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Secondary metabolites of peppermint change the morphophysiological and biochemical characteristics of tomato

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ABSTRACT

Germination and growth of plants are influenced by allelochemicals that mostly cause crops' yield reduction. In the present study, the effect of stress arising from allelopathic compounds in the water extract (WE) of peppermint (*Mentha* × *piperita* L. CV. Mitcham) on the morphophysiological and biochemical characteristics of tomato (*Lycopersicon esculentum* Mill. CV. Rio Grande) was investigated. Different concentrations (0, 2, 4, 6, 8, and 10% (v/v)) of the WE were examined. Some phenolic compounds of the WE determined by the HPLC instrument were trans-ferulic acid (10.8 mg/g), hesperidin (9.3 mg/g), ellagic acid (6.8 mg/g), and sinapic acid (4.2 mg/g). The results showed that the maximum inhibitory effect on germination and growth (dry weight, and leaf area) was obtained at the concentration of 10% (v/v) extract, and its compounds had significant effect on the amount of proline (PRO), soluble sugar and starch, as well as on the activities of tomato,s antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) at the 5% level. None of the treatments had a significant effect on the SPAD chlorophyll meter reading of tomato plants. It could be stated that the compounds present in the extract of peppermint must lead to high levels of reactive oxygen species (ROS), and subsequent oxidative stress inhibits the growth of the seedlings; however, more research is still required in this regard.

1. Introduction

Allelopathy is one kind of stress that plays a significant role in agro-ecosystems, and affects the growth, quality and quantity of the crops (Kohli et al., 1998; Singh et al., 2001). Moreover, it has emerged as a pragmatic approach to solve multiple issues in the modern agriculture. Numerous approaches including intercropping, cover crops, crop rotation, mulching, crop residue incorporation, and water extract (WE) application are being used to explore allelopathy for pest and weed management, stress abatement, and growth enhancement in crop production (Farooq et al., 2013). Aromatic plants are rich in essential oils and phenolic components; they can play an important role in plant interactions, and are considered as a major source of allelochemicals (Macias et al., 2002; Saharkhiz et al., 2010). Allelochemicals are able to alter several physiological and biochemical processes including water utilization, mineral uptake, foliar expansion, photosynthesis, amino acid metabolism, protein synthesis, glycolysis, mitochondrial respiration, and ATP synthesis (Hosseinzade et al., 2009; Soares et al., 2012). Various researchers have noted that these components may directly suppress antioxidant enzyme activity within the cell resulting in high levels of active oxygen species; eventually, the stress of oxidation inhibits the growth of the seedlings (Jinhu et al., 2012). In other words, it becomes a biotic stress, known as allelochemical stress, which can have an indirect or direct effect on the receiver plant. Thus, allelochemical stress can act as a mechanism of interference, and influence the pattern of vegetation, weed growth, and crop productivity (Romero-Romero et al., 2005). Like many other stress factors, plants, response to allelochemical stress is diverse and complex.

The family Lamiaceae is a source of phenolic compounds with strong antioxidant activities (Belmekki and Bendimerad, 2012), so there are several reports about their allelopathic properties in the literature (Mutlu et al., 2011; Batish et al., 2012; Islam and Kato-Noguchi, 2013; Taban et al., 2013). Saharkhiz et al. (2016) demonstrated that the essential oil of catnip (*Nepeta cataria* L.) from (*Lamiaceae*) has phytotoxic activity on seed germination and seedling growth of *Hordeum spontaneum* Koch, *Taraxacum officinale*, *Avena fatua* L. and three crop seeds including *Lipidium sativum*, *Nepeta cataria* and *Ocimum basilicum*. The allelopathic

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potentials of aqueous extracts and leaf powders of three Satureja species, namely: *Satureja khuzestanica* Jamzad, *Satureja bachtiarica* Bunge and *Satureja rechingeri* Jamzad (*Lamiaceae*) have been previously reported (Taban and Saharkhiz, 2015). The effect of coumarin on germination, early growth, nutrient mobilization, and some physiological parameters of faba bean (*Vicia faba* L.) was researched. Coumarin treatment significantly improved the level of primary and secondary metabolites as well as phytohormones in faba bean (Saleh et al., 2015). Some earlier studies on the effects of oxidative stress caused by allelochemicals on tomato seedlings have been documented (Kato-Noguchi et al., 2008; García-Sáncheza et al., 2012). However, according to the effects of allelochemicals on growth, productivity and yield of agricultural crops, and due to lack of information about their mechanism of action and physiological influences, in the present work as an example, tomato's morphophysiological and biochemical responses to the stress caused by allelopathic compounds from peppermint WE were determined.

2. Material and methods

2.1. Plant material

At the flowering stage (15 August 2013), the aerial parts of organic cultivation of peppermint (*Mentha* × *piperita* L. CV. Mitcham), were collected from a field located in the Darab city (1181 m above mean sea level, latitude 29°68′N and Altitude 53°2′E.) in Fars Province, Iran. The average of minimum and maximum temperatures and the relative humidity of the field in recent 20 year periods were 14.3, 39.8 °C, and 40.2%, respectively. The soil of the field was loam with pH=8.06, EC=1.64 dS m^{-1}, 21 ppm P, 220 ppm K, 2 ppm Fe, 2.1 ppm Zn, 1.9 ppm Mn and 0.38% organic matter. The plant species was identified and authenticated by Khosravi, a plant taxonomist at the Shiraz University Herbarium, Shiraz, Iran. Voucher specimen (No. 24,995) has been deposited in the herbarium.

2.2. Preparation of water extract

In order to make the required WE, the maceration method was used according to the previous described method with some modifications (Laosinwattana et al., 2009). Briefly, the aerial parts (80 g leaves and 20 g stem) of peppermint were dried under shade and powdered mechanically, using a commercial electric grinder. For making the stock extract, 100 g of the powdered plant was added to 1 l of distilled water and was placed in a closed container at room temperature (25 ± 1 °C) for 48 h with frequent agitation until the soluble matter was dissolved. The extract was filtered through three layers of cheesecloth to remove any fiber debris. The supernatant was consequently filtered using Whatman filter paper (No. 1). The concentration of the resulting stock extract was 10% (Laosinwattana et al., 2009). Then, it was appropriately diluted with distilled water to give final concentrations of 2%, 4%, 6% and 8% (v/v) along with distilled water as control. The additional fresh stock extract was transferred to a sterile glass container and stored in the refrigerator at 4 °C for future use.

2.3. Total phenols

The total phenolic contents of the plant WE and the tomato seedlings were determined separately using the method of McDonald et al. (2001). Calibration curve was prepared by mixing ethanolic solution of gallic acid (1 ml; 0.025–0.400 mg ml^{-1}) with 5 ml Folin-Ciocalteu reagent (diluted ten folds) and sodium carbonate (4 ml, 0.7 M). The absorbance was measured by using Hitachi U-2000 spectrophotometer and the calibration curve was drawn at 765 nm.

2.4. High-performance liquid chromatography (HPLC) analysis of phenolic compounds

To separate, identify and quantify the phenolic components of WE, HPLC analysis was carried out on a Agilent 1200 series (USA), equipped with a Zorbax Eclipse XDB-C18 column (10 cm × 5 μm i. d.; × 150 mm film thickness, RP), and a photodiode array detector (PAD). To prepare the injectable extract, 0.02 g of the vacuum dried residue of the plant extract was dissolved in 1 ml of methanol and the aliquots was filtered through a 0.2 μm membrane millipore chromatographic filter and 20 μL of the solution injected into the HPLC system. The flow rate was set at 1 ml min^{-1}. The elution was monitored at 280 and 320 nm. Gradient elution was selected to achieve the maximum separation and sensitivity. The elution was performed by varying the proportion of solvent A (formic acid 1% in deionized water) to solvent B (methanol (v/v)) as follows: methanol: formic acid 1% (10:90), at 0 min; methanol: formic acid 1% (25:75), at 10 min; methanol: formic acid 1% (60:40), at 20 min and finally, methanol: formic acid 1% (70:30), at 30 min. The total running time was 40 min. The column temperature was 30 °C.

2.5. Plant culture and application of water extract treatments

This research was performed under greenhouse conditions and in a controlled environment. Polyethylene pots (10 × 30 cm) were filled with 1.5 kg of a mixture of sand, leaf mold and clay in the ratio (1:1:1, v/v/v). After sowing the tomato (*Lycopersicon esculentum* Mill. CV. Rio Grande) seeds (The seeds were provided by Pakan Bazr Co. in Isfahan), they were irrigated (the first irrigation) with peppermint WE prepared at concentrations of 2%, 4%, 6%, 8% and 10% and also distilled water as a control to the final volume of 200 cc (field capacity level of the mixture). In the next irrigations, to eliminate the effect of WE leachate according to their need, the pots were irrigated with distilled water based on the field capacity level of the mixture every 2 days (Eghbali et al., 2009). Seed germination was investigated every day. After 40 days, total chlorophyll content (indicated as SPAD-value) was measured by a chlorophyll meter, SPAD-502 (Minolta, Japan) (Barraclough and Kyte, 2001). After transferring the plants into the laboratory, their growth was measured in terms of leaf area (Lpi 210, England) and dry weight per treatment. Some physiological indices like relative membrane permeability (RMP), proline (PRO), total phenols, starch and soluble sugar contents, and the activity of antioxidant enzymes (SOD, APX, CAT and POX) were determined.

2.6. Proline and relative membrane permeability

Free PRO was extracted from 0.5 g fresh seedling samples in 3% (w/w) aqueous sulphosalicylic acid, and was estimated using ninhydrin reagent (Bates et al., 1973). The RMP was determined by the method of Wang et al. (2009).

2.7. Soluble sugars and starch

The content of soluble sugars in tomato was determined according to Dubois et al., 1956. The content of starch was determined by the method of McCready et al., 1950.

2.8. Enzymes activity

Catalase (CAT, EC 1.11.1.6) activity was measured by following the reduction of H_2O_2 (ε=39.4 mM^{-1} cm^{-1}) at 240 nm according to the method of Dhindsa et al. (1981). SOD (EC 1.15.1.1.) activity

was assayed by the nitroblue tetrazolium (NBT) method (Dhindsa et al., 1981). APX (EC 1.11.1.11) activity was measured based on the method of Nakano et al. (1981). Peroxidase (POX, EC 1.11.1.7) activity was determined through following the method of Chance et al. (1955).

2.9. Statistical analysis

The experiment was arranged based on a completely randomized design (CRD) with three replications for each treatment. The normality test was done by using Minitab statistical software (version 15) to assess data normality and transformation of the data performed if needed. One-way analysis of variance (ANOVA) was performed to confirm the variability and validity of the data. Differences between the treatment means were compared using LSD test at 0.05% probability level.

3. Results

3.1. Total phenol content and chemical analysis of the water extract

Results showed that the peppermint WE contains a high phenolic content (total phenol of 40.7 mg/g based on gallic acid). Also, the results of HPLC analysis (Table 1) showed 8 phenolic acids in the WE. The major phenolic components were trans-ferulic acid (10.8 mg/g), hesperidin (9.3 mg/g), ellagic acid (6.8 mg/g), and sinapic acid (4.2 mg/g). The HPLC chromatogram of the compounds is presented in Fig. 1.

3.2. Germination and growth measurements

As shown in Fig. 2, all WE concentrations significantly ($P \leq 0.05$) decreased tomato seed germination percentage compared to the control. But they were unable to stop the germination percentage completely. By increasing the concentration of WE, no significant difference was observed between the effect of different concentrations.

Growth of the seedlings was measured in terms of leaf area, dry weight of tomato roots and stems per treatment. Leaf area was significantly ($P \leq 0.05$) reduced as compared to the control (Fig. 3). The WE at 10% substantially declined the leaf area of the tomato seedlings by 91%. By increasing the concentration of the WE, leaf area of the seedlings was decreased when compared with the control. According to the results, only dry weight of the tomato roots was remarkably ($P \leq 0.05$) influenced by 10% of peppermint WE (Fig. 4). Unexpectedly, the 4% WE had stimulated effect on the dry weight of the tomato roots as compared to the control. It further resulted in decreasing the dry weights of the seedlings' stems as shown in Fig. 5. The decreasing effects of WE on the dry weight of stem showed fluctuated trends from 2% to 8%. However, the 10% WE remarkably decreased the dry weight of the seedlings stems compared to the control.

Table 1
Phenolic components (mg/g) of peppermint water extract analyzed by HPLC.

No[a]	Component	(mg/g)	Rt[b]
1	Sinapic acid	4.2	16.5
2	Gallic acid	0.3	3.3
3	Catechin	ND[c]	8.7
4	Caffeic acid	0.05	11.6
5	Chloregenic acid	1.2	10.5
6	Rutin	ND	12.6
7	Quercetin	ND	21.6
8	p-Coumaric acid	1.0	15.9
9	Coumarin	ND	17.4
10	Carvacerol	ND	28.4
11	Vanilin	ND	13.5
12	Trans-ferulic acid	10.8	16.3
13	Hesperedin	9.3	18.5
14	Ellagic acid	6.8	19.02
15	Eugenol	ND	23.7
16	Hesperetin	ND	22.4
	Total	33.35	–

[a] Number.
[b] Retention time (min).
[c] Not detected.

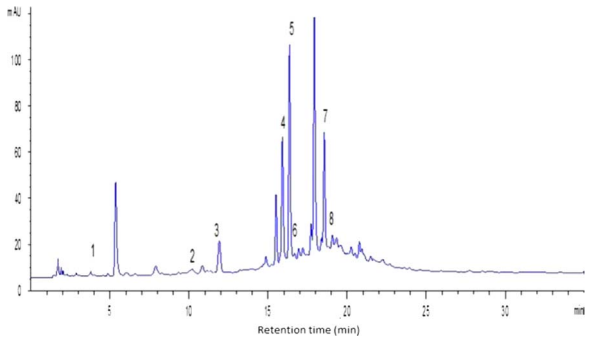

Fig. 1. HPLC chromatogram of the analyzed extract. Key: 1: gallic acid; 2: chloregenic acid; 3: caffeic acid; 4: p-coumaric acid; 5: tarns- ferulic acid; 6: sinapic acid; 7: hesperidin; 8: ellagic acid.

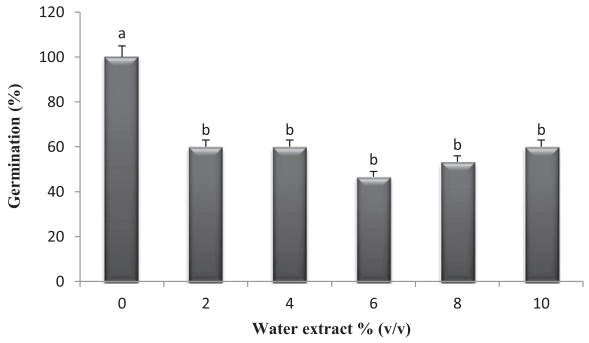

Fig. 2. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on tomato seed germination (%). Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

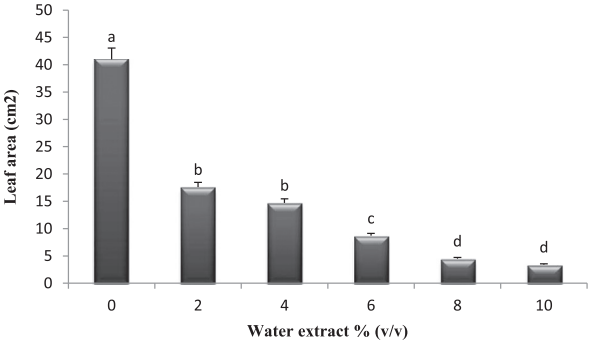

Fig. 3. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on tomato leaf area (cm^2). Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

3.3. Total chlorophyll content (SPAD units)

In comparison with the control, the WE did not have any substantial effect on the SPAD chlorophyll meter reading or the total chlorophyll content of tomato seedlings (Data not shown).

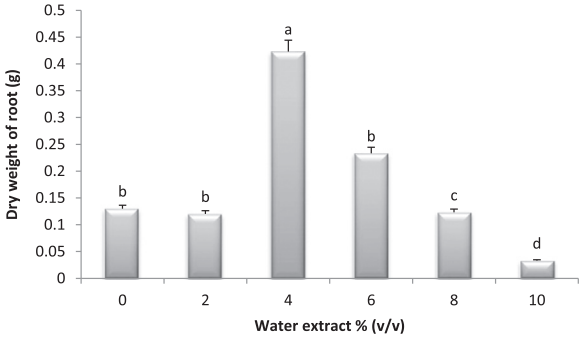

Fig. 4. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the dry weight (g) of tomato roots. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

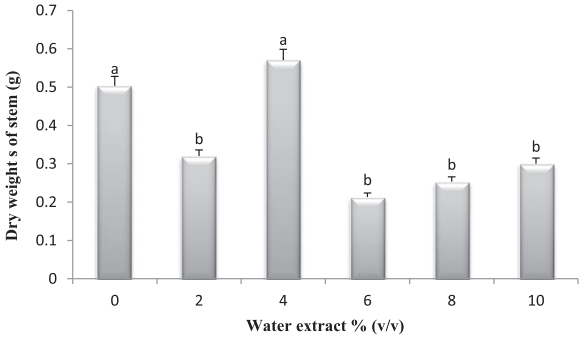

Fig. 5. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the dry weight (g) of tomato stems. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

3.4. Total phenols

The results demonstrated that, with the exception of 4% treatment plants, the content of total phenols of all tested plants was reduced with the use of the WE. However, this reduction was not notable. Only the decrease caused by 2% (v/v) WE was significant when compared with the control (Fig. 6).

3.5. Proline and relative membrane permeability

The results revealed that the peppermint WE in all concentrations had stimulatory effect on the PRO content of the seedlings (Fig. 7). In other words, the tomato seedlings exposed to concentrations of 2%, 6%, 8% and 10% of the WE contained the highest amount of PRO. Of course, there was no significant difference ($P \leq 0.05$) among them.

An increase in the amount of RMP was evident with the

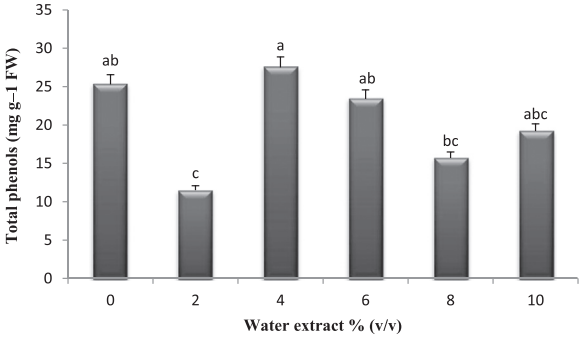

Fig. 6. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the total phenols (mg g^{-1} FW) of tomato seedlings. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

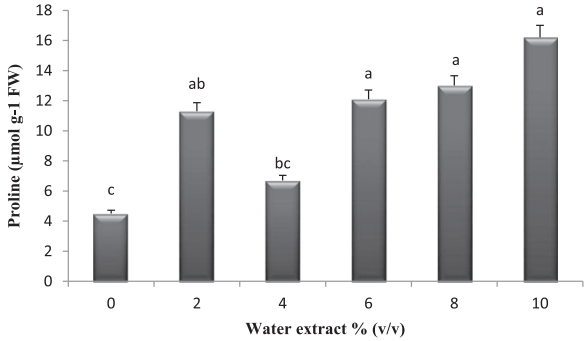

Fig. 7. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the PRO content (µmol g^{-1}) of tomato seedlings. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

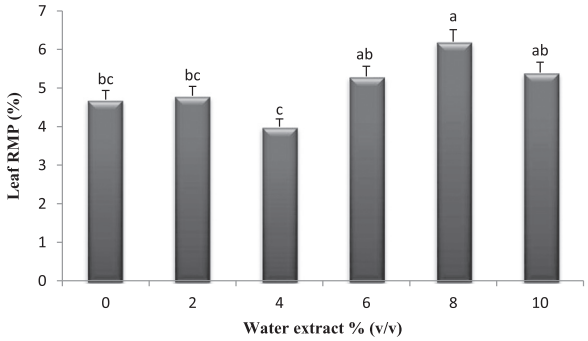

Fig. 8. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the leaf RMP (%) of tomato seedlings. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

increase in the peppermint WE concentration; however, in comparison with the control, only 8% (v/v) WE caused a considerable increase in the membrane leakage as shown in Fig. 8. The least membrane leakage (4%) was observed in the 4% peppermint WE.

3.6. Soluble sugars and starch contents

Different concentrations of WE had significant effects on the sugar and starch contents of tomato seedlings (Fig. 9). In comparison with the control, the sugar and starch contents were generally decreased in all treatments. However, the sugar content at higher concentrations (6–10%) exhibited concentration dependent increase that it was not significant.

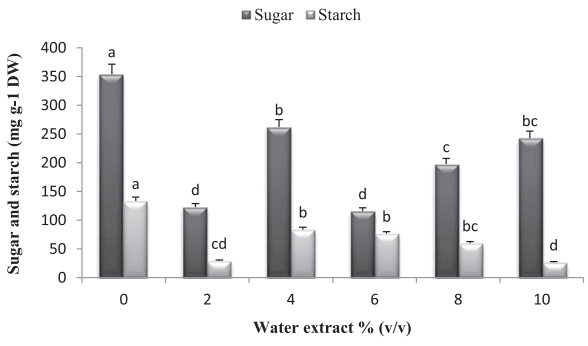

Fig. 9. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the soluble sugar and starch contents (mg g^{-1} DW) of tomato seedlings. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

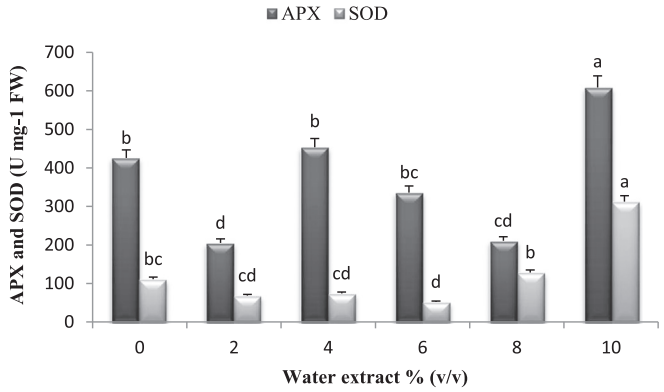

Fig. 10. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the APX and SOD activity (U mg^{-1} FW) of tomato seedlings. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

3.7. *Activities of antioxidant enzymes*

As shown in Fig. 10, the activity of SOD enzyme at 2–6% WE concentrations showed a decrease trend and at the 6% WE the decline in the enzyme activity was significant ($P \leq 0.05$). However, the seedlings treated with higher concentrations (8% and 10%) of the WE exhibited higher SOD activity in comparison with those treated with lower concentrations. According to the results (Fig. 10) with the exception of the 10% WE treatment which caused the maximum APX activity, there was inconsiderable increase in APX activity in the moderate concentrations of WE comparing to the lower concentrations.

All concentrations of the WE applied in the present research had significant effects on the activity of POD (Fig. 11). With the exception of 10% WE, the POD activity of all tested plants was reduced significantly ($P \leq 0.05$) in comparison to the control. Also the results showed that the 10% WE increased the CAT activity of tomato seedlings but other concentrations had no effect on its activity (Fig. 11).

4. Discussion

Phytotoxicity is a toxic effect by a compound on plant growth. Such damage may be caused by a wide variety of compounds, including trace metals, salinity, pesticides, phytotoxins or allelochemicals. Peppermint (*Mentha* × *piperita* L. CV. Mitcham) contains allelochemicals, which could inhibit the seed germination of some species that previously competed with it; the effect of these allelochemicals intensifies with the increase of their concentration

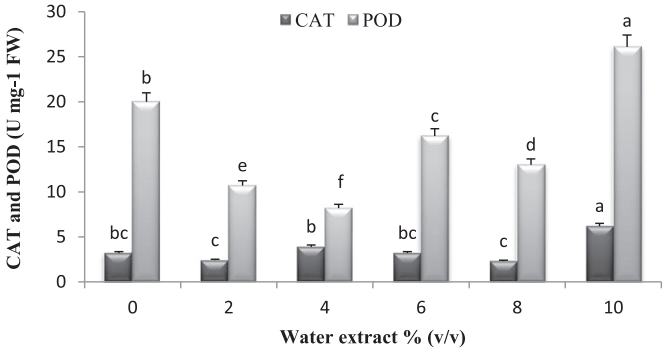

Fig. 11. Effect of different concentrations (0, 2%, 4%, 6%, 8% and 10% v/v) of peppermint WE on the CAT and POD activity (U mg^{-1} FW) of tomato seedlings. Means with the same letter are not significantly different, as indicated by the LSD test ($P \leq 0.05$).

(Dudai et al., 1999; Mahdavikia and Saharkhiz, 2015). However, it was rarely reported if there are any allelochemical effects of peppermint on noninvasive plants, especially on the horticultural crop, tomato (*Lycopersicon esculentum*). Also the role of peppermint allelochemicals in inducing conventional stress-response in the target tissue remains unknown, we intended to investigate the effect of WE from *M. piperita* on the growth and seed germination of tomato. In fact, the present study implies that peppermint allelochemicals cause oxidative stress in the aerial parts of tomato. So understanding the allelochemicals, modes of action on the growth, and analyzing the defense mechanisms including enzymatic and non-enzymatic antioxidant systems induced in the tomato seedlings exposed to the extract in order to counteract oxidative stress are very important.

Different concentrations of peppermint WE caused decrease in the seed germination and growth of tomato seedlings. Furthermore, exposure to higher concentrations of peppermint extract resulted in increased PRO accumulation and antioxidant enzymes' activities. It is to be noted that, in this study, lower concentration (4%) of the extract showed insignificant inhibitory effect and even positive stimulating effect on the dry weight of tomato roots which is in agreement with the previous reports (Taban et al., 2013; Saharkhiz et al., 2010; Tigre et al., 2012). At low concentrations, the tomato seedlings were stronger in the allelochemicals' catabolism because of low allelopathic potential of the extract, and showed better growth features after application of the treatments. The seedlings obtained from the higher concentrations of WE were more capable of countering the allelochemicals-induced stress; in order to reduce the damage caused by increase in the ROS produced by higher concentrations of the extract, both the PRO accumulation, and the activity of antioxidant enzymes were increased. So we can say that a few plants grown in the high peppermint extract concentration (10%) have been able to earn resistance against the effect of allelochemicals, and adapted to the stress conditions.

The amounts of total phenolic components and hesperidin and caffeic acid in this project were comparable to those reported in a previous study by Fecka and Turek (2007) who reported water-soluble polyphenolic compounds in peppermint though discrepancy of other components was demonstrated. Differences in the secondary metabolites might have been caused by the climatic and cultivation factors, the origin and genetic features, plant density, ontogenetic stages, seasonal changes, distillation and drying methods (Ram et al., 1997; Chauhan et al., 2009). Detection of phenolic acids in WE demonstrated that these compounds were at significantly enough concentrations to play a major role in the plant's interference and phytotoxic effects. Belmekki and Bendimerad (2012) reported that the Lamiaceae family includes rich source of polyphenol compounds, and therefore, it has antioxidant and biological properties. The phytotoxicity of phenolic compounds seems to be due to the disruption and inhibition of certain processes during the germination stage. They cause inhibition of seed germination not only by induction of oxidative stress but also by restriction on reserve mobilization; it is because phenolics can inhibit the activities of α-amylase and gibberellic acid (Politycka and Gmerek, 2008; Singh et al., 2009; Das et al., 2012). Golisz et al. (2008) noted that some alellochemicals can cause root cell death indirectly by facilitating the production of ROS, which may also act as signaling molecules leading to changes in hormonal balance during the seed germination. It was found that phenolics are able to inhibit plant cell division, root elongation, change cell ultrastructure, and interfere with the normal growth and development of the whole plant (Li et al., 2010). Singh et al. (2009) reported that the reduced chlorophyll under leachate treatments could be attributed to the inhibition of chlorophyll biosynthesis and/or the stimulation of chlorophyll degradation. Cell ultrastructure

destruction causes a reduction of photosynthesis and root activities. These abnormal physiological processes contribute to the inhibition of plant growth. There are also many reports regarding the phytotoxic effects of phenolic compounds on other plants (Abdulghader et al., 2008; Dudai et al., 2009; Singh et al., 2009; Li et al., 2010; García-Sáncheza et al., 2012); their effects are regarded as a form of biotic or allelochemical stress (Lara-Núñez et al., 2009).

In the present study, the amount of total phenolic compounds did not significantly change in response to peppermint allelochemical stress. It seems that defense mechanisms other than phenolics might be working in tomato to cope with this stress, or peppermint allelochemicals weaken the protective ability of tomato tissues against membrane lipid peroxidation and damage the whole membrane system. Batish et al. (2008) also observed that the amount of total endogenous phenolics in the mung bean hypocotyl cuttings declined significantly in response to different caffeic acid treatments (except at 1000 mM) compared with water-treated control. These results are in contradiction with the findings of Das et al. (2012).

Enhanced PRO along with increase in WE concentration suggests an allelochemical-induced stress. Accumulation of PRO indicates cellular damage in the target tissue caused by the ROS generated by peppermint allelochemicals. The present study concludes that peppermint allelochemicals induce oxidative stress in tomato through generation of ROS and upregulation of the activities of some scavenging enzymes. This result is in agreement with the findings of Batish et al. (2006). They noted that PRO enhances tolerance and provides protection against abiotic stress by avoiding ROS-induced damage to photosystems, membranes and proteins. In another study, Radish leaf PRO levels were increased by increasing the leaf extract concentration of heliotrope (*Heliotropium europaeum* L.) (Abdulghader et al., 2008).

One of the allelopathic mechanisms of phenolics is making changes in membrane permeability. Consequently, the cell content decreases, leading to increased lipid peroxidation. Finally, slow growth or death of plant tissue occurs (Li et al., 2010). RMP was also enhanced by increasing the extract's concentration. This shows that phenolic compounds can induce oxidative stress in target tomato; as a result, the cell membrane is affected by toxic ROS. Moreover, enhanced ROS can affect membrane permeability, damage DNA and proteins, induce lipid peroxidation, and ultimately, lead to programmed cellular death. García-Sáncheza et al. (2012) noted that interaction of phenolic compounds with some of their constituents, which is necessary for maintaining membrane functioning, can damage cell membrane. Several studies have shown that allelochemical stress can cause oxidative damage, as evidenced by increase in ROS-scavenging enzyme activity and membrane lipid peroxidation (Romero-Romero et al., 2005; Batish et al., 2006; Cruz-Ortega et al., 2007; Lara-Núñez et al., 2009).

In comparison with the control, the sugar and starch contents were generally decreased in all treatments. However, the sugar content at higher concentrations of WE (6–10%) exhibited concentration-dependent increase though it was not significant. In the case of decrease in sugar content, the role of phenolic allelochemicals in reduced chlorophyll content and photosynthetic rate is important. Increase in sugar content is an indication of reduction in the activity of some respiratory enzymes and reduced consumption of sugar in low-growing plants. These results are in line with the findings of Das et al. (2012) where the soluble sugar content of seeds was reduced in the presence of the leaf litter leachates of some selected tree species. In contrast, the level of soluble sugar in radish leaves was increased by heliotrope allelochemicals (Abdulghader et al., 2008). There are other reports of reduced carbohydrate content in the presence of aqueous extracts of various plants (Sadhena et al., 1999; Padhy et al., 2000). Unfortunately, most of the studies about the effect of allelochemicals on soluble sugar and starch content have been conducted during seed germination time (Kato-Noguchi and Macías, 2008; Lara-Núñez et al., 2009).

Our results showed that, at the highest concentration of the extract, there was an increase in the activity of antioxidant enzymes (SOD, APX, CAT and POX) in the aerial parts of tomato. This indicates that oxidative stress could play a role in phytotoxic phenomenon. Any increase in the activity of SOD, a major scavenger of superoxide (O_2^-) radical, demonstrates that peppermint WE exposure causes excessive generation of O_2^-, resulting in oxidative stress. Phenolic compounds can directly generate overproduction and accumulation of ROS (Cruz-Ortega et al., 2007; García-Sáncheza et al., 2012). Increased activity of these scavenging enzymes can be due to the induction of secondary defensive mechanism against oxidative stress caused by peppermint allelochemicals. Allelochemicals absorbed by plant cells should be detoxified. The detoxification and the response of plant cells to it result in increased activity of antioxidant enzymes (Ziaebrahimi et al., 2007). The impact of phenolic allelochemicals on the activity and function of certain enzymes of plants has been confirmed (Bogatek and Gniazdowska, 2007; Lara-Núñez et al., 2009; Li et al., 2010; García-Sáncheza et al., 2012). Our results clearly exhibit that the activity of SOD, CAT, APX and POD tends to decrease under the influence of low concentrations of the extract. This decrease can be due to detoxification and stress-resistance mechanisms mainly in the root cells more than in the leaf cells. This is in agreement with the findings of Cruz-Ortega et al. (2002) in maize root.

The observations made in the present study indicate that peppermint WE causes oxidative stress through generation of ROS, and activates antioxidant enzymatic and non-enzymatic machinery in the target tissue as a defense mechanism to counter the peppermint extract-induced stress. At lower concentrations (often 2%), it helps with avoiding peppermint extract toxicity; however, at higher concentrations (10%), the damage caused by ROS is more extensive, and the defense system does not function sufficiently to avoid this damage. Finally, contrary to our expectations, a few plants grown at high concentrations of peppermint WE earned more resistance to allelochemicals and adapted to the stress conditions.

5. Conclusions

In conclusion, the present study demonstrated that different concentrations of peppermint WE caused decrease in seed germination and the growth of tomato seedlings; especially the 10% (v/v) concentration showed potent allelopathic and phytotoxic effects. These inhibitory effects are, generally, due to allelochemical components of peppermint WE. The action of allelochemicals in the target plant is diverse, and affects a large number of biochemical reactions resulting in modification of various processes (Bogatek and Gniazdowska, 2007). Increase in RMP and accumulation of PRO show that phenolic compounds induce oxidative stress in tomato. It was accompanied by upregulation in the activity of scavenging enzymes including APX, CAT, POD and SOD in the aerial parts of tomato in response to exposure to the WE. Both soluble sugar and starch were decreased by increasing the WE concentration. Eventually, oxidation stress inhibited the growth of the seedlings. It can be concluded that the increased activity of antioxidant enzymes under allelochemical stress could play an important role in allelopathy phenomenon. More research is still required in this regard. It is necessary to find out the effective allelochemicals of WE from peppermint for assessing their effects on tomato under field conditions, as field experiments in natural soils can show more natural allelopathic effects of other plants including peppermint.

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