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Original Article

Effect of Foliar Application with Methyl Jasmonate on Physiological Behavior of *Mentha piperita*

Soheila Afkar[1*] and Mohsen Sharifi[2]

[1] *Department of Agriculture, Payame Noor University of Lorestan, Khorramabad P. O. Box 6815139611, Iran*
[2]*Department of Plant Biology, Faculty of Biological Sciences Tarbiat Modares University, Tehran, Iran*

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Abstract

A valuable composition of *Mentha piperita* L. essential oil is menthol that is widely used for different industries. The plants were treated with different concentrations of Methyl Jasmonate (MeJA) and after 24 h were evaluated for total soluble proteins, chlorophylls (a, b, and total), malondialdehyde, carbohydrates and antioxidant enzymes (Superoxide dismutase and Guaiacol peroxidase). Variance analysis indicated significant variation in all measured traits except for chlorophyll b that is caused by different concentration of MeJA. According to these results, MeJA could active antioxidant enzyme defense system. It can be concluded that stimulation of plant defense systems using elicitors could be a valuable and alternative strategy to protect *Mentha piperita* from stress.

Key words: *Mentha piperita,* Malondialdehyde, Methyl jasmonate, Carbohydrate, Superoxide dismutase, Guaiacol peroxidase

Abbreviations APX-Ascorbate peroxidase; CAT-Catalase; Chl a-Chlorophyll a; Chl b-Chlorophyll b; GPX-Glutathione peroxidase; GR-Glutathione reductase; H_2O_2-Hydrogen peroxide; JA-Jasmonic acid; MDA-Malondialdehyde; MeJA-Methyl jasmonate; OH-Hydroxyl free radical; O^{2-}−Superoxide anion; POD-Guaiacol peroxidase; ROS-Reactive oxygen species; SOD-Superoxide dismutase; Total Chl-Total Chlorophyll

Introduction

Medicinal and aromatic plants are a useful source of primary health care which they used by more than 80% of world's population [1]. The genus *Mentha* L. (Lamiaceae), include different species like *Mentha Arvensis* L., *M. piperita* L., *M. spicata* L. and *M. pulegium* L. [2] Mint plants have considerable commercial value so they extensively cultivated for their essential oil. Monoterpenes as a valuable composition of *Mentha* essential oil is widely used for food, pharmaceutical and cosmetic industries [3]. Menthol ($C_{10}H_{20}O$) is a terpenoid that compose the main and specific component of the essential oil of peppermint (*Mentha x piperita*) [4,5]. It has been estimated annual menthol consumption in all forms is upper 7,000 tons with a raw product value nearly US $300 million [5]. it have been defined, Jasmonates including Jasmonic acid (JA) and methyl jasmonate (MeJA) are signaling compounds with many effect on developmental processes of different plants. They have important roles in the physiological and biochemical processes [6-8] which often induce generation of ROS, inclusive H_2O_2, O^{2-} and OH [9]. Increased the rate of ROSs production in the plant cell can cause oxidative damages in cellular components like proteins, chlorophylls and lipids. Plants have antioxidant defense systems including enzymatic and non-enzymatic components, which help to maintain ROS balance within the cell [10]. Lipid peroxidation produces a cytotoxic compound

*Corresponding author: Department of Agriculture, Payame Noor University of Lorestan, Khorramabad P. O. Box 6815139611, Iran
Email Address: Soheila.afkar@gmail.com

called MDA that acts as an indicator of free radical production [11]. MDA content increases when plants are subjected to oxidative stresses. It can be conclude MDA concentration usually is a general indicator of lipid peroxidation as well as the stress level [12]. Anti-oxidative enzymes are SOD, CAT and POD that SOD enzyme coverts superoxide radicals to hydrogen peroxide and dioxygen then H_2O_2 are removed by catalase and peroxidase [9]. Previous studies carried out by Maust *et al*. [13] and Lobato *et al*. [14] showed carbohydrates accumulated under biotic and abiotic stress condition. Decrease in protein content and increase in MDA content in rice were evident 24 h after MeJA treatment [15]. Pre-treatment with MeJA improved the capacity of the antioxidative enzyme system in Barley seedling [16] and total activities of catalase, peroxidase, superoxide dismutase and glutathione reductase increased greatly in *Arabidopsis thaliana* [17]. It has been shown that MeJA is improved the drought tolerance of soybean by modulating the membrane lipid peroxidation and antioxidant activities [18] and exogenous addition of sucrose to MeJA-treated rice leaves increased endogenous sucrose and glucose contents [19].

The aim of the present study was to study the effect of various MeJA concentrations on protein, chlorophyll (a, b and total), carbohydrate, MDA content and antioxidant enzymes (POD, SOD) activity of *M. piperita* at 24 h after treatment.

Material and Methods

Rhizome cuttings with 10 cm-long of peppermint plants were transferred in to pots. The plants treated with different concentrations of MeJA (0, 0.1, 0.5 mM) and after 24 h were evaluated for their total soluble proteins, chlorophylls (a, b, and total), MDA, carbohydrates, and antioxidant enzymes (SOD and POD).

Determination of Lipid Peroxides, Carbohydrates and Chlorophyll Content in Leaf Extract

The MDA content as a level of lipid peroxidation were measured following De vos *et al*. [20]. Frozen leaf samples were homogenized in TCA solution (10% w/v) and the aliquots of filtrates were warmed in 0.25% TBA and then cooled in ice. The absorbance of solution was measured at 532 nm followed by correlation for the nonspecific absorbance at 600 nm. The amount of MDA was determined according to extinction coefficient of MDA. Carbohydrate assay using phenol and sulphuric acid, using glucose (Sigma chemicals) as standard at 490 nm as described by Dubois *et al*. [21].

Chlorophyll content was determined in 80% acetone extract. The samples were centrifuged (20000 g, 20 min) and absorbance is measured by a spectrophotometer at 663 and 645 nm. Total chlorophyll as well as chlorophyll a and b concentrations were calculated according to Arnon [22].

Measurement of Protein Content, POD and SOD Activity

Total soluble proteins were extracted from the leaves by Ausubel *et al*. [23]. Leaf samples were homogenized in a mortar and pestle with ice-cold extraction (50 mM Tris-HCl, pH 7.5; 2 mM EDTA and 0.01% (v/v) 2- mercaptoethanol) and was centrifuged (11952 g, 30 min, 4 °C). Total protein content was determined using bovine serum albumin (BSA) as a standard according to the method described by Lowery *et al*. [24] and Bradford [25].

POD activity was assayed according to Kar and Mishra [26]. The reaction mixture contained 60 mM potassium acetate buffer (pH 6.1), 5 mM H_2O_2, 28 mM guaiacol and 100 µl enzyme extract, the absorbance was measured at 470 nm every 15 sec over one minute using spectrophotometer.

Activity of SOD was measured by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm using Giannopolitis and Ries [27] method. The reaction mixture contained 50 mM phosphate buffer (pH 7.5), 50 mM carbonate sodium (pH 10.2), 0.1 mM Na-EDTA, 1 mM riboflavin, 12 mM L-methionine, 75 mM NBT and 50 µl enzyme extract. One unit of SOD was considered to be the amount of enzyme required to inhibit NBT reduction by 50%.

Statistical Analysis

The completely randomized design with three replications was used for data analyzing. Duncan's multiple range tests were used to compare MeJA treatments. Moreover, correlation coefficients were calculated among all physiological characteristics.

Results and Discussion

Analysis of variance indicated significant variation in all measured traits except chlorophyll b that is caused by different concentration of MeJA (Table 1). At 24 h, activity of antioxidant enzymes (POD, SOD) increased significantly after MeJA treatment. In our study, POD antioxidant enzyme activity was variable in *Mentha piperita* treated with the different concentrations of MeJA. The highest of POD activity was found in the leaves that treated with 0.1 mM, followed by 0.5 mM and control, The activity of POD was strongly increased by the 0.1 mM (nearly 2-fold) compared with the control (Fig. 1D). This result showed, with regard to MeJA concentration the activity of POD antioxidant enzyme is modified in a distinct manner. The SOD activity has been increased in 0.1 and 0.5 mM treated plants when compared to control and there was no statistically significant difference between 0.1 and 0.5 mM MeJA treatment (Fig. 1E). Numerous studies revealed the level of anti-oxidative enzymes was increased when plants are subjected to biotic or a-biotic stresses [28].

There is developed highly efficient antioxidant enzymes defense system like SOD and POD in plants that they scavenge active oxygen species and improved different stress factors tolerance [29,30]. In biological systems, SOD enzyme efficiently converts O^{2-} into H_2O_2 and O_2 then directly modulates the amount of ROS [31,32]. The important enzyme against oxidative stress is POD, that able to scavenge H_2O_2 produced by SOD enzyme [12]. Both SOD and POD are important enzymes associated with anti-oxidative stress in plants. Previous study indicated that foliar applications of MeJA can improve some antioxidants in romaine lettuce [33] and maximum activity of POD, CAT, GR and SOD in MeJA treated *Arabidopsis thaliana* were observed 7 days after MeJA treatment [17] that in agreement with our results. Probably activity of these enzymes is increased by up-regulation of the genes that controlling the synthesis of these enzymes [34].

No significant changes in the amount of Chlorophyll b were found. In contrast to chlorophyll b, Chlorophyll a and total chlorophyll showed significant change in plants pretreated with MeJA. The content of chlorophyll a and total chlorophyll decreased significantly in 0.5 mM MeJA-treated leaves (Fig. 1A, B respectively). This result indicates that 0.5 mM MeJA can responsible for the decline in the plant growth. In the present study, chlorophyll a content increased at 0.1 mM then in higher concentration of MeJA (0.5 mM) remained at a level similar to that of the control. The ratio of chlorophyll a/b was found to be higher in the leaf samples collected from the plants treated with 0.1 mM relative to control. When plants are exposed to biotic or a-biotic stresses, MDA as a final product of peroxidation of membrane lipids or damage to plasma lemma and organelle membranes is accumulates. So as well as the stress level, concentration of MDA is usually considered as a general index of lipid peroxidation [12,35].

Table 1 Mean squares (MS) of ANOVA for physiological characters in *Mentha piperita* L.

S.O.V.	Df	MS										
		Protein	Mannose	Chl a	Chl b	Total Chl	Glucose	MDA	POD	SOD	Xylose	Rhamnose
MeJA	2	2.996**	136.57***	2.533*	0.341^{ns}	12.741**	201.7***	0.261***	411.41***	2.275*	3893.5***	3075.5***
Error	6	0.169	1.78	0.323	0.145	0.452	13.9	0.0011	7.02	0.409	34	27.728

Table 2 Correlation coefficients between physiological characters in *Mentha piperita*

	Chl a	Chl b	Total Chl	MDA	Rhamnose	Glucose	Mannose	Protein	SOD
Chl b	0.402^{ns}	-	-	-	-	-	-	-	-
Total Chl	0.701*	0.692*	-	-	-	-	-	-	-
MDA	-0.444^{ns}	-0.608^{ns}	-0.921***	-	-	-	-	-	-
Rhamnose	-0.167^{ns}	0.487^{ns}	0.534^{ns}	-0.682*	-	-	-	-	-
Xylose	-0.167^{ns}	0.487^{ns}	0.534^{ns}	-0.682*	1***	-	-	-	-
Glucose	0.034^{ns}	0.660^{ns}	0.659^{ns}	-0.774*	0.894**	-	-	-	-
Mannose	-0.132^{ns}	0.5^{ns}	0.555^{ns}	-0.697*	0.998***	0.9**	-	-	-
protein	0.372^{ns}	-0.311^{ns}	-0.349^{ns}	0.547^{ns}	-0.905**	-0.725*	-0.88**	-	-
SOD	0.366^{ns}	-0.261^{ns}	-0.07^{ns}	0.140^{ns}	-0.706*	-0.604^{ns}	-0.685*	0.732*	-
POD	0.757*	-0.017^{ns}	0.318^{ns}	-0.205^{ns}	-0.556^{ns}	-0.318^{ns}	-0.525^{ns}	0.622^{ns}	0.748*

^{ns}, *, ** and ***, Non-significant and significant at 5%, 1% and 0.1% probability levels, respectively

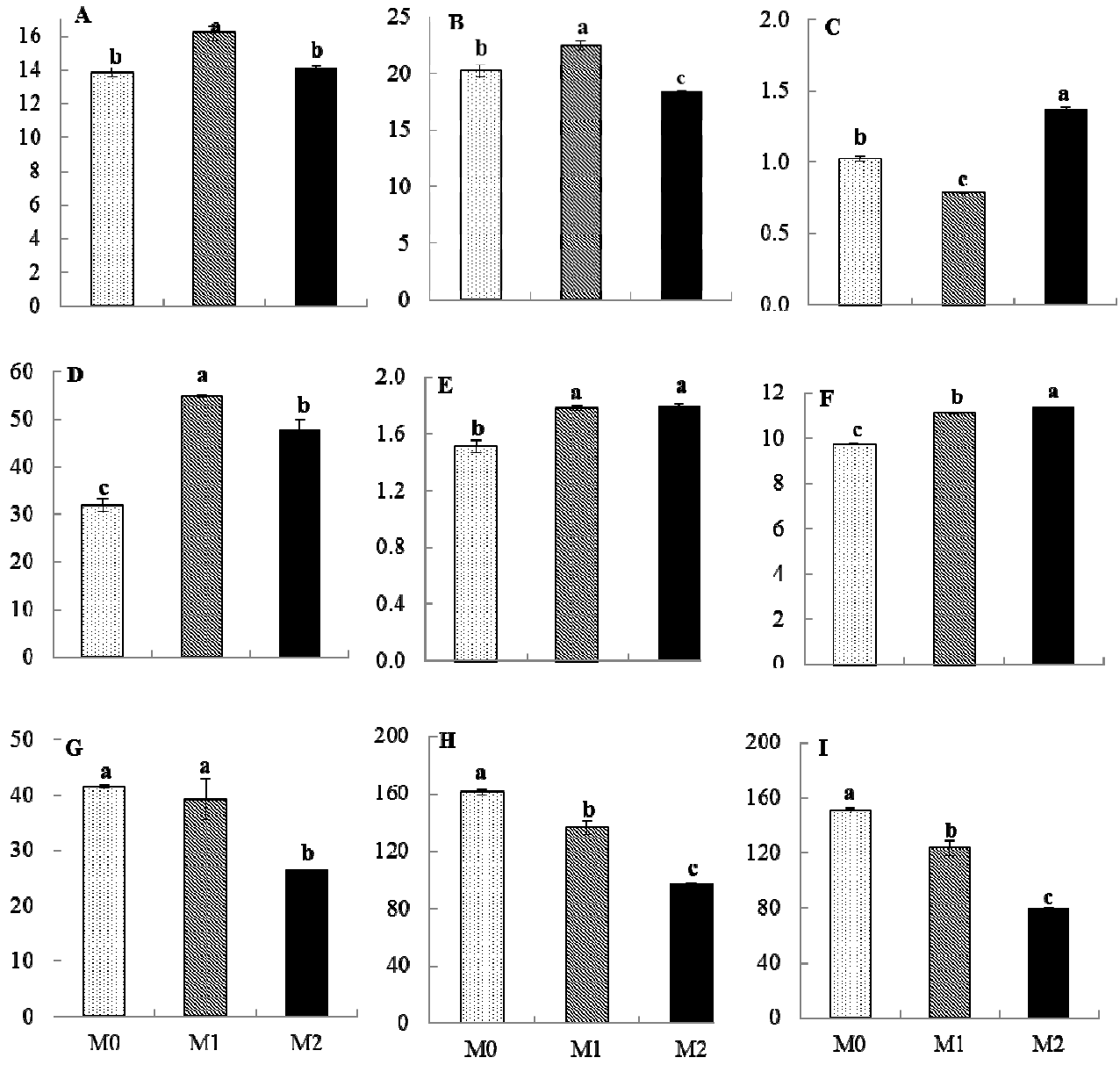

Fig. 1 Effects of methyl jasmonate (MeJA) on A) Chl a (mg g-1 F.W), B) Total Chl (mg g-1 F.W.), C) MDA (mM cm-1), D) POD (Δ A 470 mg-1 protein), E) SOD (mg mg-1 protein), F) protein (mg g-1 F.W), G) Glucose (mg g-1FW) , H) Rhamnose (mg g-1FW) and I)Xylose (mg g-1FW), in *Mentha piperita* L. (M0 = Control, M1 = 0.1 mM, M2 = 0.5 mM)

In this study, MDA content was lowest in plants exposed to 0.1 mM whereas plants that treated with 0.5 mM MeJA exhibited a higher rate of lipid peroxidation (Fig. 1C).

Enhanced the antioxidant enzymes activity in the 0.1 mM MeJA treated plants probably helps to reduce MDA contents. At 24 h after MeJA treatment in rice leaves, MDA content was increased [15] that confirmed this study. The mean comparison of carbohydrates amount in different MeJA concentrations showed that the treatment with 0.5 mM had the lowest amount (Fig. 1G, H, I). Protein content gradually enhanced during MeJA treatment (Fig. 1F). The result of this study is in contrast with the results of others that MeJA caused degradation of protein. It has been shown that physiological and biochemical changes in the organism caused by elicitors as chemicals or biological factors [36], that in agreement with these results. The obtained values of the correlation coefficient between physiological traits indicate that there was a positive relationship between SOD with protein and POD activity ($r= 0.732^{*}$ and $r= 0.748^{*}$ respectively).

A significant negative correlation was determined between MDA and total chlorophyll, Rhamnose, Glucose, Mannose ($r= -0.921^{***}$ and $r= -0.682^{*}$, $r= -0.774^{*}$, $r= -0.697^{*}$ respectively) (Table 2).

Indicating that membrane lipid peroxidation increased with increasing concentrations of methyl jasmonate and growth can be avoided.

In this study, the MDA and POD activity is decreased and increased at 0.1 mM MeJA respectively. Previous study showed with the increase of the activity of antioxidant enzymes e.g. SOD, APX (Ascorbate peroxidase), GPX (Glutathione peroxidase) and CAT, amount of MDA is decreased [35] therefore confirm our result. According to this result, It is probably increased level of POD enzyme activity and decreased content of MDA are linked at 0.1 mM MeJA treatment.

Conclusion

In conclusion we demonstrate that treatment of *Mentha piperita* with MeJA induces the POD and SOD antioxidant enzymes activity and exhibit a protective mechanism against the cellular structures from oxidative damage. These data suggest that activation of plant defenses using elicitors could be a valuable and alternative strategy to protect *Mentha piperita* from stress.

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