

## Effects of Water Stress and Mycorrhizal Fungi on Essential Oil Content and Composition of *Satureja sahendica* Bornm.

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### ABSTRACT

*Satureja sahendica* Bornm is an endemic, native, and aromatic plant in Iran, with thymol being its main and major volatile component, which is used in food and pharmaceutical industries. Increasing the oil yield and phenolic compounds in essential oil leads to increased medicinal effects. In this study, the effects of mycorrhizal fungi (*Glomus mosseae*, *G. intraradices*, and combination of *G. mosseae* and *G. intraradices*) and different levels of water stress [D<sub>1</sub>= Control (no water stress), D<sub>2</sub>= No irrigation during stem elongation till blooming, D<sub>3</sub>= No irrigation at the blooming up to start of flowering, and D<sub>4</sub>= No irrigation at 50% flowering up to full flowering] were studied on *S. sahendica* oil, in two years. When the seedlings were transferred to the field, 10 g of mycorrhiza fertilizer (containing mycorrhizal fungus spores) were added to the rhizosphere of each seedling. The plants were harvested at full flowering and the essential oils were obtained by hydro-distillation and analyzed by GC and GC/MS. The highest amount of oil yield (66.13 kg ha<sup>-1</sup>) was obtained using *G. intraradices* in non-stress conditions in the second year. The highest percentage of thymol was obtained in *G. mosseae*×control in the first year (74.59%), whereas in the second year, the highest amount of thymol was in *G. mosseae*×D<sub>4</sub> (61.97%). This study showed that with the use of mycorrhizal fungi, even in conditions of water shortage, *S. sahendica* produced more essential oil with a higher percentage of thymol. With the use of mycorrhizal fungi, the essential oil can easily be increased and the number of compounds changes.

**Keywords:** *G. intraradices*, *Glomus mosseae*, Inoculation, Medicinal plants, Thymol.

### INTRODUCTION

Based on a limited category, thirty *Satureja* species are found in the family Lamiaceae (Hyam and Rankhurst, 1995; Jamzad, 2009). Fifteen species of *Satureja* grow in Iran wildly, of which 9 species are endemic (Jamzad, 2009).

Most of *Satureja* species have aromatic and medicinal properties. In Iranian traditional medicine, the aerial parts of some *Satureja* species are used to treat diseases such as diarrhea, wounds, urinary tract

infections, and gastroenteritis (Hadian *et al.*, 2012). Isolated essential oils of *Satureja* species have shown antioxidant, antifungal, antibacterial, antidiabetic, and anti-inflammatory biological activities (Abdollahi *et al.*, 2003; Tzakou and Skaltsa, 2003; Goren *et al.*, 2004; Ghazanfari *et al.*, 2006; Eftekhari *et al.*, 2009).

*Satureja sahendica* Bornm. is one of the endemic species found in mountainous regions of western and northwestern Iran. In traditional medicine, it is used for anti-inflammatory and anti-bacterial effects (Zargari, 1990). Moreover, the *S. sahendica*

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plants and essential oil are used in food industries, which can replace artificial preservatives as a natural antioxidant (Hajhashemi *et al.*, 2002).

Previous studies showed that eight wild populations of *S. sahendica* contained 1.5 to 2.88% essential oil with thymol (19.6–41.7%), *p*-cymen (32.5–54.9%) and  $\gamma$ -terpinene (1–12.8%) as the main components (Sefidkon *et al.*, 2004). Essential oil components extracted from *S. sahendica* culture sample were reported as thymol (38.3%), *p*-cymen (21.3%) and  $\gamma$ -terpinene (30.9%) (Akbarinia and Sefidkon, 2009). Essential oil of *S. sahendica* and its major constituents can be used in antibacterial and anti-cancer applications (Yousefzadi *et al.*, 2012).

Cultivation of aromatic plants such as *S. sahendica* is indispensable for industrial uses and conservation of genetic resources in natural habitats, but there are some problems like water scarcity, which is a serious problem in many parts of the worlds, including Iran. Previous studies have shown that photosynthesis rate and transpiration decreased due to the water scarcity (Sarker *et al.*, 2005). However, increasing oil yield and phenolic compounds in the savory oil will increase its medicinal effects.

Numerous articles have shown that plants in drought stress conditions accumulate higher concentrations of secondary metabolites. Increase in essential oil of *Mentha piperita* ssp. (Charles *et al.*, 1990), *Satureja hortensis* (Baher *et al.*, 2002), *Salvia officinalis* (Bettaieb *et al.*, 2009) and *Nepeta cataria* (Manukyan, 2011) by drought stress has been observed.

However, it should be noticed that drought stress also reduces the growth of plants. It is often stated that under drought stress, the same amounts of natural products are synthesized and accumulated as in well-water conditions, but due to the reduction in biomass, their concentration increases (Selmar and Kleinwächter, 2013).

Using different fertilizers can reduce the effect of stress; for example, soil application of 30 and 60 ppm SiO<sub>2</sub> NPs can reduce the

negative effects of drought stress (Behboudi *et al.*, 2018).

Inoculating plants with mycorrhizal fungi, as a kind of biological fertilizers, can also reduce stress effects. About 95% of the plant families are known to form a mutual relationship with arbuscular mycorrhizal fungi (Trappe, 1987). These fungi are associated with the plant roots, and this relationship enhances the plant ability to adsorb water and nutrients by increasing the absorption rate through the high level of mycelium (Selosse *et al.*, 2006). These plants also produce carbohydrates, including glucose and sucrose for the fungi (Harrison, 2005). Many studies have shown that the symbiotic relationship between plants and the arbuscular mycorrhizal fungi (AMF) is a key factor in allowing plants to tolerate drought stress (Asrar *et al.*, 2012; Ruiz-Lozano and Aroca, 2010). Changes in the amount and synthesis of essential oil have also been reported for some aromatic plants such as basil (Toussaint *et al.*, 2007), mint (Freitas *et al.*, 2004) and lavender (Tsuro *et al.*, 2001). The AM coexistence of fungi with plant improves plant resistance to undesirable environmental conditions (Ruiz-Lozano, 2003).

Considering the importance of *S. sahendica* in the pharmaceutical industry, the problem of water scarcity in many regions of Iran and the promising properties of AM fungi, the purpose of this study was to determine the effect of water stress and two species of AM fungi on the *S. sahendica* oil content and composition, in two years.

## MATERIALS AND METHODS

### Plant Cultivation, Mycorrhizal Inoculation and Treatments

The experiment was conducted to evaluate the effect of water deficit and mycorrhiza on the essential oil of *S. sahendica*. This project was conducted in 2015-2017 at the Alborz Research Station (affiliated with the Research Institute of Forests and

Rangelands), Karaj, Iran (Latitude: 35° 38' N; Longitude: 51 °E; Elevation: 1,321 m asl). The soil of the experimental site was loamy with pH 7.48. The seeds were obtained from the Medicinal Plants Research Division, Research Institute of Forests and Rangeland. The experiment was conducted as a split plot in a randomized complete block design with three replications. Water stress was applied at four different levels (D<sub>1</sub>= Control, D<sub>2</sub>= No irrigation during stem elongation till blooming, D<sub>3</sub>= No irrigation in the blooming up to start of flowering, D<sub>4</sub>= No irrigation at 50% flowering up to full flowering) (Soleymani and Shahrajabian, 2012). The mycorrhiza (prepared by ATIM Resources Corporation, Onatarlo-Canada, ISIC Code: 241842) was used in four levels: M<sub>1</sub>= Non-use of mycorrhiza, M<sub>2</sub>= Inoculated with *G. intraradices*, M<sub>3</sub>= Inoculated with *G. mosseae*, and M<sub>4</sub>= Inoculated with *G. intraradices* and *G. mosseae*. When the seedlings reached 6-10 leaves, they were transferred to the field and 10 g of mycorrhiza fertilizer (containing spores of mycorrhizal fungus) was added to the rhizosphere of each seedling.

### Controlling Soil Moisture

Gravimetric method was used to control soil moisture. Regarding the determination of soil moisture content under field capacity and permanent wilting point (FC 23.41%), all plots were irrigated uniformly and more than 100% of the field capacity. One day after irrigation, soil samples were taken from the root zone (0 to 30 cm), and weighed. The samples were dried in an oven at 105°C for 24 hours. With the start of the experiment, daily sampling was done. The amount of soil water content in each irrigations (Equation 1) was determined by the following formula (DeAngelis, 2007).

$$SM\% = (WSW - DSW / DSW) \times 100 \quad (1)$$

Where, SM: Soil Moisture, WSW: Wet Soil Weight, WSD: Dry Soil Weight.

The moisture content of each plot at each irrigation interval was calculated in the

range of about 80% of the field capacity and was based on the following formula (Alizadeh, 1999).

$$V = \rho \times Z \times A \times (FC - SM) / E_i \quad (2)$$

Where, V (cm<sup>3</sup>)= Volume of irrigation water, ρ (g.cm<sup>-3</sup>)= Soil bulk density, Z(cm)= The root Zone, A(cm<sup>2</sup>)= Experimental unit Area, FC(%)= Soil moisture at Field Capacity, SM (%)= Soil Moisture, E<sub>i</sub> (%): Water utilization Efficiency.

### Isolation Procedure

The aerial parts of all *S. sahendica* treatments were harvested at full flowering stage and dried before distillation at room temperature. The essential oils were obtained by hydrodistillation method described by European Pharmacopoeia (Maissoneuve, 1983).

Approximately, 100 g of dried aerial parts were subjected to hydrodistillation for 3 hours, using an all-glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia to produce oil (Maissoneuve, 1983). The oils were dried with anhydrous sodium sulfate and placed in a dark and cold place (4°C) prior to analysis. The extracted oil was measured on a precision scale and, then, based on the amount of dry matter, the percentage of essential oil was calculated. Essential oil yield was result of multiplying percentage of essential oil in yield of shoot.

### Gas Chromatography

GC analysis was conducted according to Thermo-UFM Ultra-fast gas chromatography (Thermo Electron corporation, Serial No: 200610287), Equipped with fused silica column with PH-5 (10 m×0.1 mm id, film thickness 0.40 μm) in three replications. The oven temperature was set at 60°C for 3 minutes and then at 280°C at a rate of 40°C min<sup>-1</sup>. The detector (FID) temperature was 285°C and the



injector temperature was 285°C. Helium was used as carrier gas of 0.5 mL min<sup>-1</sup>. The oils were manually injected into GC without dilution. The percentages of compounds were calculated using the area normalization method, regardless of the response factors.

### Gas Chromatography-Mass Spectroscopy

The GC-MS analysis was carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m×0.25 mm id, film thickness 0.25 µm) to identify all the components of the oil. The oven temperature was 50-240°C at a 4°C min<sup>-1</sup> rate, transfer temperature of 260°C, helium temperature as a carrier gas with a velocity of 31.5 cm s<sup>-1</sup>, split ratio 1:60, ionization energy of 70eV, scanning time 1 s, and mass range 40-300 amu.

### Identification of Components

Essential oil components were identified by comparing their mass spectra with those made through mass spectra libraries or with authoritative combinations. The data was verified by comparing their retention indices, or with validated combinations or with published data in the literature (Adams, 2007).

### Statistical Analysis

All data were analyzed using variance analysis by SPSS software (version 18). This tool was separated by Duncan Multiple Range Test ( $P < 0.05$ ).

Due to the implementation of this project in two years, the combined analysis was initially conducted between two years, because of the importance of the components of the essential oil and the percentage of essential oil, the analysis of two-year data has taken place separately, to be examined more carefully.

## RESULTS AND DISCUSSION

Considering consumption of more than 80% of water in agriculture and water shortage in Iran (Alizadeh and Keshavarz, 2005), the main concern of experts is introduction of low water requiring plants and provision of methods to reduce water consumption and replacement of perennial plants instead of annual crops (Holmes and Rice, 1996).

*Satureja sahendica* is one of the medicinal plants that have been cultivated for the use of essential oils in various industries (Sefidkon et al., 2004).

According to the results of yield and percentage of essential oil (Table 4), it can be concluded that in the treatment with water stress condition at the blooming or flowering stages along with bio fertilizer, essential oil increased and water consumption decreased. Therefore, the amount of water saved can be used for increasing the area under cultivation. It can also produce more than 66 kg ha<sup>-1</sup> essential oil, therefore, production of *Satureja sahendica* in sufficient moisture conditions is economical compared to some other medicinal plants (Baher et al., 2002; Bettaieb et al., 2009; Manukyan, 2011).

The presence of more than 40% thymol in all treatments showed that this plant could be one of the most important medicinal plants for drug use (Sefidkon et al., 2004). The results indicated that the effect of the genetic factor on the production of thymol, *p*-cymene, and  $\gamma$ -terpinene has been more than the effect of environmental factors (Sefidkon et al., 2004), therefore, under any agronomic condition, these constituents will be the main compounds of this plant.

The oil percentage of all treatments varied from 0.91 to 2.88% and the oil yield varied from 4.93 to 60.13 kg ha<sup>-1</sup> during 2016 and 2017. Eighteen components were identified in the essential oils, with the major constituents being thymol, *p*-cymene, and  $\gamma$ -terpinene. The minor components in these oils were Sabinene (0.57%-2.04%),  $\alpha$ -terpinene (0.65-2.87%),  $\alpha$ -thujene (0.13-1%),  $\alpha$ -pinene (0.1-1.63%), Terpinolene, (0.1-0.6%), *p*-cymene-9-ol (0.2-

0.7%), E-caryophyllene (0.18-1.32%), Carvacrol (0.2-0.72%), Myrcene (0.06-0.66%), Limonene (0.12-0.34%), 1,8-cineole (0.01-0.15%), Linalool (0.12-0.63%), Cis-rose oxide (0.01-0.24%), Spathulenol (0.24-0.74%) and Caryophyllene oxide (0.33-0.98%).

Water stress mainly affected essential oil yield per hectare ( $\alpha \leq 0.01$ ) and percentage of thymol ( $\alpha \leq 0.01$ ),  $\rho$ -cymene, and sabinene ( $\alpha \leq 0.05$ ), while there was no significant difference for the essential oil percentage (Table 1). There was a significant difference between treatments inoculated with mycorrhiza on essential oil yield per hectare, content of thymol and  $\rho$ -cymene ( $\alpha \leq 0.01$ ), and essential oil percentage ( $\alpha \leq 0.05$ ).

In the first year, the interaction effect of water deficit and mycorrhiza was significant for  $\rho$ -cymene,  $\gamma$ -terpinene, thymol, essential oil percentage, and oil yield per hectare ( $\alpha \leq 0.01$ ) (Table 1). In the second year, the interaction of drought stress  $\times$  mycorrhizal fungi were significant on essential oil percentage, essential oil yield per hectare, thymol,  $\rho$ -cymene,  $\gamma$ -terpinene and some minor components such as  $\alpha$ -terpinene and Sabinene ( $\alpha \leq 0.01$ ) (Table 3). The amount of thymol (as the main phenolic compound) was the highest (74.59%) in the control  $\times$  *G. mosseae* in the first year (Table 2), whereas in the second year, the highest amount of thymol (61.97%) was obtained in

$D_4 \times G. mosseae$  (Table 4). The maximum amount of *S. sahendica* essential oil yield per hectare (37.5 kg ha<sup>-1</sup>) was obtained in control  $\times$  *G. intraradices* treatment (Table 2) in the first year. In the second year, the highest essential oil yield per hectare (66.13 kg ha<sup>-1</sup>) was observed in control  $\times$  *G. intraradices*. The highest percentage of essential oil (2.88%) was obtained in  $D_3 \times G. intraradices$  and *G. mosseae* (Table 4).

A significant difference in drought stress and biofertilizer treatments in most of the traits, except essential oil percentage (Tables 1 and 2) indicate a suitable selection of treatments. Also, the significant differences in all traits measured in the interaction of drought stress and biofertilizer (Tables 1 and 2) indicate the effect of the treatments on each

other and confirm the correct selection of simultaneous application of the two treatments on the plant. In other words, the hypothesis confirms the effectiveness of bio fertilizers on drought stress. Considering that the Essential oil yield is the result of multiplying the percentage of essential oil in the yield of shoot. In Table 2, nine treatments are in group A for essential oil percentage. However, in the case of essential oil yield, only DIM2 is in group A, which indicates the role of the shoot yield in essential oil yield. Therefore, the use of biodegradable fertilizers, especially mycorrhiza, can be very effective in reducing the effect of drought stress on the aerial parts of the plant (Trappe, 1987; Selosse *et al.*, 2006, Ruiz-Lozano, 2003), and thus increasing the percentage and yield of the essential oil (Charles *et al.*, 1990).

The results of this study indicated that the yield of essential oil per hectare significantly decreased due to the water limitation (Tables 2 and 4). In fact, water limitation decreases photosynthesis and transpiration rate (Sarker *et al.*, 2005) and the change of essential oil content in aromatic plants (Zehtab-Salmasi *et al.*, 2001). Reducing the growth of plants by water limitation can point to the reduction of root hydraulic conductivity (Augé *et al.*, 2008) and transpiration rate (Allen and Boosalis, 1983). Based on our study, irrigation under optimal conditions led to increased biosynthesis of essential oil in *S. sahendica*. Generally, water limitation increases the commercial quality of medicinal plants and content of secondary metabolites. Other studies have shown that water limitation reduced the essential oils of mints (Charles *et al.*, 1990) and sweet basil (Simon *et al.*, 1992), and our results are consistent with the results of these studies.

Thymol,  $\rho$ -cymene, and  $\gamma$ -terpinene were identified as the main components of *S. sahendica*.

All of these identified compounds accounted for more than 95% of all essential oil components in some treatments. Therefore, to consider the role of different treatments on the quality of essential



**Table 1.** Effects of drought stress on the essential oil yield and composition of *Satureja sahendica* Bormm.<sup>a</sup>

| Rep                  | SOV | Mean squares |          |                     |                |                     |          |   |
|----------------------|-----|--------------|----------|---------------------|----------------|---------------------|----------|---|
|                      |     | Df           | Sabinene | $\alpha$ -Terpinene | $\rho$ -Cymene | $\gamma$ -Terpinene | Thymol   | Yield of essential oil (kg ha <sup>-1</sup> ) |
| Drought stress       |     | 2            | 0.54ns   | 1.72**              | 118.46**       | 49.77*              | 390.24** | 497.58**                                      |
| Error (a) drought    |     | 3            | 1.21*    | 0.43**              | 50.20*         | 10.11ns             | 114.64** | 449.21**                                      |
| Mycorrhiza           |     | 6            | 0.16     | 0.02                | 6.84           | 4.65                | 1.05     | 12.04   |
| Drought stress       |     | 3            | 0.22*    | 1.04**              | 76.13**        | 0.84ns              | 100.53** | 174.56**                                      |
| Error drought stress |     | 9            | 0.36**   | 0.74**              | 88.37**        | 27.28**             | 204.11** | 237.70**                                      |
| Error                |     | 24           | 0.07     | 0.03                | 4.20           | 3.23                | 0.79     | 14.87   |
| Cv%                  |     |              | 16.86    | 9.11                | 8.70           | 10.78               | 1.73     | 17.004  |

<sup>a</sup> \*: Significant at P $\leq$  0.05; \*\*: Significant at P $\leq$  0.01, ns: Not significant.

**Table 2.** The effect of interaction between drought stress and mycorrhiza on the percentage of essential oil components of *Satureja sahendica* Bormm. in the first year.<sup>a</sup>

| Treatment <sup>b</sup>        | Sabinene          | $\alpha$ -Terpinene | $\rho$ -Cymen       | $\gamma$ -Terpinene  | Thymol             | Yield of essential oil (kg ha <sup>-1</sup> ) |   |
|-------------------------------|-------------------|---------------------|---------------------|----------------------|--------------------|---|---|
|                               |                   |                     |                     |                      |                    | Essential oil percentage                      | Yield of essential oil (kg ha <sup>-1</sup> ) |
| D <sub>1</sub> M <sub>1</sub> | 1.59ab $\pm$ 0.06 | 1.79ef $\pm$ 0.18   | 25.39bcd $\pm$ 1.12 | 13.4fg $\pm$ 0.69    | 52.38d $\pm$ 3.43  | 1.31cd $\pm$ 0.16                             | 28.36cd $\pm$ 4.5                             |
| D <sub>1</sub> M <sub>2</sub> | 1.59ab $\pm$ 0.06 | 2.13bcd $\pm$ 0.1   | 30.7a $\pm$ 1.12    | 16.51bcd $\pm$ 0.69  | 43.89k $\pm$ 2.54  | 1.62abc $\pm$ 0.17                            | 37.5a $\pm$ 6.61                              |
| D <sub>1</sub> M <sub>3</sub> | 0.57d $\pm$ 0.21  | 0.65h $\pm$ 0.18    | 9.41h $\pm$ 1.12    | 11.45g $\pm$ 0.69    | 74.59a $\pm$ 4.04  | 0.91e $\pm$ 0.15                              | 4.93g $\pm$ 1.32                              |
| D <sub>1</sub> M <sub>4</sub> | 1.82a $\pm$ 0.06  | 2.35bc $\pm$ 0.18   | 21.78ef $\pm$ 1.12  | 19.89ab $\pm$ 0.69   | 50.53ef $\pm$ 2.74 | 1.85a $\pm$ 0.15                              | 19.72f $\pm$ 2.35                             |
| D <sub>2</sub> M <sub>1</sub> | 0.95cd $\pm$ 0.52 | 2.87a $\pm$ 0.2     | 23.43de $\pm$ 1.53  | 19.29abc $\pm$ 4.68  | 45.12kj $\pm$ 2.69 | 1.82a $\pm$ 0.31                              | 19.76f $\pm$ 4.57                             |
| D <sub>2</sub> M <sub>2</sub> | 1.23bc $\pm$ 0.04 | 1.78f $\pm$ 0.2     | 17.68g $\pm$ 1.86   | 16.87bcd $\pm$ 1.04  | 55.32c $\pm$ 3.04  | 1.13de $\pm$ 0.15                             | 6.49g $\pm$ 1.64                              |
| D <sub>2</sub> M <sub>3</sub> | 1.08c $\pm$ 0.04  | 1.36g $\pm$ 0.2     | 23.58de $\pm$ 1.53  | 14.44efg $\pm$ 1.04  | 55.49c $\pm$ 2.93  | 1.05de $\pm$ 0.32                             | 9.08g $\pm$ 4.05                              |
| D <sub>2</sub> M <sub>4</sub> | 1.64ab $\pm$ 0.04 | 2.3bc $\pm$ 0.2     | 22.69de $\pm$ 1.53  | 18.16abc $\pm$ 1.04  | 52.08de $\pm$ 3.32 | 1.59abc $\pm$ 0.16                            | 21.52ef $\pm$ 2.35                            |
| D <sub>3</sub> M <sub>1</sub> | 1.71ab $\pm$ 0.2  | 2.01cdef $\pm$ 0.23 | 25.19bcd $\pm$ 1.82 | 16.48bcd $\pm$ 0.98  | 51.21de $\pm$ 2.83 | 1.74a $\pm$ 0.15                              | 23.37def $\pm$ 5.61                           |
| D <sub>3</sub> M <sub>2</sub> | 1.87a $\pm$ 0.2   | 2.14bcd $\pm$ 0.23  | 25.99bcd $\pm$ 1.82 | 16.07cdef $\pm$ 0.98 | 49.29gf $\pm$ 2.65 | 1.68ab $\pm$ 0.16                             | 29.52bcd $\pm$ 2.66                           |
| D <sub>3</sub> M <sub>3</sub> | 2.04a $\pm$ 0.2   | 2.4b $\pm$ 0.23     | 24.31cde $\pm$ 1.82 | 20.76a $\pm$ 0.98    | 46.19ij $\pm$ 2.54 | 1.85a $\pm$ 0.15                              | 26.38cdef $\pm$ 3.87                          |
| D <sub>3</sub> M <sub>4</sub> | 1.86a $\pm$ 0.2   | 2.04cdef $\pm$ 0.23 | 28.09abc $\pm$ 1.82 | 14.79def $\pm$ 1.005 | 47.15hi $\pm$ 2.46 | 1.3cd $\pm$ 0.24                              | 22.15def $\pm$ 2.43                           |
| D <sub>4</sub> M <sub>1</sub> | 1.99a $\pm$ 0.17  | 2.3bc $\pm$ 0.24    | 28.14abc $\pm$ 1.67 | 17.06bcd $\pm$ 0.89  | 46.72i $\pm$ 2.02  | 1.61abc $\pm$ 0.15                            | 35.71ab $\pm$ 6.35                            |
| D <sub>4</sub> M <sub>2</sub> | 1.79a $\pm$ 0.17  | 2.29bc $\pm$ 0.24   | 18.8fg $\pm$ 1.67   | 16.75bcd $\pm$ 0.89  | 57.48b $\pm$ 3.46  | 1.35bcd $\pm$ 0.18                            | 23.04def $\pm$ 2.85                           |
| D <sub>4</sub> M <sub>3</sub> | 1.92a $\pm$ 0.17  | 1.9def $\pm$ 0.22   | 23.02de $\pm$ 5.09  | 19.53abc $\pm$ 0.89  | 44.92kj $\pm$ 2.8  | 1.76a $\pm$ 0.15                              | 30.95abc $\pm$ 2.34                           |
| D <sub>4</sub> M <sub>4</sub> | 1.61ab $\pm$ 0.17 | 1.82def $\pm$ 0.24  | 28.54ab $\pm$ 1.67  | 15.49def $\pm$ 0.89  | 48.34gh $\pm$ 2.1  | 1.32cd $\pm$ 0.18                             | 24.13cdef $\pm$ 2.64                          |

<sup>a</sup> Means in a column followed by the same letter are not significantly different (P $\leq$  0.05). <sup>b</sup> D= Drought stress; D<sub>1</sub>= Control (no stress); D<sub>2</sub>= No irrigation during stem length till blooming; D<sub>3</sub>= No irrigation in the blooming up to start flowering; D<sub>4</sub>= No irrigation at 50% flowering up to full flowering; M= Mycorrhiza; M<sub>1</sub>= Control (no inoculation); M<sub>2</sub>= *Glomus intraradices*; M<sub>3</sub>= *Glomus mosseae*; M<sub>4</sub>= *Glomus intraradices*.

**Table 3.** Effects of drought stress on the essential oil yield and composition of *Satureja sahendica* Bornm.in the second year. <sup>a</sup>

| SOV                       | Df | Mean squares |                     |          |                     |          |                          |
|---------------------------|----|--------------|---------------------|----------|---------------------|----------|--------------------------|
|                           |    | Sabinene     | $\alpha$ -Terpinene | p-Cymene | $\gamma$ -Terpinene | Thymol   | Essential oil percentage |
| Rep                       | 2  | 0.24**       | 0.18**              | 292.09** | 34.36**             | 274.57** | 0.05ns                   |
| Drought stress            | 3  | 0.18**       | 0.18**              | 325.22** | 12.08**             | 463.66** | 0.07ns                   |
| Error (a) drought         | 6  | 0.0007       | 0.0001              | 0.60     | 0.08                | 0.45     | 0.078                    |
| Mycorrhiza                | 3  | 0.13**       | 0.27**              | 25.38**  | 16.44**             | 80.63**  | 0.12ns                   |
| Drought stress×Mycorrhiza | 9  | 0.16**       | 0.12**              | 71.76**  | 11.27**             | 90.29**  | 0.23**                   |
| Error                     |    | 0.0004       | 0.0003              | 0.67     | 0.07                | 0.50     | 0.04                     |
| Cv%                       |    | 1.48         | 1.23                | 2.42     | 1.95                | 1.67     | 15.51                    |
|                           |    |              |                     |          |                     |          | 18.97                    |

<sup>a</sup> \*\*: Significant at  $P \leq 0.05$ ; \*\*: Significant at  $P \leq 0.01$ , ns: Not significant.

**Table 4.** The effect of interaction between drought stress and mycorrhiza on the percentage of essential oil components of *Satureja sahendica* Bornm.in the second year. <sup>a</sup>

| Treatment <sup>b</sup>        | Sabinene      | $\alpha$ -Terpinene | p-Cymen        | $\gamma$ -Terpinene | Thymol         | Essential oil percentage | Yield of essential oil (kg ha <sup>-1</sup> ) |
|-------------------------------|---------------|---------------------|----------------|---------------------|----------------|--------------------------|---|
|                               |               |                     |                |                     |                |                          |   |
| D <sub>1</sub> M <sub>1</sub> | 1.68c ± 0.06  | 1.71b ± 0.06        | 40.11b ± 3.32  | 15.55c ± 0.87       | 34.1j ± 2.31   | 1.29cdef ± 0.15          | 33.02bdef ± 1.89                              |
| D <sub>1</sub> M <sub>2</sub> | 1.43g ± 0.07  | 1.55d ± 0.05        | 38.32c ± 2.47  | 14.88d ± 0.88       | 35.92hi ± 2.86 | 2.32abc ± 0.36           | 66.13a ± 7.11                                 |
| D <sub>1</sub> M <sub>3</sub> | 1.46g ± 0.06  | 1.44f ± 0.07        | 31.45g ± 2.65  | 13.35e ± 0.71       | 45.12d ± 2.65  | 1.96abcde ± 0.39         | 18.69ef ± 1.56                                |
| D <sub>1</sub> M <sub>4</sub> | 1.63de ± 0.06 | 1.77a ± 0.06        | 37.79cd ± 2.53 | 17.85a ± 1.12       | 35.4i ± 2.63   | 1.85abcde ± 0.05         | 34.51bdef ± 10.53                             |
| D <sub>2</sub> M <sub>1</sub> | 1.6de ± 0.07  | 1.62c ± 0.04        | 35.64ef ± 2.17 | 16.02c ± 0.78       | 38.48fg ± 2.57 | 1.64bdef ± 0.31          | 24.32cdef ± 5.9                               |
| D <sub>2</sub> M <sub>2</sub> | 1.32h ± 0.07  | 1.47ef ± 0.06       | 38.84bc ± 1.89 | 13.56e ± 0.75       | 38.01g ± 2.31  | 0.92ef ± 0.31            | 9.77f ± 1.84                                  |
| D <sub>2</sub> M <sub>3</sub> | 1.32h ± 0.06  | 1.16j ± 0.05        | 44.45a ± 2.68  | 10.45i ± 0.82       | 36.66h ± 1.97  | 1.7bdef ± 0.23           | 20.23def ± 2.19                               |
| D <sub>2</sub> M <sub>4</sub> | 1.54f ± 0.05  | 1.44f ± 0.06        | 36.73de ± 2.34 | 14.86d ± 0.61       | 39.65f ± 2.9   | 2.2abcd ± 0.2            | 36.75bcde ± 4.24                              |
| D <sub>3</sub> M <sub>1</sub> | 1.33h ± 0.06  | 1.26i ± 0.06        | 26.3i ± 1.96   | 14.96d ± 0.86       | 50.79b ± 2.31  | 1.07def ± 0.38           | 18.22ef ± 6.24                                |
| D <sub>3</sub> M <sub>2</sub> | 1.79a ± 0.08  | 1.56d ± 0.05        | 31.76g ± 2.04  | 16.9b ± 1.12        | 40.92e ± 2.46  | 2.15abcd ± 0.01          | 47.27abc ± 3.42                               |
| D <sub>3</sub> M <sub>3</sub> | 1.75b ± 0.08  | 1.49e ± 0.06        | 34.65f ± 2.74  | 15.9c ± 0.8         | 39.56f ± 2.42  | 2.62ab ± 0.02            | 44.83abcd ± 2.3                               |
| D <sub>3</sub> M <sub>4</sub> | 1.64d ± 0.07  | 1.35g ± 0.05        | 38.27c ± 2.91  | 12.52f ± 0.8        | 39.15fg ± 2.25 | 2.88a ± 0.89             | 52.02ab ± 20.16                               |
| D <sub>4</sub> M <sub>1</sub> | 1.52f ± 0.05  | 1.63c ± 0.05        | 29.27h ± 2.68  | 14.51d ± 0.86       | 47.46c ± 2.56  | 2.55ab ± 0.61            | 46.13abcd ± 14.95                             |
| D <sub>4</sub> M <sub>2</sub> | 1.59e ± 0.06  | 1.54d ± 0.07        | 30.44b ± 2.6   | 15.53c ± 0.89       | 44.19d ± 2.35  | 2.05abcde ± 0.08         | 43.04abcde ± 6.79                             |
| D <sub>4</sub> M <sub>3</sub> | 0.79i ± 0.07  | 0.8k ± 0.06         | 19.68j ± 1.97  | 11.33h ± 0.87       | 61.97a ± 1.58  | 0.72f ± 0.21             | 17.15ef ± 6.85                                |
| D <sub>4</sub> M <sub>4</sub> | 1.44g ± 0.03  | 1.29h ± 0.06        | 29.16h ± 2.64  | 12.03g ± 0.78       | 50.75b ± 2.28  | 1.99abcde ± 0.08         | 46.87abc ± 5.56                               |

<sup>a</sup> Means in a column followed by the same letter are not significantly different ( $P \leq 0.05$ ); <sup>b</sup> D and M are defined under Table 2.



oils, it is better to consider the variations of these three compounds.

Also, thymol and carvacrol are structurally similar to the hydroxyl group in different locations on the phenolic ring (Ultee *et al.*, 2002), so, it is better to evaluate total carvacrol and thymol in the evaluation of phenolic compounds of *S. sahendica*.

It can be assumed that the sequence in this process is as follows:  $\gamma$ -terpinene,  $p$ -cymene, thymol or carvacrol. According to our results,  $p$ -cymene, thymol, and essential oil yield of *S. sahendica* increased by using *G. mosseae* or *G. intraradices* under water stress (Tables 2 and 4). As a result, in order to increase the quality of the essential oil, the use of *G. mosseae* and *G. intraradices* has been approved (Kapoor *et al.*, 2002).

This finding is in agreement with Karagiannidis *et al.* (2011) who reported the increase of essential oil in both Oregano and Mint plants inoculated with *G. etunicatum*, and *G. lamellosum*. According to the results, thymol content in *S. sahendica* oil was affected by AM fungi. However, thymol content in plants that had been inoculated with *G. mosseae* was higher than those inoculated with *G. intraradices* (Tables 2 and 4). The highest oil yield was achieved in the application of AM fungi (D1M2) (Tables 2 and 4). Khorasaninejad *et al.* (2011) reported the highest values of biomass, essential oil yield and percentage of *Mentha piperita* L. in the control (100% field capacity) plant, compared to 85, 70, 60, and 45% of field capacity. Under water stress condition, Flexas and Medrano (2002) state that changes in photosynthesis and carbohydrate content caused plant growth failure and decrease in essential oil content, which is not in agreement with our result.

Based on the results of this study, the use of AM fungi is a promising method for the production of medicinal plants, especially in conditions of water limitation. Other researchers also reported that inoculation with fungi is effective in decreasing effects of water stress (Pirzad and Mohammadzadeh, 2018). These results help us to find new ways to increase secondary

metabolites of medicinal plants in water scarce areas.

## CONCLUSIONS

*S. sahendica*, a valuable and unique Iranian species, is used in various industries due to the presence of thymol and carvacrol in its essential oil. In the production of this plant, water consumption can be significantly reduced. However, by using bio-fertilizers, the effect of drought stress can be minimized. The results of this study showed that since this plant is perennial and produces a lot of essential oil, its production can be economical and is one of the recommended plants to produce a huge amount of biomass with less water.

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## اثر تنش آبی و قارچ میکوریزا در مقدار اسانس و ترکیبات مرزه سهندی

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### چکیده

مرزه سهندی یک گیاه دارویی انحصاری ایران است، ترکیب اصلی اسانس آن تیمول می باشد که در صنایع دارویی و غذایی استفاده می شود. افزایش عملکرد اسانس و ترکیبات فنولیک در اسانس مرزه منجر به افزایش اثرات دارویی آن می شود. در این تحقیق اثر قارچ میکوریزا (*Glomus mosseae*, ) و سطوح مختلف تنش آبی (بدون تنش آبی =  $D_1$ ، قطع آبیاری در ساقه دهی تا غنچه دهی =  $D_2$ ، قطع آبیاری در غنچه دهی تا شروع گلدهی =  $D_3$ ، قطع آبیاری در ۵۰ درصد گلدهی تا گلدهی کامل =  $D_4$ )، روی اسانس مرزه سهندی در طول ۲ سال بررسی شد. در زمان انتقال نشا به زمین اصلی در هر چاله ۱۰ گرم کود زیستی حاوی میزان مناسبی از اسپوره های فعال قارچ میکوریزا اضافه گردید. برداشت گیاهان در گلدهی کامل بود. اسانسگیری بوسیله دستگاه کلونجر و آنالیز آن با Gc-Mas انجام شد. بیشترین عملکرد اسانس ( $66/13 \text{ kg ha}^{-1}$ ) با مصرف *G. intraradices* در شرایط بدون تنش در سال دوم مشاهده شد. بیشترین درصد تیمول (۷۴/۵۹٪) با مصرف *G. mosseae* در شرایط بدون تنش خشکی در سال اول بود، در حالی که در سال دوم بیشترین مقدار تیمول (۶۱/۹۷٪) با مصرف *G. mosseae* در شرایط قطع آبیاری در ۵۰ درصد گلدهی وجود داشت. این تحقیق نشان داد که با استفاده قارچ میکوریزا حتی در شرایط کمبود آب، مقدار اسانس قابل توجه با درصد تیمول بالا در مرزه سهندی تولید شد. با مصرف قارچ میکوریزا می توان افزایش در اسانس و تغییرات در ترکیبات اسانس به دست آورد.