

## Methyl Jasmonate Mitigates Drought Stress Injuries and Affects Essential Oil of Summer Savory

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### ABSTRACT

Drought stress reduces growth and productivity of crops in arid and semiarid regions, such as Iran. Methyl Jasmonate (MeJA) is involved in regulating plants growth and their immune responses. The main purpose of this study was to evaluate the effects of MeJA on growth and some physiological responses of summer savory (*Satureja hortensis*) plant under drought stress condition. Treatments consisted of three levels of drought stress including stress free conditions, mild stress and severe stress, and four concentrations of MeJA (0, 75, 150, and 225  $\mu$ M). The results showed that drought stress and MeJA application had a significant effect on morphological, physiological, and biochemical parameters. In drought conditions, some growth parameters, namely, relative water content and essential oil yield decreased and antioxidant activity, proline content, and essential oil percentage increased. The results suggest that MeJA application under drought stress can improve growth parameters as well as relative water content, proline content, antioxidant activity, and essential oil percentage and yield. The best protection appeared to be obtained from plants treated with MeJA at 75  $\mu$ M.

**Keywords:** Antioxidant activity, Growth parameters, Proline content, Relative water content.

### INTRODUCTION

Drought stress is a major environmental factor that affects the growth and productivity of plants. Drought, or soil water deficit, can be chronic in climatic regions with low water availability, or it can be random and unpredictable due to changes in weather conditions during the plant growing season (Rosegrant and Cline, 2003). The effect of drought stress depends on multiple factors such as genotype, intensity and duration of stress, weather conditions, growth and developmental stages of plants (Robertson and Holland, 2004). The effects of drought on growth, yield, yield components, and quality characters are very different and serious. The loss of cell

turgidity is the first effect of drought stress that influences cell growth rate and its final volume. The phenomenon probably is the most sensitive drought related process, resulting in lower development rate, stem growth, leaf growth, and decreasing stomata diameter. The drought stress affects photosynthesis directly or indirectly via affecting the carbohydrate metabolism. Due to drought stress, the photosynthesis decreases and competition between vegetative and reproductive organs of plant for obtaining carbohydrates increases (Alishah and Ahmadikhah, 2009). In response to drought caused by soil water deficit, plants can exhibit drought escape or drought resistance mechanisms, with resistance further classified into drought avoidance (maintenance of tissue water

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potential) and drought tolerance (Price *et al.*, 2002).

Jasmonic Acid (JA) and Methyl Jasmonate (MeJA), collectively termed jasmonates, are regarded as endogenous regulators that play important roles in regulating stress responses, plant growth and development (Shan and Liang, 2010). MeJA, a linolenic acid derivative, is involved in plant development and defense and is overproduced during wounding, fruit ripening, and drought stress (Creelman and Mullet, 1997). Exogenously applied JA and MeJA inhibited or promoted morphological and physiological changes in plants. The pleiotropic action of JA and MeJA was in a concentration dependent manner (Zalewski *et al.*, 2010). Genes up-regulated by MeJA treatment include those involved in Jasmonate biosynthesis, secondary metabolism, cell wall formation, and those encoding stress protective and defense proteins (Cheong and Choi, 2003).

*Satureja hortensis*, a member of mentha (Lamiaceae) family, is a grassy and annual plant that has a branched stem with 10 to 30 cm heights whose color is darker than its leaf. On the leaf surface, we can see the small and abundant spots, termed as a gland, which contain the essence. Small white and colorful flowers of summer savory blossom in the summer. It is known as summer savory native to southern Europe and naturalized in parts of North America. Dispersion in Iran is mostly seen in the north-west and in some parts of Khorasan province (Yazdanpanah *et al.*, 2012). The pharmaceutical benefits of this plant point to the flowered head branch that has a strengthening effect on facilitating digestion and strengthen stomach and the digestion system and it plays an important role in removing of digestion diseases (Inze and Montago, 1995).

So far, no information has been reported on the possible role of MeJA on drought stress resistance of summer savory. Thus, the aim of this experiment was: (1) To investigate the growth inhibition of summer savory caused by drought stress; (2) To

examine whether MeJA can protect plants from drought stress, and (3) Whether the protective effect is involved in regulation of antioxidant activity, proline content, relative water content and essential oils yield. Our work may help to elucidate the physiological mechanism of drought stress mitigated by MeJA in plants.

## MATERIALS AND METHODS

### Plant Materials and Treatments

This study was conducted as a 3×4 factorial experiment using a complete randomized block design with three replications. Summer savory seeds were obtained from Pakanbazar Company, Isfahan, Iran. The seeds were planted in the pots (25 cm diameter and 25 cm height) which contained equal ratio of garden soil, fine sand, and aged animal manure (ratio 1:1:1), which were mixed and poured into the pots and kept in a greenhouse.

Treatments examined in this study were three levels of drought stress including stress free conditions (soil moisture at the field capacity level), mild stress (soil moisture at 66% of the field capacity) and severe stress (soil moisture at 33% of the field capacity), and four concentrations of MeJA (Purchased from Sigma Aldrich) including 0, 75, 150, and 225 μM. Each treatment was performed in three replicates and each replicate consisted of three pots. Seedlings were sprayed with MeJA solutions until both sides of leaves were completely wet at three growth stages: (1) Emerging of 4-6 leaves; (2) Emerging of 6-8 lateral branches, and (3) Flowering stage. Drought stress was performed in stage 6 to 8 branches according to soil changes in moisture. To calculate field capacity and soil moisture changes, all pots were weighed every day. Cumulative water losses were added to each pot to compensate for transpiration and evaporation. Accumulated water loss was

calculated as the differences in pot weight between successive weightings.

#### Growth Parameters Determination

To measure growth parameters, plants were harvested at soil surface and, immediately, plant height, stem diameter, number of lateral branches and fresh weight of the plant and leaves were determined. To obtain dry weight, the plants were weighed (fresh weight), then oven dried at 75°C for 48 hours and reweighed (dry weight).

#### Relative Water Content (RWC)

Four leaves were randomly taken from chosen plants of each pot. Leaves were weighed (FW) and then immediately floated on distilled water for 24 hours in the dark. Turgid Weights (TW) of leaves were obtained after drying excess surface water with paper towels. Dry Weights (DW) of leaves were measured after drying at 75°C for 48 hours. Relative Water Content (RWC) was calculated using the following formula (Korkmaz *et al.*, 2010):

$$RWC = \left( \frac{FW - DW}{TW - DW} \right) \times 100$$

#### Proline Content

Proline content was determined according to the method described by Batta *et al.* (1973) with some modification. Fresh leaf material (0.5 g) was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. Then, 2 mL of the supernatant was mixed with 2 mL of acid ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 hour at 100°C. The reaction mixture was extracted with 4 mL toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with an UV/visible spectrophotometer. Appropriate proline standards were

included for the calculation of proline in the samples.

#### Antioxidant Activity

Antioxidant activity was determined according to the method described by Moon and Terao (1998) with some modifications. Fresh leaf samples (2 g) from fully developed leaves were taken and homogenized with 5 mL of phosphate buffer (50 mM) using pestle and mortar and centrifuged at 13,000 rpm for 15 minutes at 4°C. The absorbance was measured with a spectrophotometer at 517 nm and the scavenging activity of DiPhenyl-2-PicrylHydrazyl (DPPH) free radical was calculated by using the following formula:

Antioxidant activity (%) =  $[1 - (\text{Absorbance of sample at 517 nm} / \text{Absorbance of control at 517 nm})] \times 100$

#### Essential Oil Percentage and Essential Oil Yield

For essential oil percentage assays, the aerial parts of summer savory were collected at full flowering stage and were dried in the shade. Essential oils were obtained from aerial parts of each sample (40 g dry matter) by hydro-distillation for 3 hours using a Clevenger. Finally, essential oil yield was determined by the following formula (Aliabadi Farahani *et al.*, 2008):

Essential oil yield = Essential oil percentage × Flowering shoot yield

#### Statistical Analysis

Data were analyzed for significant differences using a factorial analysis of variance with drought stress levels and MeJA concentrations as the main factors. Statistical analysis was performed using SAS and MSTATC software programs and the means were compared using the Duncan's Multiple Range Test at  $P < 0.05$ .



## RESULTS

### Growth Parameters

Results showed that drought stress reduced growth parameters with high reduction in plant dry weight (Figure 1-A). Foliar spray of MeJA significantly improved the growth attributes of summer savory such as plant heights (Figure 1-A), number of lateral branches (Figure 1-B), stem diameter (Figure 1-C), plant fresh weight (Figure 1-D), plant dry weight (Figure 1-E), leaf fresh weight (Figure 1-F), and leaf dry weight (Figure 1-G). As shown in Figure 1, the growth parameters of treated plants had significant differences with the control plants. Moreover, in drought stress conditions, the growth parameters were significantly lower compared to the control, while MeJA alleviated drought stress damages by improving growth parameters such as plant height, number of branches, stem diameter, and fresh and dry weight of plant and leaves (Figure 1).

### Relative Water Content

The *RWC* decreased significantly under drought stress conditions and MeJA treatment significantly increased *RWC* both in drought stress and in stress free condition (Figure 2-A). The *RWC* content in the drought stress conditions were lower ( $P < 0.05$ ) than the controls and MeJA-treated plants. Interaction between MeJA and drought stress on *RWC* content was significant at  $P < 0.05$ . MeJA treatment alleviated drought stress damages by affecting *RWC* content, especially when applied with 75  $\mu\text{M}$  concentration at mild and severe stress condition (Figure 2-A).

### Proline Content

In the drought stress conditions, proline content was higher than those in the control

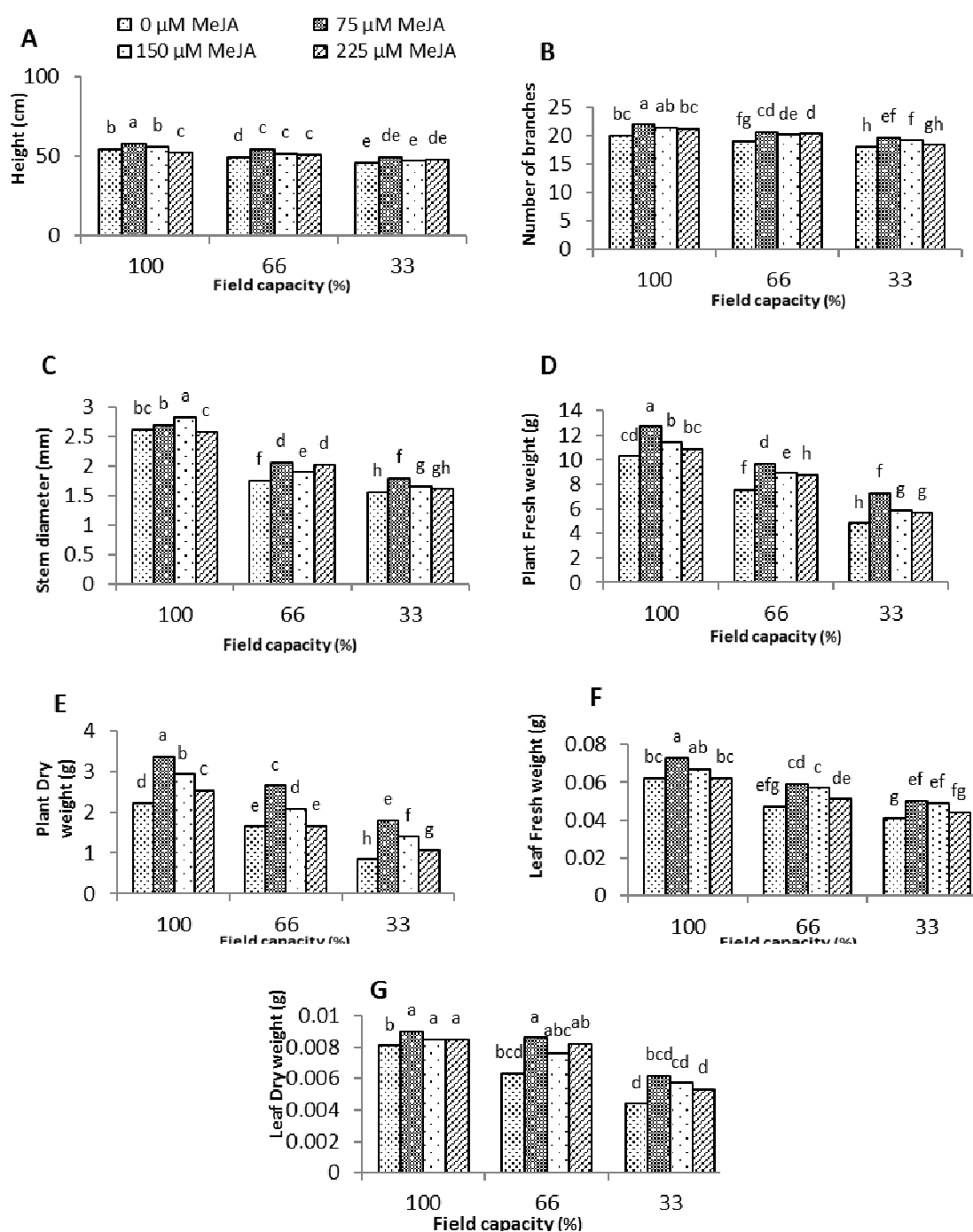
conditions (Figure 2-B). However, in the leaves of treated plants under stress condition, the proline content was higher than those grown in stress free conditions. Both exogenous MeJA and drought stress, but not their interaction, significantly affected ( $P < 0.05$ ) proline content of summer savory. Plants treated with 75  $\mu\text{M}$  MeJA showed the greatest proline content at mild and severe stress condition (Figure 2-B).

### Antioxidant Activities

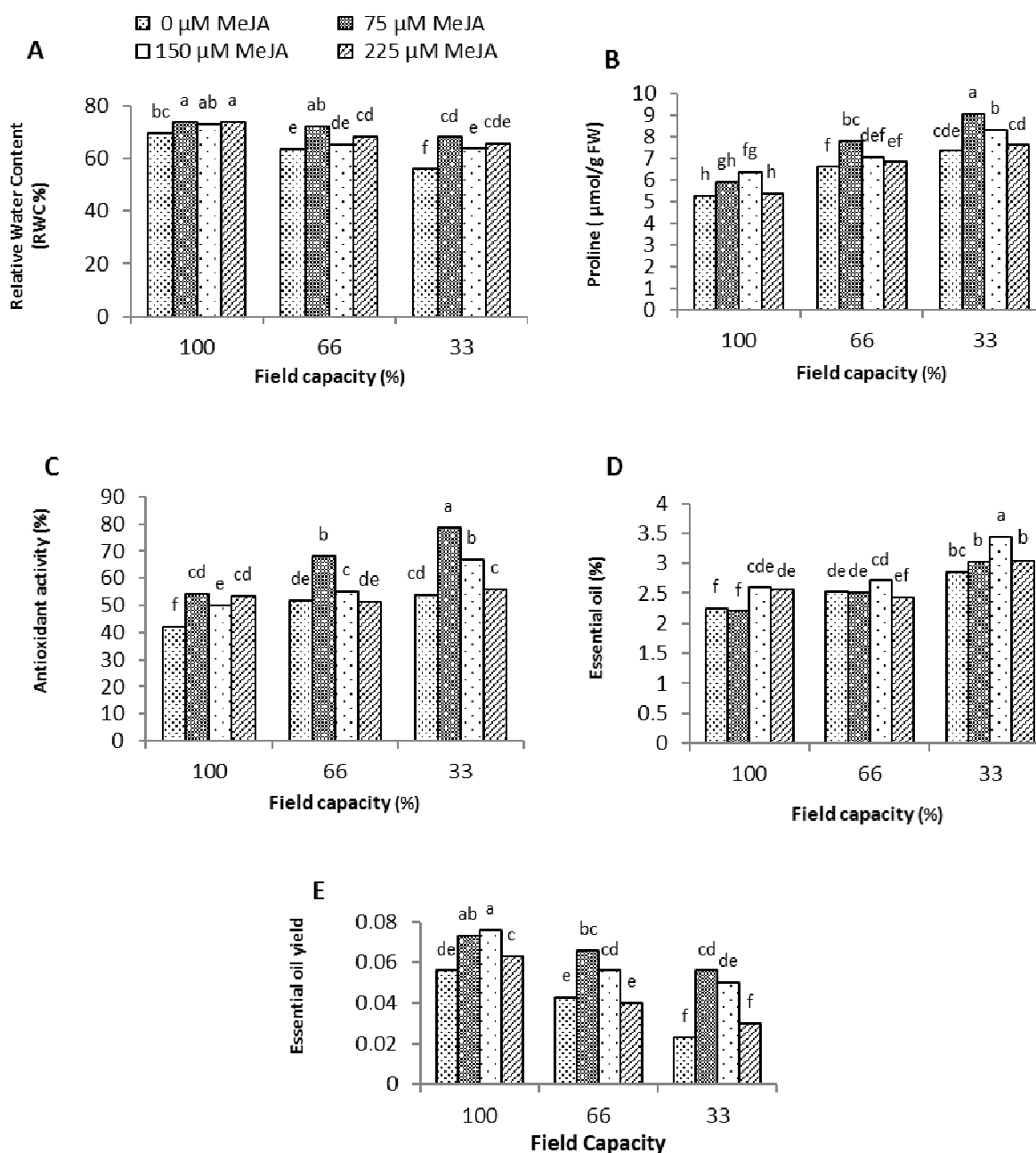
Application of MeJA significantly affected antioxidant activities both in drought stress and stress free condition (Figure 2-C). Antioxidant activities of MeJA treated plants were significantly ( $P < 0.01$ ) higher than the plants grown under stress free conditions. Moreover, drought stress caused increasing antioxidant activities compared with the control plants. According to the results, the antioxidant activities had significant difference at  $P < 0.05$  between the plants treated with MeJA and the controls. Use of MeJA under drought stress conditions caused higher antioxidant activities. In addition, interaction effects of MeJA and drought stress was significant. Plants treated with 75  $\mu\text{M}$  MeJA showed higher antioxidant activities with the highest increase when used in severe stress condition (Figure 2-C).

### Essential Oil Percentage

By imposing drought stress, the essential oil percentage increased. Application of MeJA in drought stress conditions increased essential oil percentage of the treated plants (Figure 2-D). In this study, under drought stress conditions, essential oil percentage increased and its level was significantly higher ( $P < 0.05$ ) than those kept in free stress conditions. Exogenous MeJA application, drought stress and their interaction significantly influenced the



**Figure 1.** (A-G) The reciprocal effect of drought stress, Methyl jasmonate on height, number of branches, stem diameter, plant fresh weight, plant dry weight, leaf fresh weight and leaf dry weight of summer savory, respectively. Different letters above the columns indicate significant differences ( $P < 0.05$ ) according to Duncan's multiple range test performed using SAS version 9.1 software.



**Figure 2.** (A-E) The reciprocal effect of drought stress and Methyl jasmonate on *RWC*, proline content, antioxidant activity (%), essential oil (%) and essential oil yield of summer savory, respectively. Different letters indicate significant difference according to Duncan's multiple range tests at  $P < 0.05$ .

essential oil percentage of summer savory plants. Plants treated with 150  $\mu\text{M}$  MeJA showed higher essential oil percentage in both stress and stress free conditions (Figure 2-D).

#### Essential Oil Yield

Under drought stress condition, the essential oil yield decreased, significantly ( $P < 0.05$ ). Application of MeJA alleviated

drought stress damages and improved essential oil production (Figure 2-E). Results showed significant difference ( $P < 0.01$ ) between the plants treated with MeJA and the controls. Under drought stress condition, the essential oil yield of plants were lower than those grown in stress free condition or MeJA-treated plants that grown under drought stress condition. Our results showed that the essential oil yield of MeJA-treated and the control plants had significant differences. In addition use of MeJA under drought stress conditions increased essential oil yield of plants. Under mild and severe stress conditions, applying 150  $\mu\text{M}$  of MeJA, and in stress free conditions, 75  $\mu\text{M}$  of MeJA, showed the greatest yield of essential oils (Figure 2-E).

## DISCUSSION

In this study, drought stress caused reduction of all growth parameters of summer savory plants. The reductions of plant height and dry weight under water deficit condition were perhaps due to the decline in the cell enlargement. Reduction in fresh and dry weight of the plant may also be due to a decrease in plant growth, photosynthesis, and canopy structure during the water stress as reported by Shao *et al.* (2008). The depressive effect of water stress on growth parameters may also be attributed to a drop in leaf relative water content which reduces leaf turgidity (Reddy *et al.*, 2003). The reduction in fresh and dry weight in savory under drought condition might be associated with suppression of cell expansion and cell growth due to the low turgor pressure and more leaf senescence under drought stress. In this study, it was observed that if the savory plants were exposed to drought stress, MeJA application at 75  $\mu\text{M}$  would be effective for decreasing the damage from the drought stress on growth parameters, indicating that exogenous MeJA at a certain concentrations increases the ability of summer savory plant to resist drought stress. In agreement with

our results, Mohamet and Abdu (2004) reported that drought stress in *Foeniculum vulgare* reduced growth parameters. Exogenous MeJA application can elicit a great variety of morphological and physiological responses to environmental stresses (Creelman and Mullet, 1995; Ghasem *et al.*, 2012). It has been reported that MeJA can alleviate drought stress-induced damages by changing endogenous phytohormones, polyamines, and protein banding patterns of soybean (Hassanein *et al.*, 2009). A role for MeJA in plant response to drought stress has been suggested because the stress induces the expression of jasmonate responsive genes (Agrawal *et al.*, 2003).

In this experiment, *RWC* content in summer savory decreased under drought stress in comparison to the controls. This reduction in *RWC* might have resulted in decline of plant growth attributes. Reduction of *RWC* content had a positive correlation with soil relative water content (Nautiyal *et al.*, 2002). *RWC* was decreased by increasing transpiration and reducing root growth and activity in plants (Tarumingkeng and Coto, 2003). MeJA effects on *RWC* content in this study were consistent with Huiling *et al.* (2012) results that reported the effect of MeJA compounds on Cauliflower (*Brassica oleracea*). Reduced percentage of leaf water loss in the shoot tips exposed to water stress in response to MeJA treatments could be attributed to the possible regulatory effects of MeJA on stomatal closure, leading to the enhanced ability of tissues for water maintenance (Rohwer and Erwin, 2008). Similarly, Anjum *et al.* (2011) asserted that MeJA application helped to maintain relative water content in water stressed soybean plants.

The data showed that both MeJA and Drought stress effectively increased amount of proline. High content of proline could be the reason for keeping the cellular inflammation and reduction of the membrane's damage in the savory plants. Osmotic regulation is accounted as an adaptability which increases the plant



tolerance or resistance to the drought condition (Yazdanpanah *et al.*, 2011). The proline content during drought stress would increase; in that proline as an amino acid play a key role in osmotic regulation (Redy *et al.*, 2003). Application of MeJA to the plants grown in drought stress condition caused increase in proline content. Anjum *et al.* (2011) observed that MeJA Application under drought stress condition caused proline accumulation of banana shoot tips as a physiological change and as a strategy of plant in order to improve drought stress tolerance (Mahmood *et al.*, 2012).

The antioxidant activities decreased under drought stress condition in this study. Drought stress such as other unfavorable environmental conditions often causes oxidative stress and was reported to show increase in the amounts of flavonoids and phenolic acids (Larson, 1988). Antioxidant activities may be directly linked to the content of phenols, tannins and flavonoids and, consequently, to their free radical scavenging activities (Huang *et al.*, 2006). Plant resistance to various stresses is associated with antioxidant capacity and increased levels of antioxidants may prevent stress damages (Bor *et al.*, 2003). Enhancement of the antioxidant capacity both by increasing contents and quality of phenolic compounds may play an important role in the tolerance of plants to abiotic stresses. This suggests that plants respond as an adaptive mechanism by activating genes involved in the biosynthesis of antioxidants whenever there is an environmental perturbation (Oh *et al.*, 2009). Exogenously supplied MeJA can increase the transcript levels and activities of antioxidant enzymes in plants under water stress (Shan and Liang, 2010; Ghasem *et al.*, 2012). It is not clear yet how MeJA acts to modify the antioxidant systems. It is possible that MeJA influences enzyme activities through changes in gene transcription, translation or posttranscriptional modifications. However, the organ specific nature of this hormone suggests that the effects of MeJA are tightly controlled and responsible for directing

specific sub-cellular changes in metabolism (Sylviane *et al.*, 2002).

The essential oil percentage was enhanced in plants under drought stress (Figure 2-D). Our results agree with those obtained by Bahreininejad *et al.* (2013) in *Thymus daenensis* and Khazaiea *et al.* (2008) in thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969). Simon *et al.* (1992) reported that water stress increased essential oil amount of sweet basil (*Ocimum basilicum*). Essential oils are one of the major secondary metabolites of these plants. The essential oil production depends not only on genetic factors, but also on environmental factors. Growth regulators can influence essential oil production through affecting plant growth, essential oil biosynthesis, and the number of oil storage structures. On the other hand, the time of exogenous application and kind of growth regulators can affect essential oil content (Sharafzadeh, 2011). In this study, the essential oil percentage were increased in leaves of the MeJA treated plants. Jasmonate have been reported to be elicitor signal transducers for the production of plant secondary metabolites (Gundlach *et al.*, 1992). Jasmonates have been associated with the accumulation of secondary metabolites, which are also part of the defense response. Exogenous application of jasmonates greatly stimulated the biosynthesis of a wide range of secondary metabolites in cell suspension cultures, and in intact plants (Van der Fits and Memelink, 2000).

Drought stress decreased essential oil yield of summer savory under severe water stress (Figure 2E). Reduction in essential oil yield was due to reduction in herbage yield. This indicated that essential oil yield was positively related to soil water content and herbage yield (Singh *et al.*, 1997). Essential oil percentage increased while essential oil yield decreased under drought stress. Drought stress increased essential oil



accumulation via a higher density of oil glands due to the reduction in leaf area (Simon *et al.*, 1992). Since exogenously applied jasmonates promoted plant growth and increased accumulation of essential oil percentage, essential oil yield was enhanced.

In conclusion, drought stress inhibited plant growth, decreased RWC and essential oil yield, and increased the proline content, antioxidant activity, and essential oil percentage, while MeJA application, especially when applied at 75  $\mu$ M, improved all stress-induced parameters. Therefore, MeJA application could induce drought stress tolerance in summer savory plants by increasing the proline content, RWC content, essential oil production, and antioxidant activities

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## متیل جاسمونات خسارت های تنش خشکی را کاهش داده و بر میزان اسانس مرزه تأثیر می گذارد

ب. میران شاهی، و م. سیاری

### چکیده

تنش خشکی رشد و باروری محصولات مختلف را در مناطق خشک و نیمه خشک مانند ایران کاهش می دهد. متیل جاسمونات در تنظیم رشد و پاسخ های ایمنی گیاهان مداخله می کند. هدف اصلی این مطالعه ارزیابی اثر متیل جاسمونات بر رشد و برخی از پاسخ های فیزیولوژیکی گیاه مرزه تحت شرایط تنش خشکی بود. تیمارهای مورد آزمایش در این مطالعه ۳ سطح خشکی شامل شرایط بدون تنش، تنش ملایم و تنش شدید و ۴ غلظت متیل جاسمونات (۰، ۷۵، ۱۵۰ و ۲۲۵ میکرومولار) بودند. نتایج نشان دادند که تنش خشکی و کاربرد متیل جاسمونات اثر معنی داری بر خصوصیات مورفولوژیکی، فیزیولوژیکی و بیوشیمیایی داشتند. در شرایط تنش خشکی، شاخص های رشدی، محتوای آب نسبی و عملکرد اسانس گیاهی کاهش و فعالیت آنتی اکسیدانی، محتوای پرولین و درصد اسانس افزایش یافت. نتایج پیشنهاد می کند که کاربرد متیل جاسمونات می تواند شاخص های رشدی را همانند محتوای آب نسبی، میزان پرولین، فعالیت آنتی اکسیدانی و درصد و عملکرد اسانس گیاهی در شرایط تنش خشکی بهبود بخشد. بهترین نتایج از گیاهان تیمار شده با غلظت ۷۵ میکرو مولار متیل جاسمونات حاصل شد.