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Examining the Effect of Simultaneous Use of Salicylic Acid and Cadmium on the Amount of Protein and Proline in Green Mint Plant (*Mentha Spicata*)

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Abstract: Plants are faced with multiple stresses in everywhere that they grow and these stresses limit their chance of development and survival. One of the major environmental stresses that are considered as a serious risk to humans, animals and especially plants is the stress resulted from the heavy metals. Cadmium (Cd) is one of the highly toxic and dangerous heavy metals. On the other hand, salicylic acid (SA) or ortho-hydroxy benzoic acid is one of the phenolic combinations of the plant that possess a fundamental role in the regulation of some of physiological processes of the plant. In this study the interactive influence of salicylic acid and cadmium on the amount of protein and proline in green mint plant, (Mentha spicata) which has a very high medicinal value, was investigated. In the growth room the plants were implanted under controlled conditions of temperature of 28.18 (day. night) and a photoperiod of 16 hours day and 8 hours night and the humidity of 50-65 percent and before applying the treatments, they were irrigated with nutrient solutions of Long-shtone and distilled water, then various concentrations of salicylate (0, 0.2, 0.5, 0.9, 1.5) Mm, were sprayed 2 times on the leaves of the relevant plants and then they were treated with cadmium chloride (0 ،50 ،100 ،250 ،500) micro-molar. The results showed that protein content of studied leaves was preserved at levels of 0, 0.2 and 0.5 Mm salicylic acid and various concentrations of cadmium. While the lowest protein content of total leaves of the plant is at a concentration of 1.5 Mm of salicylic acid and different concentrations of cadmium. Also, in this study, high levels of proline is at a concentration of 0.9 Mm of salicylic acid and different levels of cadmium and following this concentration, there was a reduce in the amount of proline in the studied plants as compared to the control samples.

Keywords: Salicylic Acid, Cadmium, Mint, Protein, Proline

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Introduction

Salicylic acid (SA) or ortho-hydroxy benzoic acid and related combinations also belong to a group of phenolic compounds (Popova, et al., 2003). Generally, salicylic acid affects the seed germination, photosynthesis apparatus, plant growth and plasma membrane. Also the fruit ripening, stimulation of flowering and response to stresses are among other physiological functions of salicylic acid (SA) (Coup, SA, et al. 2006). Most of the studies conducted about the role of salicylic acid in plants, are about the effect of this compound in improve or relieve of the effects caused by related stresses. For example, salicylic acid (SA) regulates expansion, division and cell death; it means that it creates a balance between growth and aging (Senaranta, et al. 2002). In most studies, it has been stated that the most important action of salicylic acid is the response and resistance against certain stresses such as heavy metals (Choudhury, Panda, SK 2004 & Pàl, et al. 2002), the heat (Dat, et al.1998), the cold (Tasgin, et al. 2003), dryness (Senaranta, et al. 2002), salinity (El-Tayeb, MA, 2005) and plant diseases (Davis, 2005) and it has been shown that, salicylic acid to a large extent reduces problems caused by these stresses. On the other hand, cadmium (Cd) is one of the heavy metals with a density of 8.6 g. cm^{-3}, which is observed with an approximate concentration of 0.04 micro-molar in non-contaminated soils and with 35 micro-molar in contaminated soils (Sanita & Gabbreielli, 1998). This element is accumulated through different activities such as metal industrial activities, industrial waste, energy production bases and production of phosphate fertilizers in the soil (Prasad, 1995; Vassilev and Yordanov, 1997). It is not only inessential for biological activities, but also it is considered as a toxic for most organisms and the amount of this toxicity is 2 to 20 times greater than other heavy metals such as copper, zinc, nickel and silver (Vassilev & Yordanov, 1997). The studies conducted in Australia have shown that the reason of increase of Cadmium in soils of the country is the use of phosphorus and zinc fertilizers and the use of other mineral fertilizers (Senn & Milham, 2000).

Unnecessary elements such as cadmium are possibly captured by the plant roots through channels or carriers of minerals that do not act completely selective. In animals, the voltage-dependent calcium channels are involved in the absorption of cadmium ions (Clemens, S., Et al. 1999). The toxicity of heavy metals in different plants is varied commensurate with factors such as the availability of metals in soils, metal uptake by plants and the amount of its displacement in plant parts. This toxicity occurs when the related metal can enter to system of plant root from the soil (Prasad & Strzatka, 2002). Usually soils contaminated with cadmium possess other polluting elements such as lead and thus further threaten the health of organisms (Alloway et al., 2004). It has been reported that the delay in the growth of plants is one of the symptoms of toxicity with cadmium (Lee, et al., 2003; Schutzendubel, A. et al., 2001). Studies have shown that cadmium affects the cell division and growth, the overall growth of plant, cell division in meristematic region and the regulation of the growth and development of plants (Das, et al. 1997). It has been reported that the negative effects of cadmium on plant growth is along with an increase in the ratio of dry weight to wet weight in all organs (Vassilev, A. & Yordanov, I. 1997). Also cadmium causes chlorosis and necrosis in leaves (Zhang et al., 2002). Studies of many researchers have shown that cadmium decreases the total chlorophyll, chlorophyll a, chlorophyll b and the carotenoids in higher plants (Sanita di Toppi, L. & Gabbrielli, R. 1999). This dangerous toxic element can disrupt the metabolism of carbohydrates (Gouia, H. et al., 2001). To confront osmotic stress caused by heavy metals, plants employ different adaptive mechanisms. A group of plants that have higher resistance, in order to maintain their osmotic balance, increase the synthesis of a series of protective metabolites of osmotic such as regenerative carbohydrates and proline (Orcutt & Nelsen, 2000; Choudhan, 2006). It has been reported that the amount of total protein in plant leaves decreases with the aging of plant and the stress level of cadmium (Khudsar T. et al., 2000).

Materials and methods

The plant tested in this study is the green mint plant (Mentha spicata L.), which belongs to the family of Labiatae. Several names have been mentioned for Mentha spicata L in various sources such as:

Silver Mint, Lamb Mint, Garden Mint, Green Mint, and Spear Mint and….

Green mint is one of the medicinal plants and due to the production of useful and relatively abundant essence it

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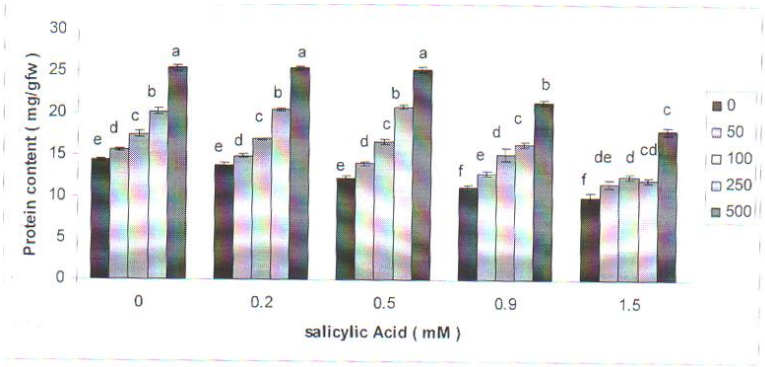
is of great economic importance. After washing with tap water and distilled water, the rhizomes of mint plant are transferred into pots containing vermiculite with 14 cm diameter (vermiculite is a kind of crushed stone and it is of the type of micas with crystals of metallic luster that its ion exchange capacity is very low in distilled water and it changes Ion concentration and pH of the used nutrient solutions lesser) (In each pot, 3 rhizomes



with the length of 4 cm were placed.)

In the growth room the plants were implanted under controlled conditions of temperature of 28.18 (day . night) and a photoperiod of 16 hours day and 8 hours night and the humidity of 50-65 percent and before applying the treatments, they were irrigated with nutrient solutions of Long-shtone and distilled water, then various concentrations of salicylate (0, 0.2, 0.5, 0.9, 1.5) Mm, were sprayed 2 times on the leaves of the relevant plants and after 6 days they were treated "three times per week "with cadmium chloride (0 ،50 ،100 ، 250 ،500) micro-molar.

Measuring the amount of proline

To measure proline, Batis et al method was used. To perform it, initially, the required reagent was prepared (derived from: Choudhary et al., 2006).

Preparation of ninhydrin reagent

1.25 grams of ninhydrin is added to 30 ml of acetic acid and the resulted solution is warmed to dissolve ninhydrin in acid. Then 20 ml of 6 molars phosphoric acid is added to the solution. The resulting solution is kept in a refrigerator at 4 ° C for 24 hours.

Measuring method of proline

0.05 grams of fresh tissue is grinded in 10 ml of 3% Sulfosalicylic acid and a smooth mixture was resulted. The resulting extract was centrifuged for 5 minutes at 10,000. Then 2 ml of zinc fluid was mixed with 2 mg of ninhydrin reagent and 2 ml of pure acetic acid and it was kept for one hour at 100 ° C in warm water bath. In order to stop the reaction, the tube containing the mixture was cooled in an ice bath. Then 4 ml of toluene was added to the mixture, and the tubes were shaken well. By keeping the tubes fixed for 15-20 minutes, two distinct layers were formed in it. The upper colored layer, containing toluene and proline, was used to measure proline concentration. Absorption of a certain amount of the dye was determined at a wavelength of 520 nm and proline content of each sample was determined by using the standard curve. The results of measuring the amount of proline was calculated and presented per gram wet weight.

Measurement of total protein content by the Bradford method

Initially, proteins were extracted at 0-4°C from plant leaves.

For this purpose, 1 g of wet tissue was completely grinded in a porcelain mortar containing 3 ml of Tris-sucrose buffer with pH=7.5. The resulted homogeneous solution was transferred into centrifuge tubes, and after 10 minutes of inactivity, it was centrifuged for 25 min at 10000. At the end of the centrifuge, the tubes were gently removed from the device and the zinc solutions of extracts were used to measure protein concentration. In order to measure the concentration of protein 1.0 ml of protein extract and 5 ml biuret reagent were added to test tubes and they were immediately vortexed. After 2 minutes and before 1 hour, absorbance was read with a spectrophotometer in wavelength of 595 nm (Bradford, 1976).

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Preparation of biuret reagent

In order to prepare the biuret reagent, 0.1 grams of Coomassie Brilliant blue 250 G was dissolved in 50 ml of 95% ethanol within one hour. Then 100 ml of 85% phosphoric acid was added to it drop by drop. At the end total volume of the solution was brought to one liter by using distilled water, the solution was filtered through Whatman No. 1 filter paper.

Statistical analysis: The study was conducted as a factorial experiment in a completely randomized design. Data analysis was performed using SPSS statistical software and variance analysis of data was performed. The Excel software was used to draw graphs.

Results

Protein content of the leaves of studied plant was preserved at levels 0, 0.2 and 0.5 Mm of salicylic acid and various concentrations of cadmium, while, the lowest total protein concentration was observed at the level of 1.5 Mm salicylic acid and different levels of cadmium (Figure 1). The highest levels of proline in this study, was observed at the level of 0.9 Mm salicylic acid and different levels of cadmium and after this concentration, reduce was observed in the amount of the proline of the studied plant (Figure 2).

Figure 1. Mean changes in total protein content with changes in amounts of cadmium and salicylic acid

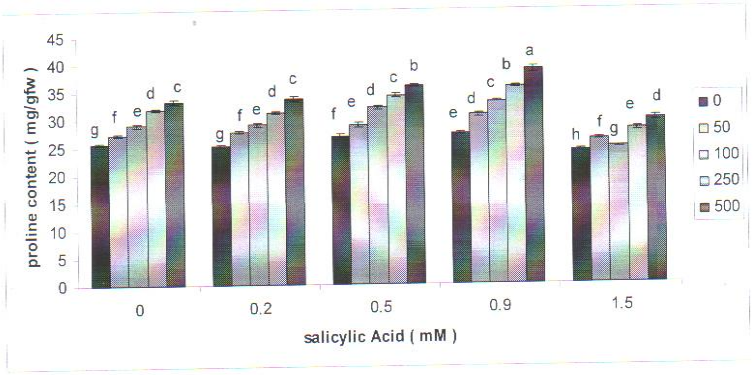

Figure 2. Mean changes in proline content with changes in amounts of cadmium and salicylic acid

Discussion and conclusions

Change in environmental conditions and creation of some environmental stresses such as salinity, drought, and the presence of heavy metals alter the osmotic conditions of plant. There are many reports regarding that the heavy metals cause defragmentation in osmotic balance of plant (Besora and Besora, 1379). It has been reported that the total protein content in plant leaves decreases with the aging of plant and the stress level of cadmium (Khudsar et al., 2000). On the other hand, salicylic acid effects on most of reactions of plant metabolism and causes some changes in them. These changes are often in form of compromises that increase the level of tolerance and adaptation of plants against environmental factors (Metwally et al., 2003). It has been said that the accumulation of osmotics (proline and sugars) has a direct and positive relationship with increasing resistance in plants exposed to abiotic stresses (Ramanjulum et al., 1998). In this study, the total protein content of the plant was preserved at concentrations less than 1.5 Mm salicylic acid and various concentrations of cadmium, but at a concentration of 1.5 Mm, salicylic acid is unable to relieve the stress caused by cadmium, as a result, total protein content of plant was decreased. Also the measured proline content in this study, showed the highest increase at concentration of 0.9Mm salicylic acid in combination with various concentrations of cadmium. It has been said that proline accumulation is observed under water deficit, high salinity, cold, heat and exposure to heavy metals. Proline accumulation which has been accepted as an indicator in environmental stresses has an important protective role against these stresses. Proline plays an important role in osmotic adjustment and osmotic tolerance of plants. Also it shows noticeable and significant protective role against heavy metal stresses. Thus, free proline in plants is one of the indicators of environmental stress (Sanita di Toppi & Gabrielli, 1999).

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References

Alloway, B. J. 2004. Zinc in Soils and croup nutrition. Review In: International Zinc Association, 128.

Avancini, G., Abreu, I. N., Saldana, M. D. A., Mohamed, R. S. and Mazzafera, P., 2003. Induction of pilocarpine formation in jaborandi leaves by salicylic acid and methyljasmonate. Phytochemistry, 63: 171-175.

Bradford, M. M. 1976. Annual. Biochem. 72: 248-254.

Choudhury, R. P., Kumar, A. and Gary, A. N. 2006. Analysis of Indian mint (*Mentha spicata*) for essential trace and toxic elements and its antioxidant behavior. Pharmaceutical and Biochemical Analysis.

Clemens, S., Antosiewicz, D. M., Schachtman, D. P. & Schroeder. J. I. 1999. The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. Plant Biology. 95(20): 12043-12048.

Das, P., Samantaray, S. & Rout, G. R. 1997. Studies of Cadmium toxicity in plants review. Environmental Pollution. 98(1): 20-36.

Dat. J. F. Foyer, C. H. and Scott, I. M., 1998. Changes in salicylic acid and antioxidants during induced thermotolerance in Mustard seedlings. Plant Physiology. 118; 1455-1461.

Davis, P. J. 2005. Plant Hormones Biosynthesis, signal transduction, action!. Springer. Chapter F2.

El-Tayeb, M. A., 2005. Response of Barley Gains to the interactive effect of salinity and salicylic acid. Plant Growth Regulation. 45: 215-225.

Gouia, H., Ghorbal, M. H. & Meyer, C. 2001. Effect of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. Plant Physiology. 38: 629-638.

Khudsar, T., Mahmooduzzafar & Iqbal, M. 2001. Cadmium induced changes in leaf epidermis, photosynthetic rate and pigment concentrations in Cajanus cajan. Biologia plantarum. 44(1): 59-64.

Lee, J., Bae, H., Jeong, J., Lee, J. Y., Yang, Y. Y. & Hwang, I. 2003. Functional expression of a bacterial heavy metal transporter in Arabidopsis enhances resistance and decrease uptake of heavy metals. Plant Physiology. 133: 589-596.

Metwally, A., Finkemeier, I., Georgi, M. and Dietz, K-J., 2003. Salicylic acid alleviates the Cadmium toxicity in Barley seedings. Physiology and Biochemistry of Plant. 132:272-281.

Orcutt, D. M. and Nelsen, E. T. 2000. Physiology of plant under stress, soil and biotic factors. Published Simultaneously in Canada, 481-518.

Pal, M., Szalai, Z. Horvath, E., Janda, T. and Paldi, E., 2002. Effect of salicylic acid during heavy metal stress. Acta Biologica Szegediensis. 46(3-4: 119-120).

Popova, L., Ananieva, V., Hristova, V.,Christov, K.,Georgieva, K., Alexieva, V. and Stoinova, Zh. 2003. Salicylic acid and Methyljasmonate induced protection on photosynthesis to paraquat oxidative stress. Bulg.J.Plant Physiol., special issue. 133-152.

Prasad, M. N. V. and Strzatka, K. 2002. (Eds.). Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Kluwer Academic Publishers, Dordrecht, p. 432.

Prasad, M. N. V. 1995. Cadmium toxicity and tolerance in vascular plants. Environmental and Experimental Botany. 33(4): 525-545.

Ramanjulum, S., Sreenivasulu, N. and Sudhakar, C. 1998. Effect of water stress on photosynthesis in two mulberry genotypes with different drought tolerance. Photosythetica. 35(2): 279-283.

Sanita di Toppi, L., Gabrielli, R. 1999. Review, Response to cadmium in higher plants. Environmental and Experimental Botany. 41: 105-130.

Schutzendubel, A., Nikolava, P., Rudolf, C. & Polle, A. 2002. Cadmium and H_2O_2 induced oxidative stress in populus canescens roots. Plant Physiology and Biochemistry. 127(3): 887-897.

Senaranta, T., Touchell D., Bum ME, Dixon K. 2002. Acetylsalicylic (aspirin) and salicylic acid induced multiple stress tolerance in bean and tomato plants. Plant Growth Regulation, 30: 157-161.

Tasgin E., Atici, O. and Nalbantoglu, B. 2003. Effect of salicylic acid and cold on freezing tolerance in winter wheat leaves. Plant Growth Regulation. 41: 231-236.

Vassilev, A. and Yordanov, I., 1997. Reductive analysis of factors limiting growth of Cadmium-treated plants: A review, BULG. J. Plant Physiology, 23(3): 114-133.

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