Industrial Crops & Products xxx (xxxx) 115429

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Essential oil composition, physiological and morphological variation in *Salvia abrotanoides* and *S. yangii* under drought stress and chitosan treatments

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ARTICLE INFO

Keywords:
Salvia
Essential oil
Drought stress
Chitosan
Physiology

ABSTRACT

Several *Salvia* species are among the most valuable aromatic herbs used for industrial and pharmaceutical applications. Hence, under greenhouse trial conditions, the effects of chitosan treatment (0, 100, and 200 mg L^{-1}) and water deficiency stress on the morphological, physiological, and biochemical features of two Salvia species were examined. Significant changes were seen in chlorophyll a, root volume, dry and fresh weight, and H_2O_2 concentration as a result of drought stress. Significant influence of chitosan was found for all studied parameters except root length and malondialdehyde (MDA) content. Hydrogen peroxide (H_2O_2), proline, and MDA were elevated, while photosynthetic pigments decreased under drought stress. The highest essential oil (EO) content (2.20% d.b.) was recorded under moderate stress condition in the absence of chitosan treatment. Using chitosan topically, it is possible to offset the impact of water scarcity on EO content decline and enhance EO compositions. The compensatory effects of chitosan application under stress conditions were observed on the abundance of EO constituents, such as 1,8-cineol, camphor, bornyl acetate, α-bisabolol, α-cadinol, and α-humulene. Moreover, present results suggested that chitosan application can alleviate the drought damage in studied *Salvia* species.

1. Introduction

The subgenus *Perovskia* Karl., consists of valuable medicinal and aromatic plants of Lamiaceae family. It includes seven species in Flora Iranica, in which three species, *P. abrotanoides* Karel., *P. atriplicifolia* Benth., and *P. artemisoides* Boiss., are found native to Iran (Ghaffari et al., 2019). The new scientific names of two mentioned species are *Salvia abrotanoides* Karel. (*Perovskia abrotanoides* Kar.) and *Salvia yangii* B.T. Drew (*Perovskia atriplicifolia* Benth.) (Bielecka et al., 2021). *S. abrotanoides*, also known as "Brazambel" in Persian, is a plant that grows in rocky places regularly from Northeastern Iran to Northern Pakistan and Northwestern India (Pourhosseini et al., 2018). Different Salvia components have been shown to be effective in treating a variety of common illnesses, including leishmaniasis, fever, typhoid, headache, toothache, atherosclerosis, liver fibrosis, and cough (Pourhosseini et al., 2018; Sairafianpour et al., 2001).

These plants produce essential oils (EOs) which are characterized by a strong aroma. EO components act as biological factors that are associated with environmental adaptation. The differences of EOs biological activities are attributed to variations in their chemical composition influenced by environmental and genetic factors (Milos et al., 2001; Zgheib et al., 2020; Morshedloo et al., 2018). Previous studies showed that *Salvia* EO contains monoterpenes as major constituents with lower contribution of sesquiterpene content (Ghaffari et al., 2019). Camphor is one of the most abundant monoterpenes while, α-humulene, δ-cadinene, β-caryophyllene, and γ-cadinene were found as its main sesquiterpene (Ghaffari et al., 2018).

Drought, salinity, temperature shocks, UV rays, and toxic metals are just a few of the environmental conditions that may have an impact on

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https://doi.org/10.1016/j.indcrop.2022.115429
Received 9 April 2022; Received in revised form 22 July 2022; Accepted 26 July 2022
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physiological processes, including the manufacture of specialized metabolites like EO components (Bistgani et al., 2017; Zhang et al., 2017; Jiao et al., 2021). Water shortages can affect agricultural productivity. Reactive oxygen species (ROS) are produced under drought stress, which may damage proteins, nucleic acids, and membrane lipids via peroxidation (Tambussi et al., 2000; Yang et al., 2009). In response, plants would increase antioxidant enzymes activities and concentrations of different small molecular weight antioxidants (Sharma and Dubey, 2005). Furthermore, changes in malondialdehyde (MDA), protein, proline, hydrogen peroxide (H_2O_2), and chlorophyll content could be related to drought stress (Karataş et al., 2014). Physiological reactions to stress may conduce adaptability to immediate water deficit for short period. However, these strategies may not be sufficient to overcome of severe stress conditions for a long time (Yang et al., 2009).

Due to plant hormones or other biologic agents known as bio-elicitors, which set off a chain reaction of pathways to respond to environmental challenges, plants have another method of responding to environmental stresses (Zandalinas et al., 2018). Bio-elicitors are used to soils or plants to regulate and enhance the physiological response efficiency (Moolphuerk and Pattanagul, 2020), The chitosan was used as a bio-stimulant to enhance the resistance of plants to water deficit.

To increase the synthesis of secondary metabolites in medicinal and aromatic plants, chitosan elicitor is often used (Bistgani et al., 2017). Chitin is converted into the polymer chitosan via alkaline N-deacetylation (Pongprayoon et al., 2022). Bittelli et al., (2001) reported that chitosan led to a decline in plant transpiration of pepper plants; therefore, water deficit (26–43%) did not decrease dry matter yield. Bistgani et al. (2017) reported that chitosan treatment reduced the negative impact of stress conditions on essential oil yield and dry matter of *Thymus daenensis* by reduction of lipid peroxidase level and proline accumulation, thus protecting the integrity of cell membranes.

Although some studies were published on the *Salvia* EO composition in different growth stages (Alamdari, 2021), various organs (Pourhosseini et al., 2018), and illuminated with different wavelengths (Ghaffari et al., 2019), to our knowledge, no study has been published on an interaction of chitosan application and drought stress in morphological and physiological traits as well as essential oil compositions of *S. abrotanoides* and *S. yangii*. Therefore, various EO compositions, physiological, and morphological traits were examined in this research. This study's primary goal was to identify the changes in these two species' physiological and morphological features as a result of drought stress and chitosan therapy.

2. Materials and methods

2.1. Experimental plant materials

The seeds of *S. abrotanoides* and *S. yangii* were collected from two provinces of Iran (Khorasan Razavi and Sistan & Baluchestan, respectively). The plants were identified by Dr. Mehdi Rahimmalek (Isfahan University of Technology, Isfahan, Iran) using the Flora Iranica and the voucher specimens, preserved in the Herbarium of Isfahan University of Technology under specimen numbers 13363 and 13368 for *S. abrotanoides* and *S. yangii*, respectively. This research was done in the summer of 2019 at the Chah-anari field at the Isfahan University of Technology in Isfahan, Iran, under greenhouse conditions (daily temperatures of 18–28 °C and photoperiod of 14 h). At the start of spring 2018, the seeds of each species were planted separately in the nursery's sandy soil, and three weeks after germination, each seedling was transplanted into a separate pot. The plants were pruned that year throughout the winter. The two-year old plants were transplanted into pots containing a mixture of sand and silt loam soil (53:33:14%) to be grown under same condition outside the greenhouse from 5th April to 5th May. After one month, plants were transferred to the greenhouse and treated by water deficit and chitosan from 5th May to 5th July.

2.2. Experimental design and treatments

The factorial experiment was performed using the randomized complete design (RCD) with three replications. Plants were subjected to three water regimes: (i) optimal regime (control), (ii) moderate regime, and (iii) severe regime combined with three levels of chitosan (0, 100, and 200 mg L^{-1}). Chitosan is soluble in acetic acid; therefore, the control samples were treated with acetic acid alone. Appropriate amounts of chitosan were dissolved in acidified water using acetic acid to create chitosan solution. Three water regimes—every other day (control), every three days (moderate), and every five days (intensive)—were used to irrigate pots (severe). It is interesting that the water supplied to each pot was determined by the pot's field capacity. Simultaneously with the irrigation regime, chitosan was sprayed on plant leaves every 5 days. Two months after treatment, sampling of the plants was done at flowering stage. No fertilizer or pesticide was utilized during the trial, and weeds were manually controlled.

2.3. Measurement of parameters

2.3.1. Morphological studies

Morphological data were recorded at harvest time and comprised of root number, root volume (mL), root length (cm), fresh root weight (g), dry root weight (g), fresh stem weight (g), and dry stem weight (g). The water replacing method was used to measure the root volume in a cylinder (Price and Tomos, 1997). The aerial and ground parts of the studied species were collected separately at flowering stage and dried at room temperature (25 °C) for ten days.

2.3.2. Malondialdehyde (MDA) content

To determine the concentration of MDA, the method of Heath and Packer (1968) was used with minor modifications. Firstly, 0.1 g of fresh leaves was homogenized in 2 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 10000g for 5 min at 4 °C. Then 0.1 mL supernatant was blended with 0.4 mL of reagent (0.5% thiobarbituric acid in 20% (w/v) TCA). Finally, the mixture was boiled for 30 min at 95 °C, immediately cooled, and centrifuged at 10000g for 10 min. The absorbance of the mixture was read at the wavelength of 450, 532 and 600 nm. The MDA contents were calculated basis as follows:

$$(\mu mol\ MDA\ g/f.w.) = 6.45 * (OD_{532}\text{-}OD_{600}) - 0.56OD_{450}.$$

2.3.3. Assay of hydrogen peroxide (H_2O_2)

H_2O_2 content was calculated according to the method of Zhou et al. (2006). Fresh leaves (0.1 g) were ground in 2 mL of 0.1% TCA. 0.1 mL of supernatant was added to 0.1 mL of 100 mM phosphate buffer (pH 5.6) and 0.2 mL of potassium iodide after the homogenate had been centrifuged at 10000g for 5 min at 4 °C (1 M). In a dark environment, the mixture was incubated for one hour before the absorbance at 390 nm was measured. The standard curve of hydrogen peroxide was plotted using specific concentrations of the H_2O_2 solution.

2.3.4. Chlorophyll contents

The chlorophyll contents were measured based on the method described by Lichtenthaler and Wellburn (1983). In brief, 0.5 g of fresh leaf samples were gently powdered and macerated with 20 mL of 80% (v/v) acetone gently in a dark room. The solution was centrifuged for 10 min at 6000g. In the next step, the absorbance of the extract was read at 645, 663 and, 470 nm, respectively, using a spectrophotometer (U-1800 UV/VIS, Hitachi, Japan). The contents of chlorophyll a, chlorophyll b, and carotenoids were expressed as mg per gram of leaf fresh weight.

2.3.5. Proline quantification

At the first, 10 mL of 3% (w/v) 5-sulfosalicylic acid was added to leaf powders (200 mg) and vortexing for 5 min. Then, the samples were

centrifuged at 10000g for 10 min and the supernatants were transferred in clean tubes and spun again. After the addition of 2 mL of acetic acid and 2 mL ninhydrin solution (0.15% (w/v) ninhydrin in glacial acetic acid), the mixture was incubated at bath water for 1 h, then transferred on the ice. The solution was then given 4 mL of toluene while being subjected to laminar air flow, and the absorbance was measured at 520 nm. Proline was measured in accordance with a standard curve that was created using proline concentrations that were known.

2.3.6. Gas Chromatography-mass spectrometry (GC/MS) analysis

The aerial parts of *Salvia* species were dried at room temperature (25 ˚C) for ten days. The obtained dry weight was used as the dry weight in calculating the percentage of EO. EOs of the samples were obtained by hydrodistillation of 50 g of plant material using a Clevenger apparatus for six hours. EOs obtained were stored in dark vials at 4 ˚C. EO content (% v/m) was calculated by following formula (Zhang et al., 2015):

$$\text{EOcontent}(\%) = \frac{\text{volumeofEOobtained}}{\text{Massofdrymatter}} \times 100$$

The quantification of volatile oil components of studied *Salvia* species was done using gas chromatography-mass spectrometry (GC-MS). GC-MS apparatus was a Varian 3400 GC/MS (California, USA) system equipped with a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 μm J&W Scientific Inc) Agilent Technologies. The separation was carried out with the following parameters: oven temperature 50–280 ˚C, increasing at a rate of 4 ˚C min^{-1}; injector temperature 240 ˚C. The carrier gas was helium with a flow rate of 2 mL min^{-1} and a split ratio of 1:60, and the ionization energy was 70 eV. The scan lasted one second and the collected mass varied from 40 to 300 amu.

EO constituents were identified based on the retention indices with reference to C9-C22 n-alkanes obtained on a DB-5MS column by comparing their mass spectra with those recorded in the NIST 08 (National Institute of Standards and Technology), Wiley275. L (ChemStation data system) and those reported in the literature (Adams, 2007). Using area percentages is considered useful to show the comparative data. Nevertheless, according the IOFI orientations, the application internal standard and response factors should be progressively applied. Quantification of the relative amounts of the individual component was performed according to the area percentage.

2.4. Statistical analysis

Data from two Salvia species were collected, and SAS version 9.4 (SAS Institute, Cary, NC, USA) was used to analyze the data using factorial experiments with three replications. The Duncan's multiple range test was used to compare means with a 95% confidence level. Using Statgraphics software version 18.2.04, cluster analysis and principal component analysis (PCA) were performed to ascertain the relationships between variables.

3. Results

3.1. Variance analysis of morphological and physiological traits

Analysis of variance indicated that the effect of drought stress was significant for root number, fresh root weight, root volume, dry root weight, H_2O_2, and chlorophyll a (Table 1). Significant effect of chitosan was also observed for all studied traits except root length and MDA. The effect of drought stress and chitosan interaction on root length and fresh stem weight was not significant. Significant interaction between species and stress observed for all studied morphological traits and H_2O_2. Finally, three-way interaction among species × stress × chitosan was significant for all studied traits except physiological characteristics (Table 1).

3.1.1. Photosynthetic pigments

According to an ANOVA study, chitosan and drought stress had a substantial impact on chlorophyll a. Additionally, the chitosan-stress interaction revealed significant variations in the contents of chlorophyll a, chlorophyll b, and carotenoids (Table 1). According to the findings of the mean comparison of the chitosan × stress interaction, the amount of chlorophyll a and carotenoids reduced as the degree of stress increased on the control level of chitosan use. The highest chlorophyll a (8.97 mg g^{-1}) was related to 100 mg L^{-1} chitosan foliar application and severe stress level, while the lowest chlorophyll a (2.31 mg g^{-1}) was found in the absence of chitosan and severe water deficit (Table 2). In high level of drought, the contents of chlorophyll-a, chlorophyll-b, and carotenoids were increased by using 100 mg L^{-1} chitosan. Therefore, the interaction of high level of drought and moderate concentration of chitosan (100 mg L^{-1}) led to greater production of the photosynthesis pigments compared to the control ones. In severe stress level, 100 and 200 mg L^{-1} of chitosan caused the enhancement (6.07 mg g^{-1}) and reduction (3.24 mg g^{-1}) values of carotenoids content, respectively (Table 2).

3.1.2. Proline content

Proline content changes were not significant under drought stress levels, but chitosan applications and the stress × chitosan interaction indicated statistical differences (Table 1). The findings showed that proline accumulation under water deficiency was substantially higher than it was in the non-stress condition (Table 2). The highest amount of proline (1060 μg/g) was under severe drought stress. Application of chitosan increased proline content in both stressed and normal plants (Table 2). Application of 100 mg L^{-1} chitosan in three stress levels

Table 1
Variance analysis of physiological and morphological traits in two *Salvia* species under water stress and chitosan treatments.

		Mean squares												
Source of variation	DF	Cars	Chl-b	Chl-a	Proline	MDA	H_2O_2	RN	RV	RL	FRW	DRW	FSW	DSW
Species	1	3.16*	0.2*	4.69 ns	1435987**	28.8**	0.39*	26.67**	2329**	100.2 ns	1659**	2194**	288.7**	255**
Stress	2	2.10 ns	0.01 ns	8.04*	48125ns	0.6ns	0.6**	59.71**	4235**	70.1ns	415.6**	23.97**	3.71ns	10.96 ns
Chitosan	2	3.57**	0.32**	9.48*	3086251**	0.51 ns	0.49**	88.81**	1335**	123.2 ns	896.6**	914.7**	22.33**	13.05*
Species × Stress	2	0.60 ns	0.05 ns	0.24 ns	175203ns	2.48 ns	0.29*	15.1*	1462**	120.8*	387.8**	11.45*	17.01**	53.32**
Species × Chitosan	2	1.78 ns	0.06 ns	1.43 ns	76210ns	2.23 ns	0.16 ns	51.36**	81.4ns	31.23 ns	19.07 ns	245.1**	8.91**	42.14**
Stress × Chitosan	4	9.74**	0.22**	35.5**	664379**	3.88**	0.5**	48.75*	1658*	112 ns	726.7*	111.68**	3.89 ns	79.39**
Species × Stress × Chitosan	4	1.70 ns	2.39 ns	3.9ns	49644ns	1.65 ns	0.14 ns	91.87**	287.8**	406**	390.7**	64.63**	3.79*	69.01**
Error	36	0.75	0.029	2.09	38065	0.93	0.089	1.95	80.54	44.35	52.12	2.42	21.1	3.88

DF: Degree of freedom; Cars: Carotenoids concentration; Chl-b: chlorophyll b concentrations; Chla: chlorophyll a concentrations; RN: Root Number; RV: Root Volume; RL: Root Length; FRW: Fresh Root weight; DRW: Dry Root Weight; FSW: Fresh Stem Weight, DSW: Dry Stem Weight; ns: Non-significant; *: Significant at $P \leq 0.05$; **: Significant at $P \leq 0.01$.

Table 2
Mean comparisons of stress and chitosan (interaction) on physiological and morphological traits of two *Salvia* species.

Treatments	Carotenoids (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Chlorophyll-a (mg g^{-1})	Proline (µg g^{-1})	MDA (µmol g^{-1})	H_2O_2 (mmol g^{-1})	Root number	Root volume (mL)	Fresh root weight (g)	Dry root weight (g)	Dry stem weight (g)
No-stress	3.75^{b}	0.96^{b}	4.81^{b}	31.59^{d}	4.6^{a}	1.78^{a}	9.66^{e}	28.83^{d}	51.91 ^{cd}	18.52^{a}	12.98^{a}
0 mgL^{-1}	3.21^{bc}	0.9^{bc}	3.33^{bcd}	350.6^{c}	4.35^{a}	1.76^{a}	20.8^{a}	84.33^{a}	57.65^{bc}	24.45^{a}	11.25^{b}
100 mgL^{-1}	3.56^{b}	1.09^{b}	4.26^{bc}	803.7^{b}	3.18^{b}	1.3 ^{cd}	13.41^{bc}	70^{b}	42.04^{e}	18.72^{c}	8.57^{d}
200 mgL^{-1}	3.32^{bc}	1.06^{ab}	3.82^{bcd}	66.73^{d}	3.81^{ab}	1.39^{bcd}	9.50^{e}	42^{c}	61.90^{b}	15.36^{d}	9.08 ^{cd}
Moderate stress	2.85^{bc}	0.65^{d}	2.89 ^{cd}	420.7^{c}	3.21^{b}	1.62^{bcd}	12^{bcd}	42.50^{c}	53.21 ^{cd}	20.20^{bc}	10.35^{bc}
0 mgL^{-1}	3.56^{b}	1.23^{a}	4.29^{bc}	736.3^{b}	4.6^{a}	1.22^{d}	11.33^{d}	35 ^{cd}	63^{b}	21.54^{b}	10.62^{b}
100 mgL^{-1}	2.44^{c}	0.75 ^{cd}	2.31^{d}	42.28^{d}	3.77^{ab}	1.96^{a}	13.50^{b}	29.58^{d}	79.97^{a}	23.98^{a}	12.88^{a}
200 mgL^{-1}	6.07^{a}	1.04^{ab}	8.97^{a}	296.7^{c}	4.08^{ab}	1.67^{ab}	14.00^{b}	28.66^{d}	49.24^{cde}	18.36^{c}	9.91^{bcd}
Severe stress	3.24^{bc}	1.04^{ab}	3.68^{bcd}	1060^{a}	4.87^{a}	1.71^{ab}	11.83 ^{cd}	26.66^{d}	48.24^{de}	18.31^{c}	8.92^{d}
0 mgL^{-1}											
100 mgL^{-1}											
200 mgL^{-1}											

In each column means followed by a same letter are not significantly different according to LSD's test at 0.05.

caused insignificant difference in proline content whereas 200 mg L^{-1} chitosan could increase the amount of proline markedly.

3.1.3. H_2O_2 and MDA

Based on variance analysis, H_2O_2 content displayed significant differences for drought stress, chitosan, and stress × chitosan interaction (Table 1). In the absence of chitosan, severe drought stress increased the content of H_2O_2 to 1.96 mmol/g. In normal conditions, unrestricted irrigation conditions, a decrease in H_2O_2 was noted with an increasing amount of chitosan concentration (1.78, 1.76, and 1.3 mmol/g of H_2O_2 for 0, 100, and 200 mg L^{-1} chitosan, respectively). Therefore, application of 200 mg L^{-1} chitosan had reducing effect on endogenous H_2O_2 accumulation (Table 2).

According to the variance analysis, neither chitosan nor stress had a discernible impact on the MDA content. Chitosan and stress had a substantial two-way interaction impact on this characteristic (Table 1). At moderate and severe water stress, MDA content rose after the administration of chitosan at a high (200 mg L-1) concentration (Table 2). Application of a high dose of chitosan reduced MDA content in comparison to the control in non-stressed conditions.

3.1.4. Morphological traits

The drought stress effect on morphology, including root number, volume, fresh and dry weight, was significant (Table 1). Based on mean comparison, root number, root volume, dry root weight, and fresh stem weight significantly reduced under drought conditions compared to non-stress conditions (Table 3). However, a considerable increase in root number and dry root weight occurred at 100 mg L^{-1} chitosan under moderate stress, compared to the no chitosan control (Table 4). With the exception of root length and stem fresh weight, the interaction between stress and chitosan was significant for all studied morphological traits. When chitosan and drought stress interacted, the highest total root fresh weight (79.97 g) was attained in extreme stress conditions without chitosan application, whereas the lowest (42.04 g) value was seen in non-stressed plants after foliar chitosan application of 200 mg L^{-1}. The results indicated that the fresh root weight of *Salvia* increased by increasing drought stress levels (Table 2). The general trend showed that increasing drought stress levels reduced root length in *S. abrotanoides*. The longest root (56.66 cm) and highest fresh root weight (69.36 g) was observed for the *S. yangii* at severe stress level. On the other hand, increasing of chitosan concentration declined dry root weight, fresh stem weight, and dry stem weight in both species and the highest ones were observed at control level of chitosan. The highest root volume was related to normal irrigation (61.66 mL) in *S. yangii* and the lowest one belonged to severe water stress (22.22 mL) in *S. abrotanoides* species.

3.2. Principal component analysis (PCA)

To differentiate between the impact of chitosan and drought stress on morphological and physiological parameters, PCA was used. As a result, PC1 accounted for 31.73% of the overall variance, whereas PC2 was responsible for 18.91% of the variation. PC_1 had high positive correlation with carotenoids, chlorophyll a, chlorophyll b, root and stem fresh and dry weight, as well as negative correlation with proline and H_2O_2. PC_2 was only positively correlated with the proline, root volume, root length, and chlorophyll-b. Identifying relationships of studied variables based on the first two PCs allowed an obvious separation between these variables (data not shown).

Under water stress condition, plants of both species were clustered together with 100 mg L^{-1} of chitosan treatment by higher amount of carotenoid, chlorophyll a, fresh root weight, fresh stem weight, and dry stem weight (Fig. 1).

Table 3
Mean comparisons of species and stress (interaction) for morphological traits of two *Salvia* species.

Species	Stress levels	Root number	Root volume (mL)	Root length (cm)	Fresh root weight (g)	Dry root weight (g)	Fresh stem weight (g)	Dry stem weight (g)
S. yangii	No-stress	14.38^{a}	61.66^{a}	48.16^{b}	50.96^{b}	4.6^{a}	33.37^{a}	13.82^{a}
	Moderate stress	12.55^{bc}	57.44^{a}	50.55^{ab}	66.46^{a}	4.35^{a}	29.82^{b}	11.97^{b}
	Severe stress	14.0^{ab}	31.11^{b}	56.66^{a}	69.36^{a}	3.18^{b}	30^{b}	13.44^{ab}
	No-stress	14.88^{a}	60.44^{a}	51.11^{ab}	48.91^{b}	3.81^{ab}	17.48^{c}	8.07^{c}
S. abrotanoides	Moderate stress	9.33^{d}	25.50^{b}	48.88^{b}	50.11^{b}	3.21^{b}	16.80^{c}	8.00^{c}
	Severe stress	12.22^{c}	22.22^{b}	46.66^{b}	52.28^{b}	4.6^{a}	17.77^{c}	8.05^{c}

Same letters indicate no significant difference according to LSD test in $p < 0.05$.

Table 4
Mean comparisons of species and chitosan (interaction) on morphological traits of two *Salvia* species.

Species	Chitosan (mg L^{-1})	Root number	Dry root weight (g)	Fresh stem weight (g)	Dry stem weight (g)
S.yangii	0	18.44[a]	69.66[a]	43.58[a]	14.94[a]
	100	11.38 [cd]	58.94[b]	26.10[b]	12.38[b]
	200	11.11[d]	58.2[b]	23.54[c]	11.60[b]
S.abrotanoides	0	10.66[d]	59.52[b]	21.19[d]	8.35[c]
	100	12.77[bc]	47.80[c]	18.10[e]	8.62[c]
	200	13.00[b]	43.98[c]	13.11 [f]	7.14[d]

In each column means followed by a same letter are not significantly different according to LSD's test at 0.05.

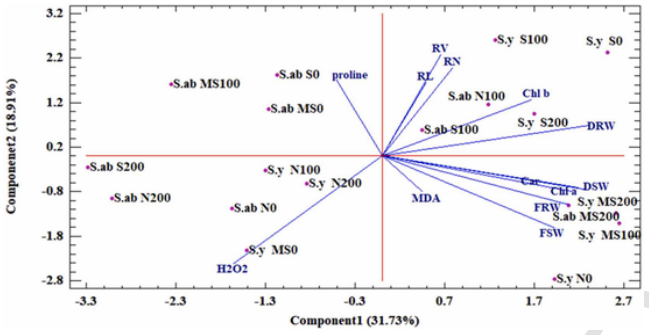

Fig. 1. PCA analysis to classification of *S. abrotanoides* and *S. yangii* based on physiological and morphological characteristics under water deficit and well-watered conditions using chitosan applications. **S. ab:** *S. abrotanoides*; **S. y:** *S. yangii*; **N:** Normal condition; **M:** moderate stress condition; **H:** Severe stress condition; **RN:** Root number; **RV:** Root volume; **RL:** Root length; **FRW:** Fresh root weight; **DRW:** Dry root weight; **FSW:** Fresh stem weight; **DSW:** dry stem weight; **Chla:** Chlorophyll-a; **Chlb:** Chlorophyll-b; **Cars:** Carotenoid content; 0, 100, 200 mg L^{-1} chitosan concentration.

3.3. Essential oil content and components under drought stress and chitosan treatment

High variations of EO content were observed for both species under different drought levels and chitosan treatments (Table 5). The implication of reduced irrigation increased EO content of *Salvia* species. In. *S. abrotanoides*, EO content ranged from 0.11% to 1%, and the high EO content was observed under severe stress condition sprayed with 200 mg L^{-1} chitosan. The highest (2.20%) content of EO in *S. yangii* was recorded under moderate stress without chitosan. variation among EO components was observed in *Salvia* species grown under drought conditions and chitosan treatments. EO composition species is presented in Table 5.

In all, there were 36 components identified by the GC-MS study, with 7 of them being the primary ones (Fig. 2). Under chitosan treatments, stress and non-stress circumstances, distinct responses for *S. abrotanoides* and *S. yangii* were seen in the current research. In comparison to well-watered settings, several components, such as 1,8-cineol, camphor, bornyl acetate, *trans*-caryophyllene, and α-bisabolol, are quantitatively enhanced.

Trans-caryophyllene, one of the main compounds of *S. yangii* was increased with severity of drought stress, whereas *δ*-3-carene and bornyl acetate decreased. In *S. abrotanoides*, the main of components including borneol, bornyl acetate, and *α*-cadinol decreased under water deficit, while the level of α-bisabolol, and camphor showed enhancement (Table 5). In addition, there were remarkable differences between chitosan concentration treatments in all volatile compounds.

Comparison of *α*-bisabolol content in the studied species under different treatments revealed the highest (58.1%) and the lowest (0.2%) *α*-bisabolol contents in *S. abrotanoides* and *S. yangii,* respectively. Furthermore, the highest 1,8-cineole (15.2%) was obtained in severe stress in absence of chitosan in *S. yangii*. Also, the highest *trans*-caryophyllene (14.8%) was observed at severe drought level and 100 mg L^{-1} chitosan application (Table 5). The highest (21.4%) and lowest (1%) borneol content was denoted for the *S. abrotanoides* EO under normal and severe stress without chitosan, respectively (Table 5). *S. abrotanoides* was found to have the lowest levels of α-cadinol (0.9%) and bornyl acetate (2.8%) while under extreme stress without chitosan. The cubenol found in plants treated with chitosan was the most noticeable point. When extreme stress and 200 mg L^{-1} chitosan were combined, the greatest concentration of cubenol (14.3%) was discovered in *S. yangii* (Table 5).

Hierarchical cluster analysis (HCA) was carried out to identify similarities between the two studied species under normal conditions and drought stress along with chitosan treatments considered by eleven major compounds and EO content. Furthermore, the samples were grouped in three main clusters (Fig. 3). The first cluster, characterized by high level of camphor and 1,8-cineol, including five samples consisting of both the tested species under the stress and non-stress conditions and absence of chitosan spraying. The second cluster consisted of three samples distinguished by high amounts of *trans*-caryophyllene, α-humulene and EO content. The third group was divided into two subgroups. The first subgroup contained *S. abrotanoides* under normal and stress conditions with chitosan treatments. This subgroup was associated with high *α*-bisabolol contents (Table 5 and Fig. 3). The samples in the second subgroup were rich in borneol, bornyl acetate, and *α* -cadinol content.

To define the correlations between the main EO components and different drought stress levels by chitosan treatments, principal component analysis (PCA) was carried out on the biochemical response of the examined plants. According to the findings, the first two main components accounted for 51.36% of all variance (Fig. 4). *Trans*-caryophyllene and α-humulene as sesquiterpene hydrocarbons showed significant and positive scores in the PC_1. By contrast, *α*-bisabolol negatively correlated with PC_1. Whereas, the second one was positively correlated with 1,8-cineole, *δ*-3-carene, and camphor (data not shown). The treatments of the two species under stress and non-stress conditions along with chitosan application was separated.

PCA biplot identified three main groups mainly discriminated by their camphor, *α*-bisabolol, *trans*-caryophyllene, and α-humulene contents. In terms of the main compounds, *S. abrotanoides* under different treatments was characterized by a high content of *α*-bisabolol. Based on the pattern, a genetic factor is suggested to play a role in the differences in EO composition between studied species. The bulk of the second group was made up of *S. yangii* samples, which were distinguished by their high camphor and 1,8-cineol concentrations both under mild and severe stress conditions, as well as their lack of chitosan. The third group's samples belonged to both species and were distinguished by chitosan treatment-induced high concentrations of *trans*-caryophyllene and α-humulene.

4. Discussion

More than 80–95% of fresh biomass of the plants is water that affects many aspects of plant growth, evolution and metabolism (Seleiman et al., 2021). Lack of ambient water has a negative impact on the yield and quality of plants (Shanazari et al., 2018). Furthermore, some factors such as genotype, plant species, evolutionary stage and duration of stress and its intensity affect the plant's response to drought stress. (Anjum et al., 2017). Various molecular pathways have been identified that are involved in transmitting the message and reacting to drought stress (Seleiman et al., 2021).

Various systems are involved in the purification system of reactive oxygen species (ROS) in plants. One of these mechanisms is the activation of the antioxidant system of enzymes including enzyme superoxide

Table 5
Volatile compounds (%) of essential oils of studied *Salvia* species under drought conditions and chitosan treatments. Empty cells indicate that the desired compound is not identified in the essential oil.

Species		*S. yangii*									*S. abrotanoides*								
Treatments		Non-stress			Moderate stress			Severe stress			Non-stress			Moderate stress			Severe stress		
Compositions (%)	RI	0	100	200	0	100	200	0	100	200	0	100	200	0	100	200	0	100	200
α-Pinene	939	4.1	2.1		1.6	1.5	1.2	4.6	0.2	0.5	1.7	1.3	1.1	2.1	2		1.9		0.5
Camphene	953	3.5	1.4		0.8	1.2	1	3.4	0.2	0.3	0.9	0.6	0.6	1.5	1.4		1.2		0.4
β-Pinene	980	0.7	0.3		0.3	0.2	0.2	0.7				0.2	0.2	0.4	0.2		0.4		0.1
β-Myrcene	991	0.4	0.7		0.3	1.4	0.1	2	0.1			0.1		0.2	0.1				0.4
δ-2-Carene	1002					0.1	0.1					0.1	0.1	0.1	0.2				
δ 3-carene	**1008**	**10.8**	**3.9**	**0.3**	**2.9**	**1.5**	**1.9**	**3.6**	**0.7**	**0.8**	**2.4**	**2.2**	**2.1**	**5.6**	**4.8**		**4.8**	**1.3**	**1.1**
p-Cymene	1026	0.8							0.1			0.2			0.3			0.2	
o-Cymene	1027	0.2	0.5		0.5	0.3	0.2	0.8					0.2	0.4					0.2
D-Limonene	1031	1.7	1.3	0.4	1.8	1.1	1.2	6.5	0.5	0.3		0.3	0.3	0.7	0.5		0.5	0.2	0.5
1,8-Cineole	**1033**	**10.2**	**9.3**	**0.9**	**9.3**	**8**	**3.7**	**15.2**	**1**	**2.8**	**4**	**4.1**	**1.9**	**6.3**	**1.5**		**2.7**	**2.3**	**3**
γ-Terpinene	1062	0.3	0.2		0.2		0.1	0.3				0.1	0.1	0.2					
Terpinolene	1063	0.7	0.2	0	0.4									0.3					0.1
Linalool	1098	0.1	0.2		0.3		0.1	0.1	0.2	0.3	0.3	0.4	0.1	1.4	0.1		0.1	0.3	0.8
Thujone	1102		0.1		0.2		0.1	0.1						0.2				0.1	0.1
Camphor	**1143**	**13.3**	**9.4**	**3.7**	**10.1**	**8.8**	**4.9**	**13.2**	**1.8**	**4.2**	**3.8**	**10**	**8**	**14.4**	**2.6**	**0.6**	**4.6**	**7**	**7.4**
Borneol	**1165**	**7.6**	**4.8**	**2.5**	**4.1**	**9.2**	**15.2**	**4**	**12.6**	**5.7**	**21.4**	**5**	**4.9**	**3.5**	**2.5**	**2.9**	**1**	**9.5**	**6.1**
Naphthalene	**1179**	**0**	**1.4**	**14**	**1.9**	**2.4**	**6.2**	**0**	**7.6**	**4**	**0**	**1.8**	**1.5**	**3.6**	**8.3**	**0.4**	**7.2**	**3.7**	**1.2**
4-Terpineol	1189	0.4	0.4		0.4	0.3	0.2	0.4	0.2	0.2		0.6	0.1	0.3				0.1	0.2
α-Terpineol	1189	0.6	0.8		0.9	0.6	0.5	0.4	0.5	1	2	1.3	0.6	0.8				0.6	0.7
Bornyl acetate	**1286**	**11.9**	**10.9**	**9.3**	**8.5**	**9.3**	**12.5**	**7.3**		**7.8**	**18.1**	**4.8**	**7**	**5.5**	**8.1**	**5.8**	**2.8**	**17**	**9.5**
α-Terpinyl acetate	1349	2.5	4	5.6	4	3.4	3	3	6.1	3.9		1.1	1	3.7	2.4	1.7	1.2		5.1
***trans*-Caryophyllene**	**1417**	**7.8**	**7.5**	**13.2**	**9.7**	**7.1**	**6.9**	**11.7**	**14.8**	**6.1**	**5.3**	**3.1**	**4.9**	**9.2**	**5.7**	**2.2**	**2.3**	**6**	**12.9**
Copaene	1432	0.2	0.2	0.7	0.4	0.2	0.2	0.5	0.7	0.3		0.2							0.3
α-Humulene	**1455**	**7**	**7.8**	**12**	**9.7**	**8.1**	**7.5**	**9.5**	**15.3**	**9**	**5.2**	**3.2**	**4.6**	**9.1**	**6.1**	**3.4**	**2.1**	**5.7**	**12.9**
γ-Muurolene	1477	1.8	2.6	4.3	2.7	3.1	0.2	2.7		2.8	3	1		1.3		1.6		1.1	6.2
β-Bisabolene	1505	0.4	0.3	0.6								1.5	0.8	0.8	0.9	0.9		1.5	
α-Calacorene	1546	0.2		1.1	0.5	0.3	0.7			0.8			0.4	0.1	0.5	0.5		0.2	0.3
Caryophyllene oxide	1561	0.5	0.6	1.3	0.8	0.7	1.1			4.1	1.1	0.5	1.5	1.3	0.9	2	0.8	1.3	
Humulenol-II	1605		0.7			0.8	1	1.6		2				2.5		1.8			2.9
Junenol	1622			0.9	0.7	3.1		0.4		1.8									
epi-α-Cubenol	1629		1.2	1.2			2.3				1.9		1.5	0.7	1.9	1.4		0.5	
α-Cadinol	**1640**	**5.6**	**8.6**	**9**	**7.3**	**12.6**	**14.9**	**2.2**		**9.2**	**16**	**3.9**	**15**	**5.1**	**13.6**	**12.2**	**0.9**	**4.1**	**14**
Cubenol	1642					6.9		1.7		14.3		5.5						2.4	2.4
α-Bisabolol	**1701**	**4**	**5.2**	**7.6**	**0.3**		**0.2**				**6.7**	**27.8**	**38**	**14.2**	**27.5**	**45**	**58.1**	**29**	**5.1**
Diazinone	1814	0.1	0.3	0.9	0.2		0.5	0.1				0.3		0.4	0.7	0.8	0.9	1.2	
Total	–	**97.4**	**87.6**	**90.7**	**81.4**	**93**	**89.5**	**97.6**	**62.6**	**85.9**	**93.8**	**81.2**	**96.5**	**98.6**	**92.8**	**87.8**	**93.5**	**95.5**	**97.3**
Essential oil content %	–	0.61	0.36	1.12	2.20	0.38	0.27	0.96	1.52	0.26	0.21	0.21	0.23	0.23	0.20	0.11	0.26	0.32	1.00

a: Retention indices on the DB-5 column.

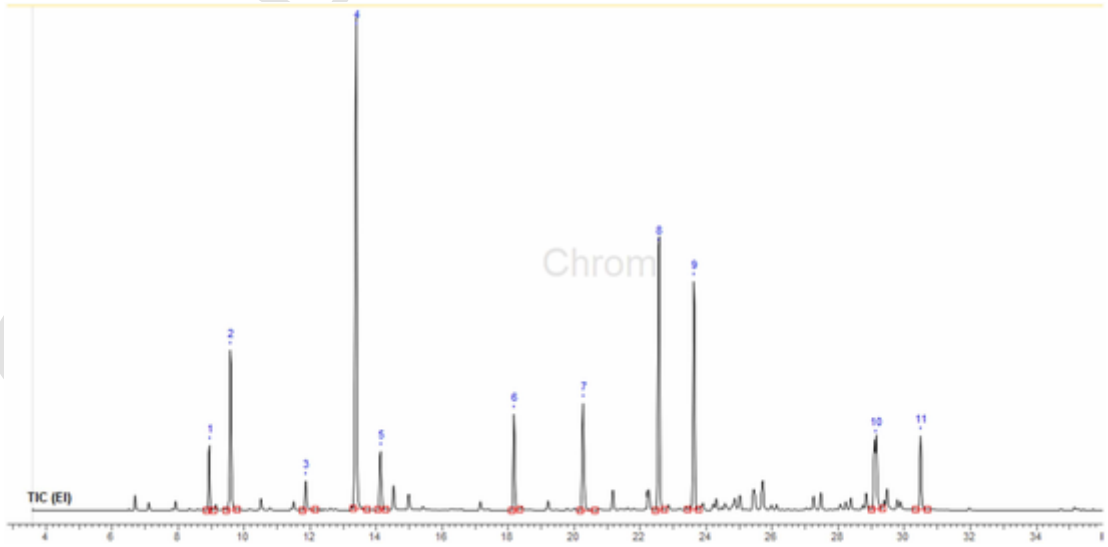

Fig. 2. GC-MS chromatogram of essential oil of Leaves of *salvia* species. 1- α-pinene, 2–1,8-cineole, 3-linalool, 4- camphor, 5- borneol, 6- bornyl acetate, 7- α-terpinyl acetate, 8- *trans*-caryophyllene, 9- α-humulene, 10- α-cadinol, 11-α-bisabolol.

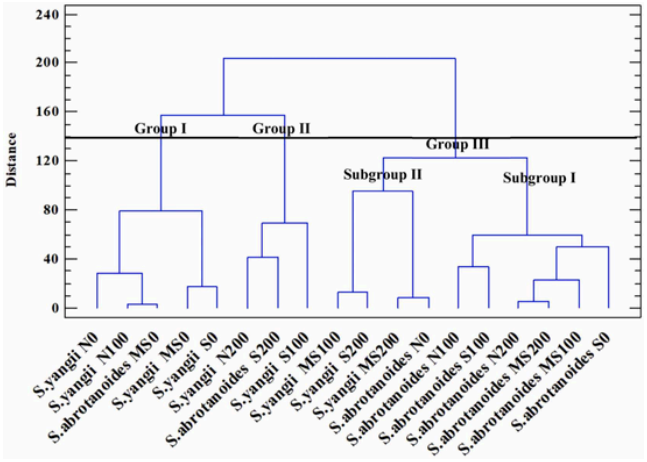

Fig. 3. Dendrogram of two *Salvia* species under drought stress environments and chitosan application based on main essential oil components and essential oil content, and using Ward clustering method. **N:** Normal condition; **M:** Moderate stress condition; **H:** Severe stress condition; 0, 100, 200 mg L^{-1} chitosan concentration.

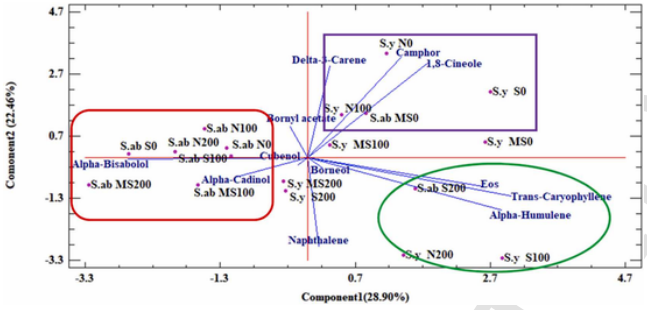

Fig. 4. Classification of studied *Salvia* species under drought conditions and chitosan application using PCA analysis. **S. ab:** *S. abrotanoides*; **S.y:** *S. yangii*; **N:** Normal condition; **M:** Moderate stress condition; **H:** Severe stress condition; **EOs:** Essential oil content; 0, 100, 200 mg L^{-1} chitosan concentration.

dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) peroxidase (POX) and glutathione reductase (GR) (Celikkol et al., 2010). On the other hand, plants that are subjected to stress may set off a signal cascade that modifies gene expression to create the desirable molecules that lessen the impacts of stress (Zargar et al., 2017). Under drought stress, some substances such glucoside, proline, and abscisic acid are elevated as non-enzymatic antioxidant metabolites in plant species (Anjum et al., 2011).

The accumulation of ROS in water-restricted conditions is a common phenomenon that leads to reduced membrane integrity and stability along with a reduction in photosynthetic pigments, such as chlorophyll content (Moolphuerk and Pattanagul, 2020; Nikolaeva et al., 2010). Enhancement of chlorophylls content with chitosan application has been reported in cowpea (Farouk and Amany, 2012). According to Dastjerd et al. (2013), chitosan treatment may stimulate chlorophyll production by raising the amount of cytokinins in plant cells, which may lessen the impacts of drought stress on photosynthetic pigments. The concentration of the photosynthetic pigments including chlorophyll a and chlorophyll b as well as carotenoids, which act as antioxidants, has grown as the severity of drought stress and the application of chitosan increase concurrently. Because chlorophyll is the first pigment engaged in the process of capturing sunlight and transforming it into chemical energy (Azhar et al., 2011), increasing the chlorophyll content in the plant can lead to increased CO_2 stabilization and photosynthesis, which ultimately leads to increase plant yield. Besides, the plant's ability to synthesize more chlorophyll under stress conditions can lead to increase resistance to drought stress (Mostafa et al., 2018). On the other hand, it seems that with increasing levels of drought stress and reactive oxygen species (ROS), the amount of chlorophyll-a decreased due to decline of chlorophyllase enzyme activity. Also, increasing the ROS may lead to oxidative injury of chloroplast lipids and proteins (Ali and Hassan, 2017), destruction of the pigment protein complexes which protect the photosynthetic apparatus (Lai et al., 2007), or reduced expression activity of enzymatic synthesis genes and transcription factors (Allakhverdiev et al., 2003). As a consequence, when any of these components are stressed, the pigments involved in photosynthesis decrease. Reduced photosynthesis owing to the destruction of chloroplasts, chlorophyll a, and chlorophyll b under drought stress might result in decreased plant output and quality (Anjum et al., 2011; Zhang et al., 2020). According to Li et al. (2006), barley's chlorophyll concentration decreased in response to drought stress, which was consistent with the findings of the current study. Also Shanazari et al. (2018) explained the difference in the rate of increase in chlorophyll content under stress conditions in wheat plant is in terms of the differences in stress conditions, drought intensity and genotypic differences (Bouchemal et al., 2017).

Proline as a regulator in plant stress conditions plays an important role in water uptake and internal physiological activities in adverse plant growth conditions (Farooq et al., 2012). Proline was proposed as an ROS inhibitor which protects plants from oxidative damage (Ali et al., 2021). Additionally, in times of famine, proline itself may serve as an internal source of nitrogen and carbon storage (Purvis and Yelenosky, 1983). Proline biosynthesis may be stimulated and/or its oxidative inhibition under conditions of water stress, leading to an increase in proline levels in the absence of moisture (Lawlor et al., 2002). According to research by Molinari et al. (2007) transgenic plants resilience to drought stress may be elevated by overexpressing the P5CS (pyrroline-5-carboxylate synthase), a key enzyme in proline production (Molinari et al., 2007). Numerous studies have confirmed that increased proline levels are a known plant response to drought stress (Kumar et al., 2013; Munawarti et al., 2014). Our findings provide evidence for the hypothesis that the use of chitosan induces proline production under drought stress.

According to Yang et al. (2009), chitosan can be a key regulator of plant responses to environmental stresses. Li et al. (2017) reported that drought-stressed plants contained more proline following chitosan treatment. Chitosan could be directly converted to other sugars and pyruvate, which is the precursor of tricarboxylic acid cycle and the biosynthesis of glutamate and other amino acids, including proline (Geng et al., 2020). It should be noted that the enhancement of proline content in stressed environments due to chitosan application might be related to the decrease in proline oxidation to glutamate, induction of proline biosynthesis, its non-involvement in protein synthesis, or a combination of these (Hidangmayum et al., 2019). Therefore, proline buildup under osmotic stress works as an osmoprotectant in addition to stopping chemical activity. The current study's findings that chitosan therapy may lessen the impact of stress by increasing proline levels were supported by earlier research (Ali et al., 2021; Bistgani et al., 2017).

Under osmotic stress, reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($\cdot OH$), hydrogen peroxide (H_2O_2), and alkoxy radicals ($RO^{\cdot}$), are produced (Al-Khayri and Al-Bahrany 2002). Hence, Increased H_2O_2 is one of the effects of water limitation (Bhattacharjee, 2005). The lower accumulation of H_2O_2 in the studied species using chitosan application could be due to the activation of ROS scavenging enzymes (Pandey et al., 2010). It was suggested that chitosan sticks to particular receptors in plant cell membranes and commences signal transduction through various secondary messengers, including H_2O_2, ROS, nitric oxide, and phytohormones (Moolphuerk and Pattanagul, 2020). The activity of antioxidant enzymes in plants by converting H_2O_2 to H_2O and thus reducing the concentration of H_2O_2

reduces the negative effects of ROS (Ahmad et al., 2022). Excessive H_2O_2 disrupts cell metabolism and damages cell membranes by the oxidation of lipids, nucleic acids, and proteins (Das and Roychoudhury, 2014).

Previous research has shown that substantial membrane lipid peroxidation occurs when the amount of free radicals rises, increasing the quantity of malondialdehyde (MDA). Thus, oxidative damage is accelerated by the amount of MDA created during the breakdown of lipid membranes (Shohael et al., 2006). Therefore, a decrease in MDA concentration under stressful circumstances results in the scavenging of reactive oxygen, the protection of biological membrane systems, the maintenance of normal biomolecular function, and the alleviating the injury (Zeng and Luo, 2012). It was also found that low concentration of chitosan treatment decreased lipid peroxidation that could be useful to recovery from drought damage. Previous study reported that at severe osmotic potential condition, application of high concentration of chitosan increased MDA accumulation over the control treatment in safflower seedling (Mahdavi et al., 2011), which is similar to the present study. Moreover, the lowest MDA content was under 100 mg L^{-1} chitosan and the Moderate stress.

Water stress condition can influence many morphological, biochemical, physiological, and molecular aspects of plant growth and development (Ali et al., 2021). The previous study reported that osmotic adjustment prevents dry matter accumulation in the hypocotyls. The roots accumulated material, which caused them to develop more quickly than roots under less stress (Rauf and Sadaqat, 2008). Anbessa and Bejiga (2002) showed that drought circumstances caused a decrease in the root volume in chickpea, which is consistent with the present study. Moreover, reducing soil moisture can reduce access to nutrients and water in terms of low moisture around the roots and limited proliferation of root biomass or limited nutrient uptake (Staniszewska et al., 2003; Razmjoo et al., 2008) and thus lead to reduced plant height, growth and yield (Staniszewska et al., 2003). The foliar application of chitosan led in some cases to considerable improvement in morphological parameters of *Salvia* species under normal or stress conditions, more prominent at the intermediate concentration of 100 mg L^{-1}. The role of chitosan in plant growth improving was reported by several previous studies (Bistgani et al., 2017; Behboudi et al., 2018). The exact method through which chitosan affects plant growth and development is yet unclear. Application of chitosan is likely to upregulate plant hormones like gibberellins, and it may also increase auxin production (Mohammadi et al., 2021).

Growth restriction under drought stress conditions can cause the stabilized carbon sources in the plant to move towards producing the secondary metabolites (Hill et al., 2021). However, various growth conditions had different effects on the EO content of the two studied species. The obtained result was in agreement with those of Bistgani et al. (2017) which reported the highest EO content in *T. daenensis* under moderate stress conditions. Additionally, Bettaieb et al. (2009) observed that increasing the intensity of the drought stress has improved the EO quality and quantity in sage. Additionally, results from earlier research showed that adding chitosan enhanced basil EO yields (Pirbalouti et al., 2017). According to Bistgani et al. (2017), chitosan treatments raised the EO content of *T. daenensis*. In *Origanum vulgare*, chitosan significantly enhanced the biomass and essential oil yield, but there was no effect on EO composition (Yin et al., 2012). Vosoughi et al. (2018) reported that the highest EO content was obtained from *Salvia officinalis* L. grown under water-stress condition by spraying with 0.50 g L^{-1} chitosan. Modification of the essential oil content with chitosan application may be in terms of the increase of CO_2 assimilation (Vosoughi et al., 2018). The most important metabolic organ are leaves; hence the foliar treatment of chitosan is the best way to affect the plant metabolism.

GC-MS analysis allowed to identify 33 compounds in essential oil. As predominated compounds included camphor, α-cadinol, α-bisabolol, δ-3-carene, 1,8-cineole, borneol, and bornyl acetate. α-Pinene can be converted into three compounds 1,8-terpine, camphene, or *p*-cymene during enzymatic processes inside the cell (Monteiro and Veloso, 2004). In the present study, only compound borneol from the above pathways are among the main compounds of essential oils, which indicates the high activity of this pathway compared to the other two pathways. According to the content of obtained from each of the three compounds α-pinene, borneol, and camphene, it can be suggested that the conversion of α-pinene to intermediate compound Viz. borneol is being carried out more quickly than the conversion of intermediate one such as borneol to camphene.

In the present research, the main compounds were α-pinene, 1,8-cineole, camphor, borneol and bornyl acetate that belonged to the monoterpene family. Furthermore, *trans*-caryophyllene and α-humolen compounds from the sesquiterpenes family were also identified in essential oil compounds (Ghaffari et al., 2019). Under drought stress conditions in *S. abrotanoides* species, borneol content decreased and camphor content increased. Borneol is converted to camphor during the enzymatic reaction by the key enzyme borneol dehydrogenase (Mendoza-Poudereux et al., 2017). On the other hand, greater borneol content may be seen when there is access to enough water. This is because the enzyme borneol dehydrogenase is less active, camphor is reduced to borneol more often, or both of these things are happening. The Meerwein-Ponndorf-Verley procedure is used to transform camphor into borneol (Mendoza-Poudereux et al., 2017; Monteiro and Veloso, 2004). It seems that increasing the enzymatic activity of borneol dehydrogenase in drought stress conditions reduces the content of borneol and increases the camphor content (Mendoza-Poudereux et al., 2017). The current finding showed a much higher level of α-bisabolol under stress conditions in the absence of chitosan in *S. abrotanoides*, probably due to stressful conditions and unknown genetic factors. Different chitosan concentrations can influence the components of EO. α-bisabolol in *S. abrotanoides* increased upon chitosan treatment under normal condition and moderate stress, but decreased under severe stress combined with chitosan. Rafieiolhossaini et al. (2010) reported that environmental conditions had great effect on bisabolol content in the chamomile EO. EO composition of the same species may vary by collecting area, according to earlier investigations. However, the greatest influence on the variance of EO components may come from the distance between species (Oreizi et al., 2014; Ghaffari et al., 2019; Tohidi et al., 2020). Additionally, according to Singh-Sangwan et al., (1994), drought stress may affect the amounts of biosynthesis for EO components either negatively or favorably. Drought has also been linked to modifications in the essential oil compositions of mints and sweet basil (Charles et al., 1990; Simon et al., 1992). Previous studies showed that 0.1% chitosan application enhanced the level of carvacrol content of Greek oregano (Yin et al., 2012) and 0.5 % chitosan increased content of linalool and eugenol in sweet basil (Kim et al., 2005). An earlier report showed that the amount of α-thujone, 1,8-cineole, and camphor increased under moderate water deficit in sage plants (Bettaieb et al., 2009). Moreover, metabolic synthesis of secondary metabolites may be affected by chitin and chitosan via enzymes activity and gene regulation (Kim et al., 2005).

The results of HCA analysis show the effect of increasing the content of monoterpenes consisting camphor and 1,8-cineol in the absence of chitosan spraying (in the first cluster) and increasing the content of *trans*-caryophyllene, α-humulene as sesquiterpenes when using chitosan in the second group. This may be in terms of the induction of two major genes, DXS and DXR, which contribute to the production of sesquiterpenes (Ahmed et al., 2020).

The PCA findings indicate that chitosan treatment may be useful in enhancing root and stem associated features for the osmotic adjustment to lessen oxidative stress brought on by drought stress when stress circumstances are present. Moreover, in previous studies, the content of *trans*-caryophyllene has a positive correlation with α-humulene contents (Ghaffari et al., 2018), which is consistent with the results ob-

tained from the PCA biplot analysis, which shows that *trans*-caryophyllene, and α-humulene compounds are in the third group belonged to both species. In the other hand, the third group in PCA biplot analysis shows that increasing the concentration of chitosan can increase the biosynthesis of sesquiterpenes. Increasing the content of sesquiterpenes may suggest that more Isopentenyl pyrophosphate (IPP) flux is from the plastid, which is the site of monoterpene biosynthesis, to the cytosol, which is the site of sesquiterpenes biosynthesis. Furthermore, chitosan use can increase the level of cytosolic activated calcium and thus activate mitogen-activated protein kinases (MAPKs), which ultimately leads to the *de-novo* expression of genes involved in metabolic pathways (Ahmed et al., 2020).

5. Conclusion

In the current research, the influence of chitosan under water restriction on the amount and composition of EO, as well as on the morphological and physiological characteristics of two *Salvia* species, was assessed for the first time. The results can be useful for further breeding initiatives for this *Salvia* subgenus. Chitosan treatments led to improve endurance of plants growth under severe stress by several mechanisms, such as enhancing proline content, decreasing lipid peroxide (H_2O_2), increasing photosynthetic pigments, and enhancing root and stem associated features. The increase in proline content and the decrease in lipid peroxide formation along with the decrease in MDA content under drought stress conditions under the influence of chitosan treatment may indicate the maintenance of cell membrane health. It may suggest that chitosan treatment has the ability to start the signaling cascade involved in the response to drought stress. Also, increasing the content of photosynthetic pigments in stress conditions due to chitosan treatment may play a significant role in reducing the negative effects of drought stress.

Moreover, stress conditions stimulated the synthesis of volatile terpenoid metabolites. Higher EO content was observed in plants under stressful conditions than from well-watered ones, indicating an elevation in the flow of fixed carbon in the plant towards the production of secondary metabolites. Also, it may be concluded from the results of the present study that chitosan treatment had relative abundance of sesquiterpenes in comparison with monoterpenes. Finally, the severity of damage under water deficit may be alleviated by chitosan application. Overall, in most cases chitosan might be suggested as well as a stimulant to elevate the drought tolerance by increasing some secondary metabolites and can be used for further metabolite improvement.

CRediT authorship contribution statement

Each of authors contributed to this study as following: farzaneh khodadadi performed the experiment and contributed to analysis and interpretation of data, and writing the manuscript Farajollah Shahriari Ahmadi* and Mehdi Rahimmalek** contributed to study conception, project design and Interpretation of data. Also, Mehdi Rahimmalek contributed to the preparation of plant samples and laboratory materials. Majid Talebi and Nasrin Moshtaghi revised the manuscript for important intellectual content. Adam Matkowski, and Antoni Szumny contributed for interpretation of results,revising and final approval of the version to be published.

Uncited references

Bittelli et al., 2001; Kumar et al., 2013; Purvis and Yelenosky, 1983; Singh-Sangwan, N., Abad Farooqi, A. H., & Singh Sangwan, 1994; Zandalinas et al., 2018.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The authors do not have permission to share data.

Acknowledgements

We appreciate Ferdowsi Mashhad University, Isfahan University of Technology and Polish National Agency for Academic Exchange (NAWA) Ulam 2021 program with BPN/ULM/2021/1/00250/U/0000 1no. for financial support and for providing scientific collaboration and advanced analyses.

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