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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