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Original Article

Prolonged Water Deficit Stress and Methyl Jasmonate-Mediated Changes in Metabolite Profile, Flavonoid Concentrations and Antioxidant Activity in Peppermint (*Mentha* × *piperita* L.)

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Abstract

Peppermint (*Mentha* × *piperita* L.) infusion is one of the main important sources of antioxidants. This antioxidant property can be enhanced by using elicitors and inducing abiotic stress. This study was conducted to evaluate the foliar application of Methyl Jasmonate (50 µM) and water deficit stress (no stress, mild stress and moderate stress as 100, 75 and 50% of field capacity, respectively) and their interactions on cultivated peppermint on plant growth, secondary metabolite and antioxidant capacity of infusions made under different treatment. Water deficit stress treatments increased flavonoid contents and total phenolic as well as antioxidant capacity significantly. Some phenolic compound such as luteolin, naringenin, coumaric acid, and quercetin were detected only in some drought and Methyl Jasmonate treatments. Also, fewer amino acids such as L-serine, Threonine, 4-aminobutiric acid, 5-oxo-L-proline and unsaturated fatty acids were identified. Also, foliar application of 50 µM Methyl Jasmonate treatment improved plant growth parameters and metabolite profile such as carbohydrates and amino acids in non-stress and stress conditions. Therefore, inducing water stress and applying MeJA is recommended to enhance the biologic properties of peppermint and improve bioactive compounds and the antioxidant capacity of peppermint infusions.

Keywords: amino acids; carbohydrate; flavanol; peppermint infusion; phenolic compounds; total flavonoids

Introduction

Human body produces oxygen-contain free radicals and other reactive oxygen species (ROS) as byproducts of physiological and biochemical processes in metabolism. There is high correlation between many chronic diseases in humans such as atherosclerosis, cancer, asthma, diabetes inflammatory joint disease, senile dementia, degenerative eye disease and other degenerative diseases with overproduction of free radicals in metabolism (Cai *et al*., 2004; Valko *et al*., 2004). Polyphenols and flavonoids have protective effect against free radicals in the human body (Moon *et al*., 2006; van Dam *et al*., 2013). Phenolic compounds are a class of secondary plant compounds have different physiological effect in human body and promote many benefits to human health. Fruits, teas, wines, vegetables, cocoa and coffee are the rich daily source of polyphenolic compounds in human diet. Also, using tea beverages is one of the common ways to add phenolic compounds to the regular diet (Perez-Jimenez *et al*., 2010). *Mentha* as a main genus of plants in *Lamiaceae* family, is the main source of world essential oil (approximately 2000 tons), making it the second most important essential oil producing genus after *Citrus* (Mucciarelli *et al*., 2001). Peppermint (*Mentha piperita* L.) is an important aromatic and medicinal plant with widespread cultivation range in many regions in the world. Its essential oil is one of the most popular and widely used oils for pharmaceutical, cosmetic, food, cleaning industries and personal care for both its flavorings and fragrance. In addition, its leaves use as a high value spice plant (Herro and Jacob, 2010).

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Peppermint oil also contain menthol, menthofuran, menthone, isomenthone, pulegone caryophyllene, neomenthol, 1,8-cineole, sabinene and limonene, with antimicrobial, cytotoxic and antioxidant activities (Hussain *et al*., 2010; Mimica-Dukic *et al*., 2003). Other important compounds found in peppermint are phenolic compounds. The proportion of phenolic compounds found in peppermint leaves is approximately 19-23% dry weight, of which 12% belongs to the flavonoids group, including eriocitrin, hesperidin and luteolin 7-O-rutinoside, among others. The 75 percent of these compounds can extract in an infusion (McKay and Blumberg, 2006). It has been reported that flavonoids extract have many beneficial effects on health which is linked to their known biological functions as antioxidants, due to their free radical scavenging and metal chelating properties (Pawlak *et al*., 2010). Fecka and Turek, (2007) reported that an infusion of peppermint provides approximately 750 mg L^{-1} of phenolic compounds. This result indicated that the amount of phenolics extracted in an infusion is very high. Phenolic compounds are involved in various plant processes such as growth and reproduction and they synthesized as a defense mechanism against biotic or abiotic stresses (Cohen and Kennedy, 2010). Several studies have demonstrated that peppermint extracts decrease glucose, total cholesterol, triacylglycerols, very low and low-density lipoprotein levels, thus decreasing the atherogenic index in diabetic rats (Barbalho *et al*., 2011; Mani *et al*., 2011).

So, due to the importance of peppermint, maintaining a constant and high essential oil production and quality, mainly in terms of chemical composition is of great importance economically for supplying market demands and industries requirements (Silva, 2002) even under the unfavorable environmental conditions including water deficit stress and drought stress. In addition, these health benefits can be enhanced using pre-harvest strategies to increase bioactive compounds in the peppermint leaves. In a wide variety of plant species, water deficit have enhancement effect on the synthesis of several plant phytochemicals, including phenolic acids, flavonoids, and tannins, as a response to the biotic or abiotic stressful conditions (Zingeretti *et al*., 2013). Under stress conditions, increasing of reactive oxygen species (ROS) production in different cellular compartments observed. Production of ROS can activate the antioxidant system, which synthesizes phenolic compounds. In addition, certain compounds such as Jasmonic Acid (JA) termed elicitors can mimic the abiotic and biotic stress and increase some secondary methabolite such as total phenolics and flavonoid concentrations in plant. Several research have been reported about the antiproliferative, antioxidant enhancement effect, and antiadipogenic activity by MeJA-mediated increases in flavonoids or phenolics in *Raphanus sativus, Ocimum basilicum, Fagopyrum esculentum*, and *Rubus sp*, respectively (Kim *et al*., 2006a; Kim *et al*., 2006b; Wang *et al*., 2008; Lee *et al*., 2013).

In addition, MeJA can enhance the production, initiation or improve the biosynthesis of specific compounds (Edreva *et al*., 2008; Ferrari, 2010). Nevertheless, the use of drought stress as a strategy to improve phytochemicals in plants should carefully applied to avoid the detrimental effects of excessive ROS production, such as cellular damage and death (Cruz 2008). Therefore, the aim of this study was to assess peppermint (*M. piperita* L.) under different levels of water deficit stress and MeJA elicitor and their interaction to evaluate the effect on plant growth as well as on the metabolite profile and the antioxidant capacity of resulting infusions.

Materials and Methods

Plant material

Plants were initiated from rhizome segments of *M. piperita* L. plants obtained from a nursery located in Persian Gulf University, Iran. The plants were grown in a greenhouse at the University of Torino in pots with a diameter of 10 cm, with irrigation every 2 days during the first 30 days. Mean daily temperature inside the greenhouse was within optimal ranges for peppermint growth (20-25 °C). Fertilization was carried out 15 and 25 days after planting. Each pot was fertilized with a solution (10 mL) containing calcium nitrate (1.12 g), magnesium sulphate (0.45 g), potassium nitrate (0.35 g), monobasic potassium phosphate (0.30 g), iron chelate (0.06 g), and manganese sulphate (0.01 g). Thirty-day-old seedlings were transferred to plastic pots with a diameter of 30 cm filled with soil, cocopeat and leaf mold (1:1:1; v/v). Pots were kept in an incubation room. The incubation room temperature was 25 °C ±1; relative humidity was 60-80% and photoperiod was 16 h/d.

Irrigation and MeJA treatments

Plants were irrigated daily based on 100% FC during establishment period. After establishment period peppermint plant were subjected to the different irrigation treatments as FC (100% field capacity), mild stress (75% FC), and moderate stress (50% FC). Throughout cultivation period, moisture levels in the growth media were controlled by daily weighting following the procedure of Yadav *et al*. (2014). Briefly, to calculate the amount of water necessary to bring each soil to determined FC, a 50 g soil sample from randomly chosen pots were collected and the water content was determined by drying at 100 °C at 24 h after the pots were watered. The percentage of soil water content was calculated according to Yadav *et al*. (2014) method. Nutrient and water leaching from pots was captured in dish placed under each pot and the leachate was returned to the soil before the addition of any water.

The experiment was conducted for 90 days. The first MeJA ((Purchased from Sigma Aldrich) treatment (50 µM) was applied to 45 days old plants by spraying aerial parts of the plants. The stock solutions of MeJA were prepared by dissolving in ethanol. After dissolving, distilled water was used for final volume maintaining. Final volume was maintained by distilled water containing 0.125% Triton X-100. Control plants were applied with distilled water including 0.125% Triton X-100 as in MeJA solutions. The second and third MeJA application was repeated at days 55 and 70 on peppermint plants exposed under three different irrigation levels (no stress, mild stress (75% FC) and moderate stress (50% FC). At the end of the experiment, all plants within each pot were harvested and then analysed.

The growth response of the plants to elicitor treatments and water deficit stress were determined by measuring the increase in shoot length (longitudinal growth) and leaf expansion, which were evaluated by measuring the length of the leaf from the base to the apex (longitudinal growth) and at the broadest part (transverse growth). All of these parameters were measured before and after treatment. Shoots (n = 6) and leaves (n = 12) were randomly chosen for each treatment. The same recommended preparation and conditions were used as commercial infusions for infusion preparation. At the end of the experiment, midlife leaves were collected from each experimental unit and dried at 45 °C for 24 h by using a convection oven after milling in an herb grinder (particle size of 0.7-1.0 mm). By adding 1 g of ground material to 100 ml of freshly boiled distilled water infusions were prepared and then filtered (0.5 mm pore size filter) after 10 min.

Total phenolic and flavonoid contents

Folin-Ciocalteu colourimetric method was used for determining the total phenolic content of the peppermint infusions (Singleton and Rossi, 1965), and the total flavonoid content was determined according to the method of the Liu *et al*., (2002). The results of total phenolic content experiment were expressed as mg of (+)-catechin equivalents per g of dry matter (μg CAE/g) and the results of total flavonoid content were expressed as milligrams of Gallic acid (GA) equivalents per g of dry matter (mg GAE/g)

Quantification and identification of phenolic compounds

Infusions sample were analysed using an Agilent ChipCube LC/MS inter-face (Agilent Technologies, Inc., Palo Alto, CA, USA). The samples were injected into a reversed-phase column [Zorbax octadecylsilane (ODS-C18), 15 × 4.6 mm] operated at room temperature. A total of 10 μL of sample was injected, and the compounds were eluted at 1 mL/min using a linear gradient system consisting of two solvents: (A) 2:98 (v/v) acetic acid/water and (B) (2:30:68, v/v/v) acetic acid/acetonitrile/water. The ratios of mobile phases were 90% A and 10% B at t = 0 min and 0% A and 100% B at t= 30 min. The absorbance was set at λmax of 260, 280, and 320 nm. Negative ion mode was operated in the mass spectrometer, under the following conditions: octapolerf, 150 V; capillary voltage, 4000 V; gas temperature, 300 °C; drying gas flow rate, 10 L/min; skimmer voltage, 50 V; nebulizer pressure, 40 psi; and fragmentor voltage, 130 V. Liquid chromatography-mass spectrometry (LC–MS) accurate mass spectra were recorded across the range of m/z 50-1000. Quantification was carried out using phenolic acid and flavonoid standards (coumaric, sinapic, eriocitrin, naringenin, rutin, vanillin, luteolin, quercetin, caffeic, rosmarinic acids, and hesperidin).

Low-molecular-weight metabolites identification

A sample solution (10 mg/mL) was prepared in methanol with lyophilized infusions, which was concentrated under N2 stream. Then, 50 μL of derivatizing agent, N, O-bis (trimethylsilyl)- trifluoroacetamide + 1% rimethylchlorosilane, was added. Then solution was stirred at room temperature condition (2 min). Finally, 1 μL of sample was injected into an Agilent GC series 6890 series coupled to an Agilent single quadrupole MS detector. Electron energy set at 70 eV and mass range at m/z 50-700. An HP-5MS capillary column (30 m × 0.25 mm inner diameter × 0.25 μm) was used. The injector temperature was fitted at at 250 °C. Also, the oven program was as follows: the initial temperature was 100 °C for 1 min, and followed by a ramp of 6 °C min^{-1} to 220 °C, which was held for 1.23 min by the second ramp 10 °C min^{-1} to 290 °C, then raised to 310 °C at 40 °C/min, and held for 7.5 min. helium gas was used as carrier gas with the 1 mL min^{-1} maintaining flow rate. Chem Station (Agilent Technologies) software was used for gas chromatography-mass spectrometry (GC-MS) control and data processing.

Free radical scavenging assays

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging was determined as described by Brand-Williams *et al*. (1995) method and The 2, 2′-azinobis (3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was conducted according to the method by Re *et al*. (1999). Also, Marcocci and Parker (1994) method with some modifications was used for determine the nitric oxide radical scavenging capacity. For make up a volume of 3 mL, 5 mM sodium nitroprusside in phosphate-buffered saline was added to the sample at different concentrations. Then sample incubated at room temperature (27 °C) for 90 min. Then, 1.5 mL of incubated solution was added to 1.5 mL of Greiss reagent. The inhibition of nitrite formation was determined by the decrease in absorbance at 546 nm. The results were expressed as IC50 value (the half maximal inhibitory concentration) and compared with a (+)-catechin standard.

Statistical analysis

All results were analysed using one-way analysis of variance (ANOVA), and Tukey's test or Dennett's test ($p < 0.05$) were used for determine the differences between treatments (6 replicates per treatment and one plant per replicates). Pearson correlation was performed to verify the association between measurements ($p < 0.01$).

Results and Discussion

Growth parameters

Table 1 shows the effect of different water deficit stress alone or in combination with MeJA on the growth parameters, total phenolic and flavonoid content of peppermint plants. The plants grown at 75 and 50% FC decreased in leaf width, leaf length and shoot length compared to the control plants. A similar trend was observed in the fresh weight of leaves. Also, the dry weight was affected by water deficit treatments. Alishah *et al*. (2006) demonstrated a significant decrease in plant height, number of leaves, leaf area, stem diameter and leaf area index as a consequence of soil moisture stress in purple basil (*Ocimum basilicum*). The same effects has been reported by Moeini *et al*. (2006) who observed 2-, 6-, and 4-fold decreases in plant height, leaf area, and fresh yield, respectively, in basil (*Ocimum basilicum*) plants cultivated under constant water stress (at 60% of field capacity) for 6

weeks. During the vegetative period, drought stress resulted in shorter plant and smaller leaf area in mint (*Mentha arvensis*) (Abbaszadeh *et al*., 2006), yarrow (*Achillea millefolium*) (Ashoorabadi *et al*., 2006) and chicory (*Cichorium intybus*) (Taheri *et al*., 2008). Likewise, plant height reduction in calendula (*Calendula officinalis*) (Rahmani *et al*., 2008) and vegetative dry matter of balm (*Melissa officinalis*) (Farahani *et al*., 2009b) was ascribed to reduced water use by the plants under water deficit. Growth and essential oil content of fennel (*Foeniculum vulgare*) plants were also adversely affected by unfavourable irrigation schedules (Patel *et al*., 2000). Letchamo *et al*. (1995) reported a significant reduction in relative growth rate and dry matter of thyme (*Thymus vulgaris*) grown under drought stress. Khorasaninejad *et al*. (2011) also observed a significant reduction in growth parameters of peppermint (*Mentha piperita* L.) under water stress. Water deficit stress reduced leaf length and area, plant height, plant moisture content, fresh and dry weight and the percent essential oil content of lemongrass species, namely, *Cymbopogon nardus* and *C. pendulus* (Sangwan *et al*., 1994). Water stress is one of the major abiotic stresses that adversely effect on most agricultural crop production worldwide. Water stress changes the balance between the ROS production and the antioxidant defence, causing the ROS accumulation in plant. ROS induces oxidative stress to membrane lipids, proteins, and other cellular components such as chloroplast. Water stress can damage to photosynthetic apparatus by ROS production. Also, water stress has harshly effect on stomatal functioning and change in cell turgor according to the severity and duration of the stress period. Loss of turgor is the absence of cell growth resulted in a reduction in plant biomass production (Yordanov *et al*., 2003; Blum, 2011). This may explain the decrease of fresh and dry weight observed in drought treatments. MeJA treatment in non-stress and even in stress conditions showed a significant effect on the transverse and longitudinal size of leaf and shoot length, as compared to the control. Also, the dry weight of leaves treated with MeJA under water deficit stress condition was lower than water deficit alone treatments, which may be of industrial importance, because infusions are prepared from dry material.

Also, MeJA treated plant in moderate stress condition improved growth of peppermint. Maximum leaf dry and fresh weight was observed in non-stress condition with foliar application of MeJA. These results are in parallel with other reports who claimed that jamonates improves growth measurements of tomato, *Arabidopsis*, *Artemisia* and sweet basil (Baldi and Dixit, 2007; Boughton *et al*., 2005; Sorial *et al*., 2010; Traw and Bergelson, 2003). Baldi and Dixit (2007) also reported that foliar spry of methyl jasmonate on *Artemisia annua* increased total plant biomass. Moreover, there are several reports about the positive effect of Jasmonic acid on increasing the shoot growth and number of flowers in *Phaseolus lunatus* and *Lemna minor* (Heil, 2004; Krajncic *et al*., 2006). Sorial *et al*. (2010) reported that, shoot and leaf dry weight, the number of stalk, total pigment content in basil plants were increased under MeJA treatment. Swiatek *et al*. (2003) claimed that JA is effective hormone on cell size and cell division. Ding *et al*. (2002) noted that methyl jasmonate increased the plant resistance and thus it improves growth at low concentrations significantly.

Mahmood *et al*. (2012) reported that MeJA could ameliorate water stress tolerance in banana by regulating the growth, chlorophyll levels, proline accumulation, proliferation rate, oxidative stress and tissue water status and membrane lipid peroxidation. It is well known that jasmonic acid involve a wide spectrum of primary and secondary metabolic responses in plants and also affects water processes in plants (Hayat *et al*., 2009).

The enhancement effect of exogenous JA application on endogenous JA may relate to the defence gene expression that induced from activates of JA signalling pathways without any damage to the nucleic acids (Nafie *et al*., 2009), and also may be in charge of other bio molecular processes. Our results are in parallel with some previous studies on JA; suggest that via modulating phenolic and polyphenolic metabolism, total flavonoids and ROS-detoxification enzyme systems JA can potentiate peppermint cells defence responses. Peppermint treated with MeJA or drought stress showed an increase in total phenolic and flavonoid content (Table 1). The stimulation of Jasmonic acid treatment total phenolic compounds in plant tissues were reported earlier. Our results is also similar with Kim *et al*. (2006a) study where at the second day after the treatment by 0.1 and 0.5 M methyl jasmonate total phenolic contents were 27 and 57%, respectively, in comparison with the control treatment. Also, Cai *et al*. (2012, reported that elicitor's application enhanced biosynthesis of phenolic compounds in *Vitis vinifera* efficiently. In general, JA as plant signalling molecules are involved in many signal transduction systems, and induce secondary metabolic pathway gene expression

Table 1. Effect of foliar spray of MeJA on growth parameters and total phenolic and flavonoid content of peppermint plants grown at different level of FC

Treatments	Leaf Transverse Growth (cm)	Leaf Longitudinal Growth (cm)	Shoot Growth (cm)	Fresh Weight (mg leaf^{-1})	Dry Weight (mg leaf^{-1})	Total Phenolic Content (µg GAE/ml)	Flavonoid Content (µg CAE/ml)
100% FC (Control)	3.1±0.1 a*	5.4±0.2a	58.1±1.8b	161.5±5.1b	48.8±0.1a	22.4± 1.8e	8.11 ± 0.3f
Mild stress	2.5±0.0b	4.61±0.1b	46.9±4.14c	134.8±7d	42.2±1.6b	30.2±3.23d	10.21±1.6e
Moderate stress	2.1±0.1c	3.93±0.2b	29.4±1.11e	125.2±6e	39.1±0.1b	40.7±1.79c	13.97±1.1d
Control+MeJA	3.4±0.1a	5.9±0.2a	61.4±4.23a	167.2±4.1a	49±1.2a	71.31 ± 8.2a	18.11 ± 1.3ab
Mild stress+MeJA	2.6±0.1b	4.68±0.21b	47.8±3.12c	153.4±5.2c	44.5±1.1b	52.18±4.1b	17.41±2.1c
Moderate stress+MeJA	2.4±0.1 b	4.23±0.11b	39.4±2.11d	132.1±3.4e	41.2±2.1b	47.30 ± 2.1b	19.21±1.8a

Note: *Results are the average of three independent determinations ± SE. Different letters in each column indicate significant differences ($p < 0.05$, Tukey's test).

levels such as phenylalanine ammonia lyase. Phenylalanine ammonia lyase is an involve enzyme in the synthesis of phenolic compounds via phenylpropanoid pathway and increasing the related gene expression level increase the amount of phenolic and polyphenolic compounds (Ding *et al*., 2002). Likewise, JA induces other related genes in phenolics biosynthesis pathway such as polyphenol oxidase (López-Vélez *et al*., 2003). Total flavonoid was increased in water deficit stress condition and MeJA treated plants in our study. Flavonoids are the main bioactive secondary metabolites and they can act as secondary antioxidant system in plants. Depletion in antioxidant enzyme activity activated this secondary antioxidant system (Agati *et al*., 2012). Also, they can serve as scavengers of ROS by neutralizing and locating radicals before they damage the cells under adverse environmental conditions (Løvdal *et al*., 2010). A considerable increase in flavonoid content following abiotic and biotic stresses has been reported, such as natural or mechanical wounding, drought stress, nutrient deprivation and metal toxicity (Agati *et al*., 2012). Ascorbate-dependent related enzymes have key role in flavonoids biosynthesis and reported those enzymes are to be under the JA control (Wolucka *et al*., 2005). Significant increase in total flavonoids obtained in our present work may be modulated and controlled by the ascorbic acid availability. Also, many flavonoid biosynthesis genes are also induced under stressful conditions.

Quantification of individual phenolic compounds

The main compounds identified in the peppermint infusions were phenolic acids, flavan-3-ols, flavonols and flavonones (Table 2). Infusions made from MeJA-treated or non-MeJA treated plants under different water deficit stress treatments showed an increase in the majority of these compounds as compared to control plants. The most of phenolic acids and flavonoids were increased in infusions obtained from stressed plants, and this effect was greater in the mild treatment in most cases except in rosmarinic acid, cumaric acid, coffeic acid and hesperidin. The maximum level of rosmarinic acid and hesperidin were observed in moderate water deficit stress treatment. Those compounds are the most abundant compound with beneficial health effects in peppermint infusions. Also, MeJA treated plant enhanced producing the coumaric acid, luteolin, quercetin, naringenin, and vanillin were found in infusions of stressed leaves but not in controls. Marley *et al*. (2014), found that peppermint plants treated with different concentrations of elicitors increased the hesperidin and rosmarinic acid in leaves, among other phenolic compounds, and also they found some compounds, such as coumaric acid, luteolin, quercetin, naringenin, and vanillin, in elicit-treated plants but not in controls. These results suggest that MeJA application may cause a state of oxidative stress in the plant, resulting in increased production and synthesis of different phenolic compounds. Using exogenous phytohormone in plants exposed to water stress increased the amount of abscisic acid in different plant organ, which induces several environmental responses, such as the accelerated production of ROS in mitochondria, pigments contain organelles like chloroplasts, and peroxisomes. Abscisic acid may stimulate the antioxidant enzyme activity and the synthesis of phenolic compounds. This mechanism is used in plants as an adaptation strategy to overcome oxidative stress (Cruz, 2008). Foliar spry of MeJA on cultivated peppermint in stress condition may effect indirectly by stimulate the level of ABA in plant. The Addition of 50 µM MeJA on cultivated plant in non-stress and even in stress condition increased the amount of rosmaric acid (RA). MeJA application in the *Coleus blumei* suspension cultures enhanced the amount of the RA by three times (Szabo *et al*., 1999). Also, Georgiev *et al*. (2007), reported RA accumulation (2.4-fold) in *Lavandula vera* suspension cultures by Addition of 50 µM MeJA. Exogenous application of MeJA (100 µM) in cell suspension cultures of *Lithospermum erythrorhizon* showed the maximum RA concentration (ten times) in comparison to the control treatment (Mizukami *et al*., 1993). Similar enhancement effect by MeJA addition on RA accumulation in *L. erythrorhizon* suspension cultures reported by Ogata *et al*. (2004).

Identification of Low-Molecular-Weight Compounds.

Table 3 shows the identification of low-molecular-weight polar compounds obtained by GC-MSD analysis. Water deficit stress as well as foliar application of MeJA resulted to considerable changes in the metabolite profile of infusions compared to the control treatment. Some of amino acids, such as aminobutiric acid, alanine, leucine, isoleucine, proline, and Serine were identified in the control plants, but, were not detected in some stressed or MeJA treated plants infusions. Similar results reported by Marly et al. (2014). They found fewer amino acids in peppermint plants treated with different levels of drought stress 85 (control), 65, 35, 24 and 12% soil moisture. Also, Ferrario-Mery *et al*. (1998) reported that tobacco (*Nicotiana tabacum* L.) leaves where decreases of were observed in the concentrations of some amino acids, such as Asp, Glu, Ser, and Gly, as well as a decrease in NO^{-3} (approximately 40-70%) after 4 days drought stress.

Many carbohydrate and nitrogen contain compounds have high relation with water stress tolerance in plants including some amino acid like proline, total soluble sugars, glycine betaines, anthocyanins and soluble proteins (Monreal *et al*., 2007). Changing turgor pressure and transpiration rate by water deficit stress can explain the amino acid profile change in our study because some amino acids were not detected under moderate or severe water deficit stress. Water deficit stress leads to a decrease in root absorption of NO^{-3} and transport of NO^{-3} to the leaves, and it can cause to decreasing amino acid synthesis (*Ferrario-Mery et al*., 1998). In response to the different environmental stresses, plants accumulate large quantities of different types of compatible solutes in cells or organelles (Serraj and Sinclair, 2002). These compatible solutes provide high protection to the plants under environmental stress by ROS detoxification, osmotic adjustment of cell, membrane integrity protection of and stabilization of enzymes/protein (Ashraf and Foolad, 2007; Bohnert and Jensen, 1996; Yancey, 1994). Results showed that the amount of proline amino acid affected by the level of water deficit stress and MeJA treatments.

Both MeJA and Drought stress increased the amount of proline content in this study. High level of proline may relate cellular inflammation keeping and reduction of the

membrane's damage in plants. Application of MeJA to the plants grown in drought stress condition increased the proline content in our study. Anjum *et al*. (2011) reported proline accumulation of banana shoot tips culture under drought stress condition which treated with MeJA. It seems that increasing the proline level is a common physiological strategy of plant in order to improve stress tolerance (Naidu *et al*., 1991; Bassi and Sharma, 1993; Schat *et al*., 1997; Hare *et al*., 1998; Rhodes and Nadolska-Orczyk, 2002; Munns, 2005; Sharma and Dietz, 2006; Mahmood *et al*., 2012). Overall, results indicated that water deficit stress affected amino acid biosynthesis in the leaves. MeJA treatment led to the presence of metabolites not detected in the control; for instance, some amino acids were detected in infusions obtained from plants treated with 50 μM MeJA, but not in the control sample. Phenolic acid pathway uses carbohydrate metabolism products as precursors and increase in carbohydrate content may relate to the phenolic acid synthesis in plants (Ghasemzadeh and Jaafar, 2012; Ibrahim and Jaafar, 2011). These effects may be related to the increase in growth parameters of the plants treated with 50μM MeJA especially in control irrigation treatment. The identified carbohydrates in this study include Arabinose and D-glucose in both control and treated plants infusions. The amount of Arabinose and D-glucose were increased in stressed plant and foliar spry of MeJA showed enhance effect. Also, some organic and fatty acids were identified in both control and treated plants infusions. However, compounds such as oleic and citric acids were identified in stressed plants. Also, compound such as pantothenic acid was not detected in infusions made from stressed plant. Like phenolic compounds, some organic acids (such as citric acid) and fatty acids may also play a protective role against various oxidative diseases because of their antioxidant properties or singlet oxygen quencher and scavenger of OH• radicals (Oliveira *et al*., 2008; Polumbryk *et al*., 2013). Moreover, these compounds play main role in nutrient deficiency condition and plant microbe interactions (Lopez-Bucio *et al*., 2000). Pantothenic, linoleic, and α-linolenic acids were identified only in control treatments. This result indicates that water deficit stress conditions decrease the amount of these compounds in leaves. Also, some compounds were found in treated. These results may indicate that water deficit stress conditions increase the amount of these compounds in leaves. Similar results were reported by Benhassaine-Kesri *et al*. (2002) who reported decreases in linoleic and α-linolenic acid contents *Brassica napus* leaves subjected to water stress condition. Altering the membrane lipid composition is also a common respond of plant species under environmental stress condition (Gigon *et al*., 2004). 3-fold decreases in total lipid content of Arabidopsis thaliana reported by Gigon *et al*. (2004) under drought stress for 14 days. Indeed, there is a correlation between inhibition of lipid biosynthesis and decrease in the membrane lipid content. However, the response may be different according to the type of lipid studied (Harwood, 1996; Benhassaine-Kesri *et al*., 2002; Gigon *et al*., 2004).

Evaluation of antioxidant capacity

Excess oxygen species can increase the lipid peroxidation cells membrane and resulting even in cell death (Yamamoto *et al*., 2002; Ma *et al*., 2007). Plants activate the antioxidant systems in counteract with excess oxygen species (Wang *et al*., 1999). Methyl jasmonate (MeJA), synthesized from derived fatty acids from cyclopentanone-based compounds of the jasmonates (Creelman and Mullet, 1995 1997; Schaller and Stintzi, 2009). Plants under drought stress stimulates the synthesis and activity of antioxidant metabolites and antioxidant enzymes like SOD that can protect plant organelle, cells, tissues and organs. Table 4 shows the DPPH, ABTS, and nitric oxide scavenging assays of peppermint infusions for antioxidant capacity in different level of water deficit stress and MeJA treatments. Water deficit stress and foliar application of MeJA on peppermint improved the content of individual phenolics and flavonoids as well as antioxidative capacity in our study.

There are several reports about the exogenous application of jasmonates and response of plants to water deficit stress. Bandurska *et al.* (2003), reported that JA increased the antioxidative capacity of plants under water stress. Also, Li *et al.* (1998) found that JAs play a key role in signalling drought-induced antioxidant responses, including ascorbate metabolism. Nafie *et al.* (2011) pointed out that JA have protective effect in plant by enhances the activity of antioxidant enzymes in drought-induced oxidative damage. Vatanparast *et al.* (2012), reported phenolic compounds and antioxidant activity increase pomegranate (*Punica*

Table 2. Chromatographic profile of phenolic compounds of infusions prepared from MeJA treated or non MeJA treated peppermint (*M. piperita*) leaves grown at different levels of FC

Compound	RT (min)	Control	75% FC	50%FC	Control + MeJA	75%FC+MeJA	50%FC+MeJA
Caffeic acid	12.71	0.31 ± 0.0 d	0.51 ± 0.0 c	0.81 ± 0.0 b	0.51 ± 0.0 c	2.14 ± 0.0 a	1.11 ± 0.2 b
Coumaric acid	16.59	LDL[b]	1.11 ± 0.0 c	1.42 ± 0.0 b	0.34 ± 0.0 d	1.31 ± 0.0 b	2.23 ± 0.4 a
Luteolin	17.43	LDL	1.52 ± 0.0 a	LDL	LDL	1.92 ± 0.11 a	LDL
Eriocitrin	19.43	0.28 ± 0.0 c	8.3 ± 0.0 a	5.63 ± 0.1 b	0.72 ± 0.2 c	14.7 ± 0.1 a	8.0 ± 0.2 b
Rutin	20.38	14.4 ± 1.1 d	55.2 ± 0.6 a	31.5 ± 0.8 b	17.2 ± 0.4 d	63.1 ± 9.4 a	39.3 ± 4.1 c
Sinapic acid	21.25	0.2 ± 0.0 b	2.1 ± 0.0 b	3.3 ± 0.1 a	0.28 ± 0.0 b	3.1 ± 0.0 b	1.79 ± 0.1 b
Rosmarinic acid	22.45	42. 6 ± 2.8 f	58. 6 ± 2.3 e	88.3 ± 1.5 c	111.2 ± 11.4 a	77.4 ± 5.2 d	103.1 ± 6.4 b
Hesperedin	23.21	27.7 ±3.2 f	35.1 ± 2.1 e	74.1 ± 3.5 c	83.81 ± 1.8 b	63.9 ± 4.5 d	92.2 ± 2.6 a
Quercetin	28.44	LDL	LDL	0.51 ± 0.0 d	3.9 ± 0.14 c	7.17 ± 1.21 a	6.08 ± 0.2 b
Naringenin	31.81	LDL	LDL	0.8 ± 0.0 d	63.11 ± 7.5 a	5.32 ± 1.2 d	2.94 ± 0.4 c
Vanillin	33.49	LDL	LDL	0.51 ± 0.0 b	0.14 ± 0.0 b	8.1 ± 1.1 a	0.43 ± 0.0 b

Note: [a]Results are expressed as ng μL^{-1} of peppermint infusion and are the average of three independent determinations ± SE. Different letters indicate significant statistical differences for each compound ($p < 0.05$; Tukey's test). [b]LDL = lower than the detection limit.

Table 3. Low-molecular-weight metabolites of infusions prepared from MeJA treated or non MeJA treated peppermint (*M. piperita*) leaves grown at different levels of FC[a]

Proposed compound and their nature	RT (min)	Control	75% FC	50% FC	Control+ MeJA	75% FC+MeJA	50%FC+ MeJA
L-alanine (AA)[b]	4.31	0.67	0.48	LDL[c]	0.94	0.55	0.42
L-leucine (AA)	6.83	2.47	0.33	LDL	LDL	LDL	LDL
Isoleucine (AA)	7.28	3.01	0.89	0.37	LDL	1.17	1.43
L-proline (AA)	7.3	2.31	4.84	8.92	2.17	4.84	9.21
L-serine (AA)	8.59	1.88	LDL	LDL	2.03	LDL	LDL
Threonine (AA)	9.11	0.93	LDL	LDL	1.17	LDL	LDL
4-aminobutiric acid (AA)	11.66	0.88	LDL	LDL	0.97	LDL	LDL
5-oxo-L-proline (AA)	11.6	9.98	LDL	LDL	10.21	LDL	LDL
Glutamine(AA)	13.45	0.02	0.04	0.05	0.04	0.05	0.08
Phenylalanine (AA)	13.53	0.9	LDL	LDL	0.94	LDL	LDL
L-asparagine (AA)	14.42	0.01	LDL	LDL	0.03	LDL	LDL
L-tyrosine (AA)	19.06	0.05	LDL	LDL	0.05	0.04	0.08
Tryptophan (AA)	23.5	0.04	LDL	LDL	0.03	0.22	0.43
Arabinose (C)	18.78	1.75	2.79	5.91	5.1	6.11	8.24
D-glucose (C)	20.1	0.07	5.2	10.15	13.17	15.14	18.82
Lactic acid (OA)	3.66	1.68	5.66	10.11	9.15	12.97	19.28
Glycolic acid (OA)	3.79	1.15	3.81	8.44	1.38	6.72	7.39
Malonic acid (OA)	5.58	0.93	1.18	0.54	1.84	0.52	0.67
Succinic acid (OA)	7.52	3.41	2.51	1.05	7.88	5.31	2.98
2,3-hidroxipropionic acid (OA)	7.8	1.28	5.24	3.94	2.28	7.74	9.11
Fumaric acid (OA)	8.23	0.68	0.54	1.05	LDL	1.88	LDL
Malic acid (OA)	11.0	5.31	2.2	0.83	10.46	5.72	2.23
Threonic acid (OA)	12.33	0.33	0.51	LDL	1.29	LDL	LDL
Lauric acid (OA)	13.87	0.01	1.11	LDL	0.93	LDL	0.7
Tartaric acid (OA)	14.06	0.02	0.37	0.73	0.44	1.32	2.51
Citric acid (OA)	17.27	LDL	LDL	2.11	1.48	3.15	4.01
Myristic acid (OA)	17.31	0.01	LDL	1.44	LDL	1.04	1.71
Pantothenic acid (OA)	19.08	0.01	LDL	LDL	0.03	LDL	0.034
Palmitic acid (FA)	20.51	0.11	11.91	5.43	5.01	7.33	9.14
Linoleic acid (FA)	23.02	0.01	LDL	LDL	0.01	LDL	LDL
Oleic acid (FA)	23.04	LDL	1.33	3.87	1.11	4.95	6.84
Stearic acid (FA)	24.78	1.03	3.68	1.58	1.35	1.73	1.51
α-linolenic acid (FA)	23.64	0.05	LDL	LDL	0.09	0.03	0.06
Glycerol (AL)	6.88	10.32	5.21	1.58	14.22	7.45	4.18
Tyrosol (AL)	12.54	0.19	1.71	0.41	0.43	1.62	1.11

Note: [a]Results are expressed as a percentage of the total area. [b] Nature of compound: Amino Acid (AA), Carbohydrate (C) Organic Acid(OA), Fatty Acid (FA), Alcohol(AL). [c]LDL = lower than the detection limit.

Table 4. Antioxidant capacity of infusions prepared from peppermint (*M. piperita*) leaves grown at different levels of water deficit stress alone or treated with MeJA, for Inhibition of $ABTS^+$, $DPPH^\bullet$, and $NO^\bullet$ Radicals[a]

Treatment	IC_{50} $DPPH^\bullet$	IC_{50} $ABTS^+$	IC_{50} NO
100% FC (Control)	60.4± 1.2a	16.5± 0.3a	56.2± 2.8a
Mild stress	34.2± 3.1c	11.2± 0.4c	41.3± 1.3b
Moderate stress	21.2± 2.1e	8.4± 0.2e	30.3± 1.7c
Control +MeJA	43.2± 3.8b	13.4± 1.5b	52.1± 3.2a
Mild stress +MeJA	27.1± 2.2d	9.7± 0.5d	38.9± 2.9b
Moderate stress +MeJA	18.5± 1.16e	8.3± 0.2e	20.1± 1.4d

Note:[a]Results are expressed as μg/mL and reflect averages of triplicate assays ± SE. Different letters indicate significant statistical differences for each treatment ($p < 0.05$; Tukey's test).

granatum L.) foliage treated with 0.5-2.0 mM MeJA. MeJA application reduced the adverse effects of drought and oxidative stresses in *Arabidopsis thaliana*, strawberry and barley seedling (Wang, 1999; Popova *et al*., 2003; Jung, 2004). Application of Methyl jasmonate in tobacco and *Arabidopsis* suspension cell culture enhanced the amount of ascorbic acid (Wolucka *et al*., 2005) and influences the metabolism of ascorbic acid (Maksymiec and Krupa, 2002; Wolucka *et al*., 2005).

The infusions obtained from peppermint subjected to water deficit stress exhibited an important and significant decrease in IC50 values, as compared to the control in both assays. Sever water stress had the greatest antioxidant activity, because IC50 values were reduced significantly for ABTS and DPPH assays, as compared to the control treatment. Also, the maximum inhibition percentage obtained approximately 91 and 94% for DDPH and ABTS assay, respectively (data not shown). Increasing the amount of some phenolic compounds, such as coumaric acid, luteolin, rutin, rosmarinic acid, naringenin, and caffeic acid, in water deficit stressed plants and also MeJA treated plants could attributed with this affects. There are many well documented scientific reports about the physiological and chemical effects of peppermint flavonoids on biological systems. For example, anti-HIV and radical scavenging properties of luteolin 7-O-b-glucuronide has been documented by Fecka and Turek (2007).

Luteolin and luteolin-7-O-glucoside potently strengthen the haem oxygenase- 1-mediated antioxidative potential by the modulation of the Nrf2/mitogen-activated protein kinase signaling pathways (Song and Park, 2014). Also, the physiological effects of the eriocitrin as a main phenolic acid compound have been widely pointed out in many research literatures (Ballester *et al*., 2013). Obtained results in our study are in accordance with a previous report, which reported that higher phenolic compound levels resulted to increase in plant antioxidant activity (Kim et al., 2006) and also showed a linear correlation between antioxidant activity and phenolics contents (Li *et al*., 2008). The antioxidant activity in peppermint is attributed to the soluble phenolic fractions of infusions. The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure, which can play an important role in neutralizing ROS, such as free radicals, singlet and triplet oxygen and peroxides. Sodium nitroprusside (SNP) is one of the most widely studied nitric oxide donors. This donor property was used to demonstrate the indirect NO scavenging effect of infusions of MeJA treated plants under different water deficit stress. Moderate deficit irrigation peppermint the IC50 value reduction was significant in three assays compared to the control treatment.

Conclusions

Improving and enhancing effects of exogenous application of JAs on plant stress tolerance in different plants species under abiotic stresses conditions reported previously. Also, the effectiveness of JAs entirely depends on its concentration and the tested plant species type. The antioxidant activities increased under water deficit stress condition and SA application in this study. MeJA has protective effect in stress condition especially in the growth measurements that is very important to constant peppermint production in water stress condition. Water deficit stress often causes oxidative stress such as other environmental stress and show increase in the amounts of flavonoids and phenolic acids. Antioxidant activities may be directly linked to the content of total phenolic and flavonoids compound and, indirectly, associated with free radical scavenging activities. The results of this study suggest that water deficit stress and MeJA plays an important role in the production of phenolic compounds, carbohydrates and amino acids in the peppermint plant, which is reflected in the antioxidant capacity of resulting infusions. However, water deficit stress affected negatively on some metabolites, such as amino acids and unsaturated fatty acids; therefore, further research is required in order to determine the biological significance of this increase in metabolites and antioxidant capacity in the peppermint infusions.

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References

Abbaszadeh B, Aliabadi FH, Morteza E (2009). Effects of irrigation levels on essential oil of balm (*Melissa officinalis* L.). American Eurasian Journal of Sustainable Agriculture 3:53-56.

Abbaszadeh B, Sharifi AE, Ardakani MR, Lebaschi MH, Safikhani F, Naderi HBM (2006). Effect of application methods of nitrogen fertilizer on essential oil content and composition of balm (*Melissa officinalis* L.) under field condition. Iran Journal of Medicinal and Aromatic Plants Research 22:124-131.

Agati G, Azzarello E, Pollastri S, Tattini M (2012). Flavonoids as antioxidants in plants: location and functional significance. Plant Science 196:67-76.

Alishah BH, Heidari R, Hassani A, Dizaji AA (2006). Effect of water stress on some morphological and biochemical characteristics of purple basil (*Ocimum basilicum*). Journal of Biological Science 6:763-767.

Anjum SA, Wang L, Farooq M, Khan I, Xue L (2011). Methyl jasmonate-induced alternation in lipid peroxidation, antioxidative defence system and yield in soybean under drought. Journal of Agronomy Crop Science 197:296-301.

Ashoorabadi Sharifi E, Matin M, Lebaschi H, Abbaszadeh B, Naderi B (2011). Effects of water stress on quantity yield in *Achillea millefolium*. Abstracts Book of the First International Conference on the Theory and Practices in Biological Water Saving, Beijing.

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental Experimental Botany 59:206-216.

Ashraf M, Harris PJC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Science 166:3-16.

Baldi V, Dixit VK (2007). Yield enhancement strategies for artemisinin production by suspension cultures of *Artemisia annua*. Bio Technology 99:4609-4614.

Ballester AR, Teresa Lafuente M, Gonzalez-Candelas L (2013). Citrus phenylpropanoids and defence against pathogens. Part II: Gene expression and metabolite accumulation in the response of fruits to *Penicillium digitatum* infection. Food Chemistry 136(1):285-291.

Bandurska H, Stroiński A, Kubiś J (2003). The effect of jasmonic acid on the accumulation of ABA, proline and spermidine and its influence on membrane injury under water deficit in two barley genotypes. Acta Physiologia Plantarum 25:279-285.

Benhassaine-Kesri G, Aid F, Demandre C, Kader JC, Mazliak P (2002). Drought stress affects chloroplast lipid metabolism in rape (*Brassica napus*) leaves. Physiology Plant 115:221-227.

Blum A (2011). Plant breeding for water-limited environments. Springer: New York.

Bohnert HJ, Jensen RG (1996). Strategies for engineering water-stress tolerance in plants. Trends Biotechnology 14:89-97.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28(1):25-30.

Cai Z, Kastell A, Mewis I, Knorr Smetanskal D (2012). Polysaccharide elicitors enhance anthocyanin and phenolic acid accumulation in cell suspension cultures of *Vitis vinifera*. Plant Cell Tissue and Organ Culture 108(3):401-409.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science 74:2157-2184.

Creelman RA, Mullet JE (1995). Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. Proceedings of the National Academy of Sciences of the United States of America 92:4114-4119.

Creelman RA, Mullet JE (1997). Oligosaccharins, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. Plant Cell 9:1211-1223.

Cruz, M (2008). Drought stress and reactive oxygen species. Plant Signaling Behaviour 3:156-165.

Dam NMV, Jong TJD, Iwasa Y, Kubo T (1996). Optimal distribution of defences: Are plants smart investors? Functional Ecology 10:128-136.

Ding CK, Wang CY, Gross KC, Smith DL (2002). Jasmonate and salicylate induce the expression of pathogenesis-relatedprotein genes and increase resistance to chilling injury in tomato fruit. Planta 214:895-901.

Edreva A, Velikova V, Tsonev T, Gürel A, Aktas L, Gesheva E (2008). Stress-protective role of secondary metabolites: Diversity of functions and mechanisms. General and Applied Plant Physiology 34:67-78.

Fecka I, Turek S (2007). Determination of water soluble polyphenolic compounds in commercial herbal teas from *Lamiaceae*: Peppermint, Melissa, and sage. Journal of Agriculture and Food Chemistry 55:10908-10917.

Ferrari S (2010). Biological elicitors of plant secondary metabolites: mode of action and use in the production of nutraceutics. Advance in Experimental Medicinal and Biology 698:152-166.

Ferrario-Mery S, Valadier MH, Foyer CH (1998). Overexpression of nitrate reductase in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. Plant Physiology 117:293-302.

Georgiev MI, Kuzeva SL, Pavlov AI, Kovacheva EG, Ilieva MP (2007). Elicitation of rosmarinic acid by *Lavandula vera* MM cell suspension culture with abiotic elicitors. World Journal of Microbiology and Biotechnology 23:301-304.

Ghasemzadeh A, Jaafar H (2012). Effect of salicylic acid application on biochemical changes in ginger (*Zingiber officinale* Roscoe). Journal Medicinal Plants Research 6:790-795.

Gigon A, Matos AR, Laffray D, Zuily-Fodil Y (2004). Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (ecotype Columbia). Annual Botany 94:345-351.

Hare PD, Cress WA, Van Staden J (1998). Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environment 21:535-53.

Harwood JL (1996). Recent advances in biosynthesis of plant fatty acids. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1301:7-56.

Hayat Q, Hayat S, Ali B, Ahmad A (2009). Auxin analogues and nitrogen metabolism, photosynthesis, and yield of chickpea. Journal Plant Nature 32:1469-1485.

Hayat S, Ahmad A (2007). Salicylic acid: A plant hormone. The Netherlands: Spring. Dordrecht.

Heil M (2004). Induction of two indirect defences benefits lima bean (*Phaseolus lunatus, Fabaceae*) in nature. Journal of Ecology 92:527-536.

Herro E, Jacob SE (2010). *Mentha piperita* (peppermint). Dermatitis 21 (6):327-329.

Jung S (2004). Effect of chlorophyll reduction in *Arabidopsis thaliana* by methyl jasmonate or norflurazon on antioxidant systems. Plant Physiology and Biochemistry 42:225- 231.

Khorasaninejad S, Mousavi A, Soltanloo H, Hemmati K, Khalighi A (2011). The effect of drought stress on growth parameters, essential oil yield and constituent of peppermint (*Mentha piperita* L.). Journal of Medicinal Plants Research 5:5360-5365.

Kim HJ, Chen F, Wang X, Choi JH (2006a). Effect of methyl jasmonate on phenolics, isothiocyanate, and metabolic enzymes in radish sprout (*Raphanus sativus* L.). Journal of Agricultural and Food Chemistry 54:7263-7269.

Kim HJ, Chen F, Wang X, Rajapakse NC (2006b). Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). Journal of Agricultural and Food Chemistry 54:2327-2332.

Krajncic B, Kristl J, Janzekovic I (2006). Possible role of jasmonic acid in the regulation of floral induction, evocation and floral differentiation in *Lemna minor* L. Plant Physiology and Biochemistry 44:752-758.

Lee YJ, Kim KJ, Park KJ, Yoon BR, Lim JH, Lee OH (2013). Buckwheat (*Fagopyrum esculentum* M.) sprout treated with methyl jasmonate (MeJA) improved anti-adipogenic activity associated with the oxidative stress system in 3T3-L1 adipocytes. International Journal of Molecular Science 14:1428-1442.

Li Z, Wang X, Chen F, Kim HJ (2007). Chemical changes and over expressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. Journal of Agriculture and Food Chemistry 55:706-713.

Lopez-Bucio J, Nieto-Jacobo F, Ramírez-Rodriguez V, Herrera-Estrella L (2000). Organic acid metabolism in plants: From adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Science 160:1-13.

López-Vélez M, Martínez-Martínez F, Del Valle-Ribes L (2003). The study of phenolic compounds as natural antioxidants in wine. Critical Review of Food Science Nutrition 43:233-244.

Løvdal T, Olsen, KM, Slimestad R, Verheul M, Lillo C (2010). Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato. Phytochemistry 71:605-613.

Ma B, Wan J, Shen ZG (2007). H_2O_2 production and antioxidant responses in seeds and early seedlings of two different rice varieties exposed to aluminium. Plant Growth Regulation 52:91-100.

Mahmood M, Bidabadi SS, Ghobadi C, Gray DJ (2012). Effect of methyl jasmonate treatments on alleviation of polyethylene glycol-mediated water stress in *banana (Musa acuminata* cv. 'Berangan', AAA) shoot tip cultures. Plant Growth Regulator 68:161-169.

Maksymiec W, Krupa Z (2002) The *in vivo* and *in vitro* influence of methyl jasmonate on oxidative processes in *Arabidopsis thaliana* leaves. Acta Physiologia Plantarum 24:351-357.

McKay DL, Blumberg JB (2006). A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytotherapy Research 20:619-633.

Mimica-Dukić N, Božin B, Soković M, Mihajlović B, Matavulj M (2003). Antimicrobial and antioxidant activities of three *Mentha* species essential oils. Planta Medica 69(5):413-419.

Mizukami H, Tabira Y, Ellis BE (1993). Methyl jasmonate-induced rosmarinic acid biosynthesis in *Lithospermum erythrorhizon* cell suspension cultures. Plant Cell Report 12:706-709.

Moeini H, Heidari A, Dizaji A (2006). Effect of water stress on some morphological and biochemical characteristics of purple basil (*Ocimum basilicum*). Journal of Biological Science 6:763-767.

Monreal JA, Jimenez ET, Remesal E, Morillo-Velarde R, Garcia Maurino S, Echevarria C (2007). Proline content of sugar beet storage roots: response to water deficit and nitrogen fertilization at field conditions. Environmental Experimental Botany 60:257-267.

Moon JK, Shibamoto T (2009). Antioxidant assays for plant and food components. Journal of Agriculture and Food Chemistry 57:1655-1666.

Mucciarelli M, Camusso W, Bertea CM, Bossi S, Maffei M (2001). Effect of (+)-pulegone and other oil components of *Mentha* × *piperita* on cucumber respiration. Phytochemistry 57:91-98.

Munns R (2005). Genes and salt tolerance: bringing them together. New Phytology 167(3):645-663.

Nafie E, Hathout T, Mokadem ASA (2011). Jasmonic acid elicits oxidative defense and detoxification systems in *Cucumis melo* L. cells. Brazilian Journal of Plant Physiology 23:161-174.

Naidu BP, Paleg LG, Aspinall D, Jennings AC, Jones GP (1991). Amino acid and glycine betaine accumulation in cold-stressed wheat seedlings. Phytochemistry 30:407-409.

Ogata A, Tsuruga A, Matsunob M, Mizukami H (2004). Elicitor induced rosmarinic acid biosynthesis in *Lithospermum erythrorhizon* cell suspension cultures: Activities of rosmarinic acid synthase and the final two cytochrome P450-catalyzed hydroxylations. Plant Biotechnology 21:393-396.

Oliveira A, Pereira J, Andradec P, Valentaoc P, Seabrac R, Silva B (2008). Organic acids composition of *Cydonia oblonga* Miller leaf. Food Chemistry 111:393-399.

Patel BS, Patel KP, Patel ID, Patel M (2000) Response of fennel (*Foeniculum vulgare*) to irrigation, nitrogen and phosphorus. Indian Journal of Agronomy 45:429-432.

Pawlak K, Bylka W, Jazurek B, Matlawska I, Sikorska M, Manikowski H, Bialek-Bylka G (2010). Antioxidant activity of flavonoids of different polarity, assayed by modified ABTS cation radical decolorization and EPR technique. Acta Biologica Cracoviensia Series Botanica 52(1):97-104.

Perez-Jimenez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: An application of the Phenol-Explorer database. European Journal of Clinical Nutrition 64:112-120.

Polumbryk M, Ivanov S, Polumbryk O (2013). Antioxidants in food systems. Mechanism of action. Ukraine Journal of Food Science 1:15-40.

Popova L, Ananieva E, Hristova V, Christov K, Georgieva K, Alexieva V, Stoinova ZH (2003). Salicylic acid and methyl jasmonate induced protection on photosynthesis to paraquat oxidative stress. Bulgarian Journal of Plant Physiology (Sp Iss):133-152.

Rhodes D, Hanson AD (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. Annual Review of Plant Physiology and Plant Molecular Biology 44:357-384.

Rhodes D, Nadolska-Orczyk A, Rich PJ (2002). Salinity, osmolytes and compatible solutes In: Lauchli A, Luttge U (Eds). Salinity, Environment, Plant, Molecules. Netherlands: Al-Kluwer Academic Publishers pp 181-204.

Schaller A, Stintzi A (2009). Enzymes in jasmonate biosynthesis - Structure, function, regulation. Phytochemistry 70:1532-1538.

Silva AS (2002). Análise técnica econômica e de tendências da indústria brasileirade óleos essenciais. Papel Virtual, Rio de Janeiro.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic and phosphotungstic acid reagents. American Journal of Enology and Viticulture 16:144-153.

Song YS, Park CM (2014). Luteolin and luteolin-7-O-glucoside strengthen antioxidative potential through the modulation of Nrf2/MAPK mediated HO^1 signaling cascade in RAW 264.7 cells. Food and Chemistry of Toxicology 65:70-75.

Sorial ME, El-Gamal SM, Gendy AA (2010). Response of sweet basil to jasmonic acid application in relation to different water supplies. Bio Research 7(1):39-47.

Strizhov N, Abrahám E, Okrész L, Blickling S, Zilberstein A, Schell J, ... Szabados L (1997). Differential expression of two P5CS genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in *Arabidopsis*. Plant Journal 12:557-569.

Swiatek A, Azmi A, Witters E, Van-Onckelen H (2003). Stress messengers jasmonic acid and abscisic acid negatively regulate plant cell cycle. Bulgarian Journal of Plant Physiology 29:172-178.

Szabo E, Thelen A, Petersen M (1999). Fungal elicitor preparation and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. Plant Cell Report 18:485-489.

Taheri AM, Daneshian J, Valadabadi SAR, Aliabadi FH (2008). Effects of water deficit and plant density on morphological characteristics of chicory (*Cichorium intybus* L.). Abstracts Book of 5th International Crop Science Congress and Exhibition.

Traw MB, Bergelson J (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in Arabidopsis. Plant Physiology 133:1367-1375.

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004). Role of oxygen radicals in DNA damage and cancer incidence. Molecular Cell Biochemistry 266:37-56.

van Dam RM, Naidoo N, Landberg R (2013). Dietary flavonoids and the development of type 2 diabetes and cardiovascular diseases: Review of recent findings. Current Opinion in Lipidology 24:25-33.

Vatanparast G, Mirdehghan H, Karimi H, Vazifeshenas M (2012). Foliar application of salicylic acid, methyl jasmonate and potassium sulfateon photosynthetic characteristics and fruit quality of pomegranate. Iran Agriculture Research 31:23-34.

Wang SY, Bowman L, Ding M (2008). Methyl jasmonate enhances antioxidant activity and flavonoid content in blackberries (*Rubus sp.*) and promotes antiproliferation of human cancer cells. Food Chemistry 107:1261-1269.

Wang CY (1999). Methyl jasmonate reduces water stress in strawberry. Journal of Plant Growth Regulation 18:127-134.

Wolucka BA, Goosens A, Inze D (2005). Methyl jasmonate stimulates the *de novo* biosynthesis of vitamin C in plant cell suspensions. Journal of Experimental Botany 56:2527-2538.

Yadav RK, Sangwan RS, Sabir F, Srivastava AK, Sangwan NS (2014). Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L. Plant Physiology and Biochemistry 74:70-83.

Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H (2002). Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. Plant Physiology 128:63-72.

Yancey PH (1994). Compatible and counteracting solutes. In: Strange K (Ed). Cellular and Molecular Physiology of Cell Volume Regulation. Boca Raton, FL: CRC Press pp 81-109.

Yao HJ, Tian SP (2005). Effect of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biology and Technology 35:253-262.

Yordanov I, Velikova V, Tsonev T (2003). Plant responses to drought and stress tolerance. Bulgarian Journal of Plant Physiology 2003:187-206.