

## Water Stress Modifies Essential Oil Yield and Composition, Glandular Trichomes and Stomatal Features of Lemongrass (*Cymbopogon citratus* L.) Inoculated with Arbuscular Mycorrhizal Fungi

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### ABSTRACT

We evaluated the effects of Arbuscular Mycorrhizal Fungi (AMF) on percentage of Essential Oil (EO) content, EO yield, EO composition, and anatomical characteristics of lemongrass at four levels of water availability [100, 75, 50, and 25% Field Capacity (FC)]. EO composition was determined by GC-MS analysis, and scanning electron microscopy was used to investigate the anatomical parameters (stomatal density and size as well as glandular trichomes). EO content and yield significantly increased under moderate water stress (50% FC) and AMF inoculation. The highest EO content (1.09%) and yield (0.26 g plant<sup>-1</sup>) occurred in 50% FC combined with AMF inoculation treatment. In contrast, the lowest EO content (0.68%) and yield (g plant<sup>-1</sup>) were observed at 25% FC and no AMF inoculation. The main EO components were geranial, neral,  $\beta$ -pinene and nerol. Water stress increased the content of geranial,  $\beta$ -pinene, whereas it decreased the neral and nerol content. In general, AMF inoculation enhanced the geranial, neral, and  $\beta$ -pinene, but nerol was slightly decreased in total. The highest geranial (48.02%) and  $\beta$ -pinene (7.72%) was observed in AMF inoculated plants at 50% FC. However, the maximum content of neral (35.02%) was found in inoculated plants at 100% FC. Water stress changed the stomatal density and size as well as the number of glandular trichomes. The highest stomatal density was observed in 50% FC (148.1 stomata number mm<sup>-2</sup>). Water stress decreased the stomatal size, with the lowest stomatal length (24.1  $\mu$ m) and width (14.1  $\mu$ m) observed at 25% FC. In addition, the number of glandular trichomes at 50% FC (24.6 trichome number mm<sup>-2</sup>) was greater than the other treatments. This study suggests inoculating lemongrass plants with AMF and maintaining a moderate water stress to obtain the optimum EO.

**Keywords:** Geranial trichomes, Glandular trichomes, *Glomus mosseae*, Stomatal density, Water deficiency.

### INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is a perennial plant that belongs to the family Poaceae and originates from tropical regions in Southeast Asia (Vaqrar *et al.*, 2007, Babarinde *et al.*, 2016). The lemon scent

flavor of lemongrass has made it popular in Asian cooking (Ajayi *et al.*, 2016). The strong lemony scent results from a high concentration of citral, with the two main geometric isomers being geranial and neral (Oliveira *et al.*, 2018). In addition, lemongrass contains a variety of other compounds (e.g. terpenes, flavonoids, and

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alkaloids) whose composition varies depending upon the growing conditions (Ajayi et al., 2016). In addition to cooking, the Essential Oil (EO) of lemongrass is used in the perfume, cosmetic, and food industries. Lastly, it has medicinal value as it is frequently used to treat cough, cold, rheumatism, digestive problems, bladder issues, toothache and swollen gums (Balakrishnan et al., 2014, Ahmad and Viljoen, 2015).

Water stress can significantly influence the biochemical, physiological, and anatomical characteristics of plants like lemongrass (Liwani et al., 2018). To help cope with increased stress, plants frequently form a symbiotic relationship with mycorrhizae fungi (Ruíz-Lozano et al., 2012). Arbuscular Mycorrhizal Fungi (AMF) frequently colonize the roots of plants located in ecosystems with water and salt stress, thereby improving plant health (Ruíz-Lozano et al., 2012; Rydlová et al., 2015). In aromatic/medicinal plants like lemongrass, AMF inoculation is a natural and effective way to induce plant growth and improve EOs (Rydlová et al., 2015). Latef et al. (2016) demonstrated that AMF inoculation promotes growth in plants exposed to water stress by improving root distribution, leaf area, nutrients uptake, and soil development (Latef et al., 2016). It is hypothesized that secondary metabolites, such as EOs and phenolic compounds in aromatic and medicinal plants, might also be improved by AMF inoculation (Urcoviche et al., 2015).

Water stress and AMF inncoulation may improve EO production in lemongrass. Improvement in EO quality as a result of water stress has been reported in *Pelargonium graveolens* L. (Amiri et al., 2017), *Thymus daenensis* Celak (Bistgani et al., 2018), *Foeniculum vulgare* L. (Zardak et al., 2017), *Dracocephalum moldavica* L. (Fadaee et al., 2018), *Thymus Vulgaris* L. (Arpanahi and Feizian, 2019). Water stress can alter anatomical properties of plants, such as stomatal characteristics, but the response varies by species (Haworth et al., 2018). For example, the change of stomatal density and

size under water stress was reported in several studies (Yang and Wang, 2001; Zhang et al., 2004; Haworth et al., 2018). Several researchers have reported the effect of mycorrhizal inoculation and water stress on essential EO quantity and quality (Amiri et al., 2015; Zardak et al., 2017; Amiri et al., 2017; Fadaee et al., 2018; Arpanahi and Feizian, 2019). They mostly concluded that AMF colonization enhances the plant ability to cope with different environmental stresses. However, the combined impact of AMF inoculation and water stress on anatomical attributes of lemongrass are still unknown. Therefore, the objectives of our study were to evaluate the effects of AMF inoculation and water stress on EO quantity and quality, and anatomical properties of lemongrass.

## MATERIALS AND METHODS

### Growth Conditions and Treatments

Six-month old lemongrass seedlings were obtained from the Research Institute of Forests and Rangelands, Iran. The pot experiment was conducted in a greenhouse with a photoperiod of 16/8 hours (lightness/darkness) and relative humidity of 65-80% in University of Tehran, Karaj, Iran. The experimental pots had a top diameter of 19 cm, a base diameter of 13 cm, and a height of 10 cm. The soil used for the study was sandy loam with a pH: 7.2, EC: 1.1 dS m<sup>-1</sup>, N: 0.24%, P: 12.7 mg kg<sup>-1</sup>; and K: 213 mg kg<sup>-1</sup>. This work was conducted as factorial based on Randomized Complete Block Design (RCBD) with three replications of each treatment. Water stress was applied at four levels [100, 75, 50, and 25% Field Capacity (FC)] and inoculation with AMF (*Glomus mosseae*) was done at two levels (inoculated and non-inoculated).

### AMF Application

Due to its high and common symbiosis with most plant species, *Glomus mosseae*

was applied in rhizosphere of the lemongrass seedlings. The mycorrhizal inoculant was provided by Soil and Water Research Institute of Iran. The inoculum suspension was prepared by wet sieving technique including colonized root fragment, hyphae, and 1,000 spores per pot. The experimental soil was sterilized by autoclave at 121°C for 60 minutes (Gerdemann and Nicolson, 1963).

### Irrigation

All plants were irrigated for one month prior to the start of the experiment. The plants were then randomly selected for one of four water treatments [100, 75, 50, and 25% Field Capacity (FC)] that lasted 80 days. The amount of irrigation water required for each treatment was determined using Equation (1) (Benami and Ofen, 1984):

$$V_n = (FC - PWP) / 100 \times \rho_b \times V_p \times MAD$$

Where,  $V_n$  is the irrigation water required before irrigation ( $\text{cm}^3$ ), FC is Field Capacity (%), PWP is the Wilting Point (%),  $\rho_b$  is bulk density ( $\text{gr cm}^{-3}$ ),  $V_p$  is the pot Volume ( $\text{cm}^3$ ), MAD is Management Allowed Depletion. FC and PWP were measured by pressure plate.

### Essential oil (EO) Content and Yield

Lemongrass EO content was quantified by using the method described by the European Pharmacopoeia for oil production (European Pharmacopoeia, 1983). Briefly, 100 g of dried aboveground plant parts were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The oil samples were dehydrated by placing them in dark glass bottles containing anhydrous sodium sulfate. The samples were maintained at 4°C until they were analyzed by gas chromatography and/or mass spectroscopy. EO yield was determined as the amount of EO extracted from total dry weight of one plant.

### Gas Chromatography (GC)

EO was determined using Thermo-UFM ultrafast gas chromatograph equipment with a ph-5 fused silica column (10 m length  $\times$  0.1 mm id., 0.4  $\mu\text{m}$  film thickness). Oven temperature was maintained at 60°C for 5 minutes and then programmed to 285°C at a rate of 5°C  $\text{min}^{-1}$ . The Flame Ionization Detector (FID) and injector temperature were 290 and 280°C, respectively. Helium was applied as carrier gas with an inlet pressure of 0.5  $\text{kg cm}^{-2}$ .

### Gas Chromatography-Mass Spectroscopy (GC/MS)

GC-MS analyses was conducted using a Varian 3400 GC-MS system equipment with AOC-5000 auto injector and DB-5 fused silica capillary column (30 m  $\times$  0.25 mm i.d.; 0.25  $\mu\text{m}$  film thicknesses). Temperature was programmed to increase from 60 to 250°C at a rate of 3°C  $\text{min}^{-1}$ . The injector and interface temperatures were maintained at 260 and 270°C, respectively. The acquisition mass range was 40–340 amu, the ionization voltage was 70eV and the carrier gas was helium at a velocity of 45  $\text{cm s}^{-1}$ .

### Identification of Components

The homologous series of n-alkanes was applied to calculate the Retention Index (RI) for all volatile Constituents (C7–C25). The EO components were identified by matching their RI and mass spectra with those reported in literature (Rather, 2017). Identification of EO components was done by gas chromatography/mass spectroscopy.

### Anatomical Analyses

For investigation of the anatomical parameters viz. stomatal density and size as well as glandular trichomes, dry leaf samples were analyzed via scanning electron



microscopy (SEM, SU 3500, Hitachi, Japan). In this regard, the gold was used for coating the dry samples in the vacuum coating unit (SG 110, Iran) according to the method reported by Robinson *et al.* (2012). Image J software was applied to measure the anatomical properties.

### Statistical Analysis

The data were analyzed by SAS software package for Windows (SAS, Version 9.3, SAS Institute, Cary, NC). The means were compared using a Duncan multiple range test with \* and \*\* representing significant differences of  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

## RESULTS

### Essential oil (EO) Content and Yield

EO percentage and EO yield significantly increased with moderate water stress and AMF inoculation. The highest EO content (1.09%) was found at 50% FC and AMF inoculation (Table 1). In contrast, the combination of 25% FC and no inoculation produced the lowest EO content (0.68%) (Table 1). At high water contents (100 and 75% FC) AMF inoculation had no significant effect (Table 1). However, when water stress (50 and 25% FC) increased, AMF inoculation significantly increased EO content (Table 1). The maximum EO yield ( $0.26 \text{ g plant}^{-1}$ ) was in the 50% FC inoculated

plants, whereas the minimum ( $0.07 \text{ g plant}^{-1}$ ) was in 25% FC non-inoculated plants (Table 1). In 50 and 25% FC treatments, EO yield in AMF-inoculated plants was significantly greater than that of non-inoculated plants. In contrast, there was no significant difference in EO yield between AMF-inoculated and non-inoculated plants at the lower water stress treatments. (75 and 25% FC) (Table 1).

### EO Composition

The GC and GC/MS analysis of lemongrass EO identified 17 compounds (Table 2), most of which belonged to oxygenated monoterpenes. In all treatments, geranial, neral,  $\beta$ -pinene and nerol were the main constituents (Table 2). Water stress increased the content of geranial,  $\beta$ -pinene, whereas it decreased the neral and nerol content. In general, AMF inoculation enhanced the geranial, neral, and  $\beta$ -pinene, but nerol was slightly decreased in total. The highest geranial (48.02%) and  $\beta$ -pinene (7.72%) contents were observed in 50% FC and inoculated plants. However, the maximum content of neral (35.02%) was found in 100% FC and inoculated plants. Nerol content in the 100% FC non-inoculated plants was the greatest as 5.89%.

### Anatomy

Water stress significantly altered anatomy of the stomata and glandular trichomes.

**Table 1.** The effects of water stress and Arbuscular Mycorrhiza Fungi (AMF) inoculation on Essential Oil (EO) percentage and EO yield of lemongrass.

Water stress	AMF	EO (%)	EO yield ( $\text{g plant}^{-1}$ )
100% FC	Uninoculated	0.71 <sup>ed</sup>	0.19 <sup>c</sup>
	Inoculated	0.78 <sup>cd</sup>	0.23 <sup>b</sup>
75% FC	Uninoculated	0.74 <sup>c-e</sup>	0.20 <sup>bc</sup>
	Inoculated	0.79 <sup>c</sup>	0.22 <sup>b</sup>
50% FC	Uninoculated	0.96 <sup>b</sup>	0.26 <sup>bc</sup>
	Inoculated	1.09 <sup>a</sup>	0.26 <sup>a</sup>
25% FC	Uninoculated	0.6 <sup>f</sup>	0.07 <sup>d</sup>
	Inoculated	0.68 <sup>e</sup>	0.09 <sup>d</sup>

**Table 2.** The effects of water stress and Arbuscular Mycorrhiza Fungi (AMF) inoculation (+) and no inoculation (-) on Essential Oil (EO) composition of lemongrass.

Compound	RI	100% FC		75% FC		50% FC		25% FC	
		-	+	-	+	-	+	-	+
6-Methyl-5-hepten-2-one	987	0.53	0.55	0.45	0.34	0.16	0.19	0.21	0.34
β-Pinene	990	6.36	6.39	7.16	7.32	7.35	7.72	7.3	7.65
Limonene	1029	0.42	0.35	0.34	0.33	0.07	0.09	0.1	0.12
cis-β-Ocimene	1036	0.57	0.53	0.51	0.52	0.72	0.78	0.78	0.84
trans-β-Ocimene	1048	0.24	0.26	0.26	0.27	0.29	0.32	0.38	0.37
Linalool	1102	1.61	1.82	1.76	1.73	1.46	1.48	1.76	1.88
Citronellal	1155	0.33	0.14	0.23	0.15	0.18	0.14	0.27	0.25
isoNeral	1166	1.64	0.87	0.98	0.86	1.66	1.55	1.96	1.75
Rose furan epoxide	1177	0.68	0.24	0.27	0.32	0.21	0.26	0.55	0.59
isoGeranial	1184	2.15	2.17	2.85	2.5	1.56	1.59	3.25	3.32
β-Citronellol	1238	1.39	1.3	1.23	1.09	0.74	0.71	0.78	0.89
Neral	1250	34.92	34.78	33.1	35.2	30.39	32.21	29.34	32.8
Nerol	1264	5.89	5.24	5.12	5.28	3.06	3.09	3.11	3.23
Geranial	1282	39.02	40.95	40.05	40.7	46.4	48.02	40.4	41.2
Geranyl acetate	1385	2.61	2.12	2.09	2.15	1.67	1.27	1.89	1.66
trans-Caryophyllene	1421	0.07	0.05	0.09	0.04	0.22	0.28	0.25	0.22
Caryophyllene oxide	1786	0.19	0.11	0.15	0.12	0.2	0.25	0.24	0.27
Total (%)		99.62	97.87	96.64	98.92	96.34	99.95	92.57	97.38

With increase in water stress, width and length significantly declined (Table 2). In contrast, the 50% FC produced a stomatal density that was significantly higher than the other water stress treatments. However, the increase in stomatal density only resulted in a shift from 142.6-145.1 stomata mm<sup>-2</sup> for the 100, 75, and 25% FC treatments to 148.1 stomata/mm<sup>2</sup> at the 50% FC treatment (Table 3). The number of glandular trichomes in 50% FC was significantly greater than other treatments (Table 3, Figure 1). There was no significant effect of AMF inoculation for the measured leaves

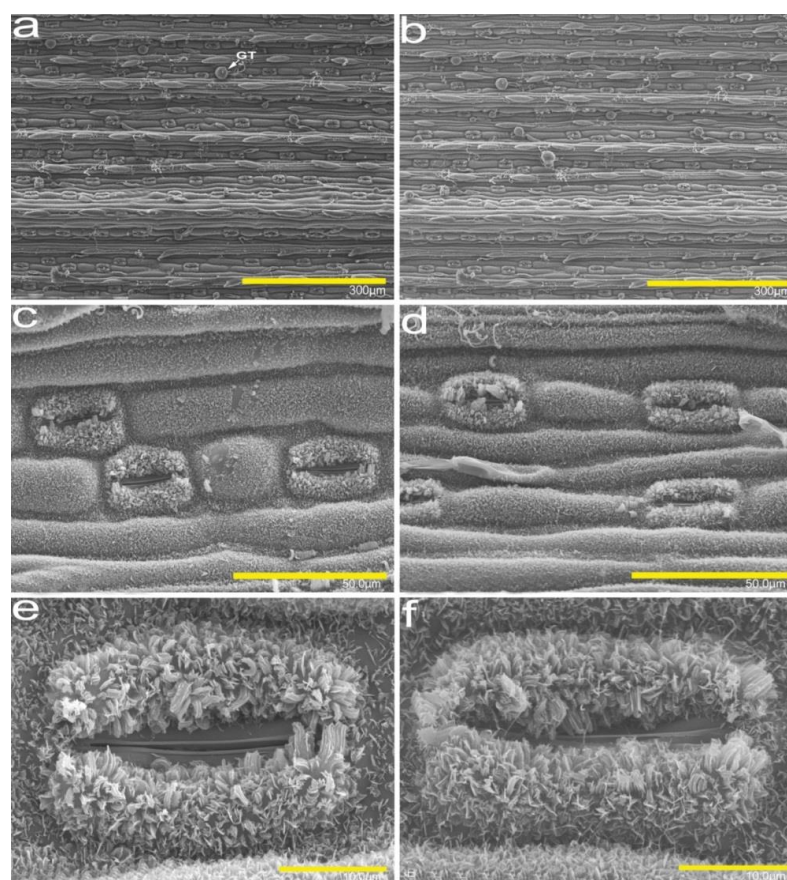
variables.

## DISCUSSION

Exposing lemongrass plants to moderate water stress (50% FC) led to a significant increase in their EO content and yield. Water stress induces the accumulation of lemongrass EO likely because the density of oil glands on leaf surface increases (Pirbalouti *et al.*, 2014) and a higher terpene production. The reduction of soil moisture can increase EO because more metabolites

**Table 3.** The effects of water stress on stomatal density and size and the number of glandular trichomes of lemongrass.

Water stress	Stomatal density (Number mm <sup>-2</sup> )	Stomatal width (μm)	Stomatal length (μm)	glandular trichomes density (Number mm <sup>-2</sup> )
100% FC	142.6 <sup>c</sup>	16.83 <sup>a</sup>	30.1 <sup>a</sup>	21.3 <sup>b</sup>
75% FC	145.1 <sup>b</sup>	16.83 <sup>a</sup>	29.3 <sup>a</sup>	22.5 <sup>b</sup>
50% FC	148.1 <sup>a</sup>	15.5 <sup>b</sup>	25.8 <sup>b</sup>	24.6 <sup>a</sup>
25% FC	143.3b <sup>c</sup>	14.16 <sup>c</sup>	24.1 <sup>c</sup>	22.3 <sup>b</sup>



**Figure 1.** Images of anatomical properties of lemongrass leaf taken by Scanning Electron Microscopy (SEM). Stomata density and size, and Glandular Trichomes (GT) density at 100 (right side) and 50% FC (left side). Scale bars of (a, b)= 300 µm; (c, d)= 50 µm, (e, f)= 10 µm.

are produced by plants under water stress in an effort to impede cell damage from oxidization stress (Farahani *et al.*, 2009; Lermen *et al.*, 2015). Hence, water stress has been associated with an increase in EO production in *Calendula officinalis* (Anderson *et al.*, 2016), *Pelargonium graveolens* L. (Amiri *et al.*, 2017), *Salvia officinalis* L. (Vosoughi *et al.*, 2018), *Thymus Vulgaris* L. (Arpanahi and Feizian, 2019).

AMF inoculation of medicinal plants increased EO production in lemongrass (Lermen *et al.*, 2015), *Coriandrum sativum* and *Anethum graveolens* (Rydlová *et al.*, 2015), *Mentha crisper* (Urcovich *et al.*, 2015), *Coriandrum sativum* L. (Oliveira *et al.*, 2016), *Pelargonium graveolens* L. (Amiri *et al.*, 2017), *Leptospermum scoparium* (Wicaksono *et al.*, 2018). The

biosynthesis of different secondary metabolites, like monoterpenes and sesquiterpenes, can be enhanced by the AMF symbiosis in medicinal plants (Lermen *et al.*, 2015, Amiri *et al.*, 2017). In most aromatic plants, monoterpenes are the most common terpene present. The main compounds (geraniol, neral,  $\beta$ -pinene, and nerol) obtained in the present study were monoterpenes. The biosynthesis of monoterpenes in plants can be increased by soils inoculated with AMF. The Mevalonate (MVA) pathway is the main process for biosynthesis of monoterpenes (Amiri *et al.*, 2017). The expression of genes corresponding with monoterpene biosynthesis may be improved by AMF. For example, transcriptome analysis of some enzymes in the MVA and Methyl Erythritol Phosphate (MEP) pathways indicated that

AMF increased isoprenoids via induction of the MEP pathway. Hence, AMF symbiosis can boost the IPP/DMAPP pool by the MEP pathway (Mandal *et al.*, 2015). Eventually, AMF inoculation expands the quantity of carbohydrates and the net photosynthesis rate, the great precursors in monoterpene biosynthesis (Mandal *et al.*, 2015). Therefore, an increase in some monoterpenes can be due to AMF inoculation. Terpenes are a structurally complex group of secondary metabolites that are found in many EOs (Tetali, 2019). The EO content of lemongrass is characterized by a high amount of monoterpenes that includes geraniol and neral, and lower concentration of  $\beta$ -pinene and nerol (Hanaa *et al.*, 2012). Geraniol is a powerful aromatic compound that is widely used in the perfume and food industries. It possesses antimicrobial and insecticide activities (Amiri *et al.*, 2017). In our work, the geraniol content increased as a result of water stress and AMF symbiosis. Neral also has strong antimicrobial qualities. It is used in the synthesis of vitamin A, ionone, and methylionone (Robacker and Hendry, 1977). Moderate water stress (50% FC) resulted in the highest content of geraniol and  $\beta$ -pinene and a 16 and 11% increase of, respectively, geraniol and  $\beta$ -pinene relative to the 100% FC treatment. However, we observed a 12 and 44% reduction of, respectively, neral and nerol in 25% FC in comparison with 100% FC. AMF inoculation resulted in an increase of the main compounds found in lemongrass (Table 2). For example, geraniol in AMF-inoculated plants (42.71%) was greater than that in non-inoculated ones (41.4%). Our results are similar to Lermen *et al.* (2015), who reported that geraniol increased from 43% in non-inoculated plants to 62% in AMF-inoculated plants under Pb stress.

Stomata are important structures that control the exchange of gases between atmosphere and plant. Their anatomy maybe influenced by many environmental stresses induced by salinity, temperature, CO<sub>2</sub> concentration, and water deficit

(Steinhorsdottir *et al.*, 2019). Under water stress, photosynthesis is reduced by both stomatal and non-stomatal components (Boussadia, 2018). Several studies have indicated that the stomatal density was enhanced under water stress (Yang and Wang, 2001; Zhang *et al.*, 2004; Haworth *et al.*, 2018), while the stomata number per leaf was reduced (Quarrie and Jones, 1977). Similar to our study, Xu and Zhou (2008) reported an increase in stomatal density under moderate water stress, but a reduction with increased water stress severity. As with our study, it is commonly reported that increased water stress results in a decrease in stomatal size (width and length) (Quarrie and Jones, 1977, Xu and Zhou, 2008). However, not all researchers have consistently reported a decrease in stomatal size with increasing water stress. For example, Zhang *et al.* (2004) reported that stomatal length increased under water stress, whereas width decreased.

Plants have species-specific changes in stomatal size as a result of abiotic changes (Liu *et al.*, 2006). Water deficient can initially inhibit leaf growth and development, decreasing leaf area and subsequently stomatal characteristics (Liu *et al.*, 2006; Comas *et al.*, 2019). In our study, shifting from abundant water availability (100% FC) to moderate stress (50% FC) increased stomatal density. However, increased water stress (25% FC) reduced stomatal density. If water stress becomes too great, guard cell division is impeded and stomatal density declines (Comas *et al.*, 2019).

The density of glandular trichomes increased from 100 to 50% FC, but it decreased at 25% FC. Many metabolites are stored and released by glandular trichomes at the leaf surface (Shi *et al.*, 2018). High concentrations of more volatile terpenoids and other non-volatile defense components like proteins and sugars are usually associated with high glandular trichome density (Li *et al.*, 2018). Plants have different responses to water stress depending on the type of trichomes present. For



example, Thitz *et al.* (2017) showed that severe water stress decreased the density of glandular trichomes on the adaxial leaf surface in silver birch. The presence of capitate and peltate glandular trichomes might result from responses to chemical compounds synthesized in various trichomes under stressful conditions. Peltate glandular trichomes are the main site for producing semi-volatile compounds and they support large oil glands, in which these compounds are stored (Li *et al.*, 2018). In our study, the moderate water stress treatment significantly increased glandular trichomes and EO content.

## CONCLUSIONS

We investigated the effect of water stress and AMF inoculation on EO content and composition and anatomical characteristics of lemongrass. EO yield increased under moderate water stress and AMF inoculation. The highest EO content and yield were observed at 50% FC and AMF inoculation. Geranial, neral,  $\beta$ -pinene, and nerol were the main components of EO composition, which were influenced by water stress and AMF. Water stress increased the content of geranial,  $\beta$ -pinene, while it decreased the neral and nerol content. In general, AMF inoculation enhanced the geranial, neral, and  $\beta$ -pinene. Water stress changed the anatomical characteristics. The highest stomatal density was found in 50% FC. Increasing water stress decreased the width and length of the stomata. Lastly, the number of glandular trichomes in 50% FC was greater than other treatments. Therefore, to obtain optimum EO quality and quantity for lemongrass, we suggest maintaining moderate water stress (50% FC) combined with AMF inoculation.

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## تأثیر قارچ آربسکولار مایکوریزا بر عملکرد و اجزای اسانس، کرک‌های غده‌ای و پارامترهای روزنه‌ای گیاه علف لیمو تحت تنش خشکی

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### چکیده

پژوهش حاضر به منظور بررسی تأثیر قارچ مایکوریزا بر عملکرد و اجزای اسانس و ویژگی‌های آناتومیکی گیاه علف لیمو تحت تنش (۱۰۰٪، ۷۵٪، ۵۰٪ و ۲۵٪ ظرفیت زراعی خاک) خشکی انجام شد. نتایج نشان داد که محتوای اسانس و عملکرد اسانس تحت تنش متوسط و تلقیح مایکوریزی افزایش یافت. بیشترین مقدار و عملکرد اسانس در تیمار برهمکنش ۵۰٪ ظرفیت زراعی و کاربرد مایکوریزا حاصل شد، اما کمترین مقدار آنها در ۲۵٪ ظرفیت زراعی و عدم تلقیح مایکوریزا انجام شد. ژرانیال، نرال، بتاپین و نرول اجزای اصلی اسانس علف لیمو بودند. تنش خشکی سبب افزایش ژرانیال و بتاپین و کاهش نرول و نرال شد. بیشترین مقدار ژرانیول و بتاپین در تیمار ۵۰ درصد ظرفیت زراعی و تلقیح مایکوریزی انجام بدست آمد. درحالیکه بیشترین مقدار نرال در تیمار ۱۰۰ درصد ظرفیت زراعی و تلقیح مایکوریزی مشاهده شد. تنش خشکی سبب تغییر در تراکم و اندازه روزنه و همچنین تراکم کرک‌های غده‌ای شد. بیشترین تراکم روزنه در تیمار ۵۰ درصد ظرفیت زراعی مشاهده شد. تنش سبب کاهش اندازه روزنه شد به‌طوری‌که کمترین طول و عرض روزنه در ۳۵ درصد ظرفیت زراعی بدست آمد. همچنین تعداد کرک‌های غده‌ای در ۵۰ درصد ظرفیت زراعی بیشتر از سایر تیمارها گزارش شد. نتیجه کلی تحقیق نشان داد که تنش ملایم و کاربرد قارچ مایکوریزی بهترین شرایط جهت دستیابی به اسانس بهینه می‌باشد.