalked capitate in other treated samples. Our findings revealed that the kind and density were significantly influenced by salinity stress and also nanoparticles treatment. However, the maximum and minimum numbers of trichomes were detected in samples treated with 1.5 µm ZnO and 40 µm Fe_2O_3 nanoparticle-treated samples, respectively. Since different types of stress induce the creation of trichomes in plants, our findings indicated that 1.5 µm ZnO nanoparticles created high stress in the evaluated plants. Our findings are consistent with previous investigations on *Hordeum sativum* L. (Rajput et al., 2021).

Furthermore, Moghimipour et al. (2017) investigated the effect of ZnO nanoparticles on leaf glandular trichome in *Ocimum sanctum* L., and reported the highest number of trichomes in plants treated with 1.5 g/l of these nanoparticles.

However, a reverse condition was found for the treatment of 40 µm Fe_2O_3 nanoparticles. Askary et al. (2016) reported similar results, in which Fe_2O_3 nanoparticles significantly decreased trichomes density in *Mentha* × *piperita* L.

Although the type of major oil components was the same among the treated samples, their percentages differed. 1.8-cineole was detected as the main oil constituent in all examined samples. It is a natural monoterpene and is also known as eucalyptol. Due to its pleasant aroma and taste, this compound is usually used in food, cosmetics, and fragrances. Eucalyptol is used in traditional and modern medicine for respiratory tract infections, such as bronchitis, common cold, and sinusitis. Investigations revealed that the compound improves the frequency of ciliary beats of the mucus membrane and shows secretolytic and bronchospasmolytic, antiseptic and antimicrobial properties (Juergens, 2014). The percentage of 1.8-cineole changed by more than 17% between samples. Its lowest amount was recorded in 1.5 µm ZnO nanoparticle-treated samples, while the highest amount belonged to 1.5 µm ZnO nanoparticle-treated samples under 120 mM salinity stress. However, 1.8-cineole percentage in most treated plants were less than the control plants. It demonstrated that application of different nanoparticles in the presence or absence of salinity stress decreases 1.8-cineole amount, which reduces the quality of essential oil.

Similar results were reported by Moghimipour et al. (2017), who evaluated the essential oil composition of *Ocimum sanctum* treated with various concentrations of ZnO nanoparticles. They reported 1.8-cineole as the second main oil compound, and its lowest amount was recorded in plants treated with 1.5 g/l ZnO nanoparticles.

Camphor is the second main oil compound in some treated plants and its percentage varied by more than 30% among the treated plants. It is a toxic compound that can be fatal to children by ingestion even in a very small amount (Narayan and Singh, 2012). Our finding revealed that the amount of camphor in most nanoparticles-treated samples under salinity stress is less than that of the control plants. Therefore, these treatments increased the quality of lavender oil.

Hosseinpour et al. (2020) indicated that various molecular markers can detect genetic polymorphisms and diversity among plants individuals which are created by environmental stress such as salinity (Saleh, 2016), heavy metals (Hossein-Pour et al., 2019), pesticides (Taspinar et al., 2017) and nanoparticles (Hosseinpour et al. 2020). Therefore, we used ISSR molecular markers to detected genetic diversity and polymorphism within and among the treated samples. We found a high level of genetic diversity among the treated samples, which its great part belonged to among samples. This finding revealed the creation of genetic diversity in plants by salinity stress and nanoparticles treatments. However, samples treated with the same type of nanoparticles at different concentrations did not cluster closely. It revealed that the rate of induced genetic polymorphism is directly related to the concentration of the nanoparticles. Similar results have been reported for *Solanum lycopersicum* L., samples were treated with ZnO nanoparticles (Hosseinpour et al., 2020).

It seems that the gene conferring salinity tolerance has been overexpressed under 120 mM salinity stress. However, the treatment of plants with various types and concentrations of nanoparticles can moderate the damaging effects of salinity stress to some extent. The observed genetic diversity among the treated samples was closely related to various levels of expression of salinity stress gene. The ISSR molecular markers can detect polymorphism in DNA regions (Zietkiewicz et al., 1994). Similar results were reported by Abbas et al. (2021), which suggested that some microsatellite salinity tolerance markers showed polymorphism in *Aegilops tauschii* Coss. populations.

5. Conclusion

We examined the effects of various concentrations of two nanoparticles, ZnO and Fe_2O_3, in the presence/absence of NaCl salinity stress on some physiological

characteristics and parameters of genetic diversity of lavender plants to observe plant responses to these treatments. The results revealed that intracellular amounts of Zn^{2+} and Fe^{2+} decreased under salinity stress, while the application of ZnO and Fe_2O_3 nanoparticles increased these ions concentrations in plants. We recorded four types of glandular trichomes, and most of them varied significantly among the treated plant samples. However, the short-stalked capitate and digitate trichomes were detected as the most frequent types among the samples. Although the type of main and trace oils compounds was the same among the treated samples, their percentages differed and affected the oil quality. The parameters of genetic diversity and polymorphism differed between samples. Therefore, we found that nanoparticles and salinity treatments lead to a significant genetic diversity, which its great part is assigned among the samples. Our findings indicated that the treatment of nanoparticles and salinity has significant effects on the evaluated physiological traits and genetic structures evaluated in lavender.

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