The Interaction of Cadmium and Salicylic Acid on Some Pigments of Mentha Spicata L

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Abstract: Because of the high toxicity of cadmium and the importance of Spearmint (Mentha spicata L.) in many cases such as use of this plant in medicinal, food and economical industries, we studied the interaction of different concentrations of cadmium (Cd) and salicylic acid (SA) on some of Mentha spicata's pigments. Spearmint plants were grown in a controlled environment and before the treatments, the plants watering with Long-shtone solution. Then, different concentrations of salicylic acid (0, 0.2, 0.5, 0.9, 1.5mM) spraied on the leave of plant and treated by cadmium chloride (0, 50, 100, 250, 500µM) for six days. The results indicate that the interaction that of 1.5 mM SA and 500µM Cd decreased the amount of chlorophyll a, chlorophyll b and total chlorophyll compare to the control. Our investigation show that the anthocyanin content was at 500 µM Cd and also the increasing of arotenoids content was on the concentration of 0.5mM SA and the different concentrations of Cd together. In addition, the accumulation of Cd in Spearmint roots were greater than shoots, indicating significant mental immobilization by the roots. Data suggest that the activity of SA may decrease the toxicity of Cd in Spearmint plants.

Keywords: Mentha Spicata, Cadmium, Salicylic Acid, Chlorophyll, Anthocyanin

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Introduction

Cadmium is one of the important environmental pollutant heavy metals with high toxicity for plants and animals that releases mostly from phosphate fertilizers (Sanitadi Toppi, L. & Gabrielli, R. 1999). On the other hand,salicylic acid is a phenolic compound that regulates some of the plant processes such as defense responses to pathogen's attacks and stresses (Prasad,1995; Vassilev and Yardanov,1997). Furthermore, evidences show the role of salicylic acid on responses regulation of some abiotic stresses such as heat and cold (Papova, L. et al., 2003). The most amount of salicylic acid was reported in pathogen's attacking that create necrosis in plants (Raskin, I. 1992). The amount of heavy metal toxicity in different plants change by some factors such as the existing of metal in the soil, the amount of metal absorbance by plant and its transition in the plant's organs. This toxicity occurs when the relevant metal can enter the root system from the soil (Prasad & Strzatka, 2002). Usually, the pollutant soils by Cd have the other pollutant elements such as Pb,thus, this factor threats living beings health(Alloway, et al.,2004).

According to the other researches, Cd decreases the amount of photosynthesis and transpiration and also increasing the amount of respiration. On the other hand, different species of plants and agricultural variety have diverse capability for absorption, keeping, accumulation and tolerance to Cd. Cadmium entrance to the roots and it's transition to the shoot is the principal mechanism that different species of plants use for accumulating and tolerance of Cd (Clements, S. et al., 1999). Probably, the non-essential element like Cd enters to the plant by channels or inorganic matter transporters that act non-selective (Clements, S. et al., 1999).

Nigam and Strivastava (2001) reported that metal chellats formation or complexes formation in the soil can cause simple absorption of the soil Cd and its transition in the plant. Also, it is improved that the existence of some organic acids and amino acids on the surrounding of the root can effect on the amount of cadmium absorption. The interaction of cadmium with organic ligands causes the formation of mobile organic compounds that connect to cadmium. Finally, the formation of these complexes cause the greatest absorption of Cd by the root (Nigam, R. & Strivastava, M. N.2001).

On the other hand, salicylic acid supports from photosynthetic system via the increasing of ability of cell antioxidants and also synthesis of new proteins (Avacini, G. et al., 2003). In the most researches, the most important role of salicylic acid is explained for resistance to some stresses such as heavy metals (Choudhury, S., Panda, S. K. 2004 & Pal, M. et al.,2002), heat (Dat, J.F. et al., 1998), cold (Tasgin, E. et al., 2003), salinity (El-Tayeb, M.A. 2005) and plant diseases (Davis, P.J. 2005) and it is indicated that SA decreases the problems that are resulting from these stresses. Therefore, because of the entrance of heavy metals like Cd to the plants life circle and the other living beings and also the increase of environmental pollution, we decided to use salicylic acid for decreasing the toxicity of cadmium in *Mentha spicata L.*

Material and methods

Plant material

In this research, rhizomes of *Mentha spicata* washed with strilled water and then sown in pots containing vermiculite and allowed to grow with photoperiod 28/18 (day/night) and 50-65 percent humidity. Before the treatments, the plants watering with Long-shtone solution. The pH of the nutrient solution was adjusted to 5.7. Then different concentrations of salicylic acid (0, 0.2, 0.5, 0.9, 1.5 mM) spraied on the leave of plants twice a week and after six days the plants treated with $CdCl_2$ (0, 50, 100, 250, 500 µM) three times a week. At the weekend, the pots were washed with water or remove deleterious substances from the vermiculite as well as from the roots surfaces.

All determinations were carried out in triplicate and data were tasted for significance.

Pigment determination

Chlorophyll pigments and carotenoids were extracted in 80% aqueous acetone (v/v), using four 1cm^2 fresh leaf discs taken from the distal ends of an upper mature leaf and were extracted in 10 ml glass

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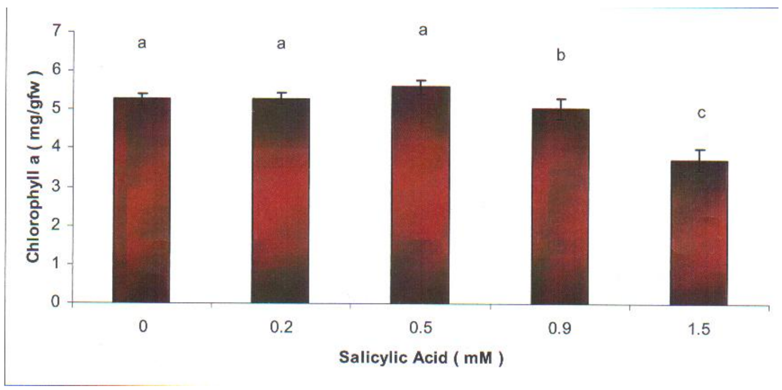
centrifuge tubes containing 8 ml of acetone. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content were determined by measuring the absorbance of chlorophylls at 646.8, 663.2 and 470 nm and then using equations described by Lichtenthaler (1987).

$Chla = (12.25\,A_{663.2} - 2.79\,A_{646.8})$
$Chlb = (21.21\,A_{646.8} - 5.1\,A_{663.2})$
$ChlT = Chla + chlb$
$Car = \left(\frac{1000A\,470 - 1.8\,Chla - 85.02\,Chlb}{198}\right)$



Anthocyanin determination

To determine the concentration of anthocyanines, four 1 cm^2 fresh leaf discs were taken from the distal ends of an upper mature leaf as above and were extracted in 15 ml glass centrifuge tubes containing 5 ml of acidified methanol (methanol : HCl, 99: 1, v:v) and kept at night in the dark for 24 hours.

Metal determination

For measuring of cadmium ions, we used atomic absorption method.For this, a sup sample (0.5 g) was dried at 70°c for 72 hours, and then 10 ml concentrated nitric acid added to the residue and the samples kept in the laboratory temperature for 24 hours for better solving of plant texture. After 24 hours, the mixture was heated to reduce to 5ml volume. The solution was then brought to 20ml volume with distilled water and then filtered by Wattman ≠1. Total concentrations of Cd were analyzed by Shimadzu (model 610, Japan).

Results

In this report, we examined the interaction of SA and Cd on chlorophylls, anthocyanins, carotenoids contents and the accumulation of Cd in roots and shoots of *Mentha spicata L.*

The results indicate that SA sometimes decreased the amount of chlorophyll a and other times increased it (figure 1). The amount of chlorophyll a, chlorophyll b and carotenoids decreased in the plants that are treated by Cd. The reason for this is that the interference of pigments metabolism with heavy metals cause the inhibition of chlorophyll biosynthesis on the level of protochlorophyllide reductase enzyme (Sposito, G., 1985).

On the other hand, with increasing of cadmium, a greatly decrease observed on the content of chlorophyll a in the plant's leave (figure 2). However, the interaction of 0.5mM SA and the different concentrations of Cd showed the amount of chlorophyll b (figure 3) and total chlorophyll (figure 4) increased. As the increasing of Cd concentration, the amount of anthocyanin significantly increase in the *Mentha spicata*'s leave, so that above parameter increased greatly on the concentration of 500 µM Cd (figure 5). The increasing of carotenoids content is on the concentration of 0.5 mM SA and the different concentrations of Cd together (figure 6). In addition, increasing Cadmium concentration showed Cd accumulations in roots greater than shoots (figure 7, 8, 9).

It's necessary to mention that by increasing of Cd concentration, the absorbance and accumulation of this element in the plant's root showed a significant increase.

Discussion

One of the environmental stresses sign is the decrease of photosynthetic pigments that is also dependent

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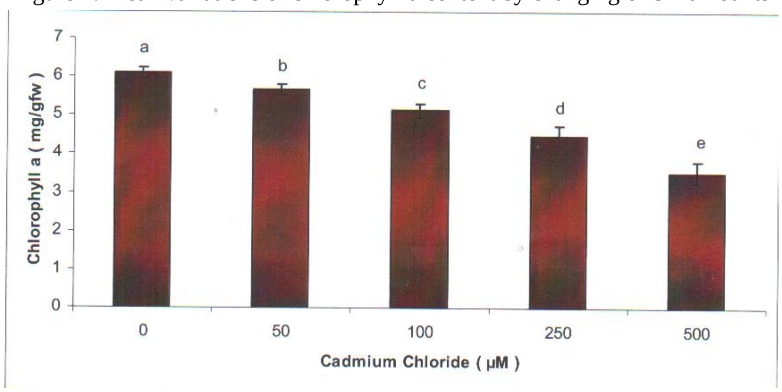
upon plant's genotype (Colom, M.R. & Vazzana, C.2001).

When stress happens, salicylic acid supports from photosynthetic apparatus by the increasing of cell antioxidants and new protein synthesis (Avacini, G. et al., 2003). On the other hand, there are numerous reports about the decrease of photosynthetic pigments synthesis by Cd which is sometimes related to the inhabiting effect of Cd on Fe and Mn absorbtion and also the inhibition of sulphidril groups by cadmium (Khudsar, 2001 ; Prasad, 2002 ;Azevedo et al., 2005). It is improved that intensive effects of Cd on photosynthesis is in several ways and one of those ways is the deficiency in the chlorophyll biosynthesis (Haag-Kerwer, A. et al., 1999).Thus, damaging of Fe and Mg pools in leave is the reason for the above effects (Greger & Landberg, 1999). In addition, Cd causes the lack of Fe in some plants (Iqbal and Khudsar, 2000).Therefore, chlorophyll content maybe decrease due to decreasing of Fe absorbtion by the roots. On the other hand, Spearmint plants increase the amount of carotenoids to reduce the damage that is caused Cd stress to protect photosynthetic system from oxygen radicles (Vartika Rai et al., 2005).

Figure 1. Mean variations of chlorophyll a content by changing of SA amounts



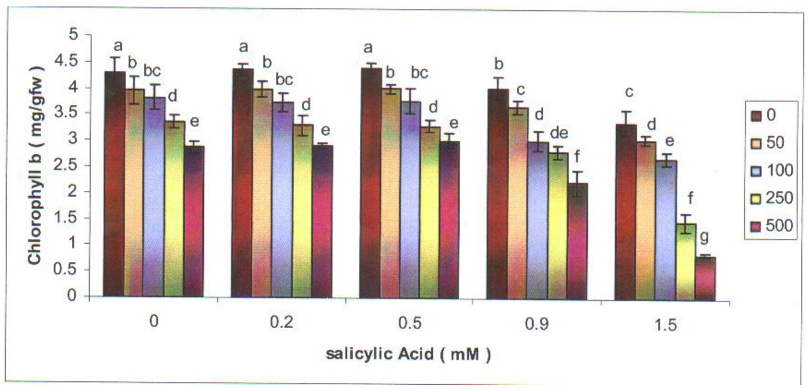
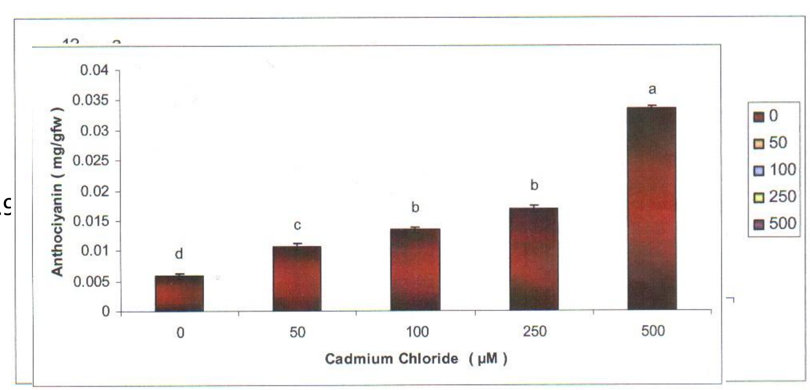


Figure 2. Mean variations of chlorophyll a content by changing of Cd amounts

Figure 3. Mean variations of chlorophyll b content by changing of SA and Cd amounts

Figure 4. Mean variations of total chlorophyll content by changing of SA and Cd amounts

Figure 5. Mean variations of anthociyanin content by changing of Cd amounts.



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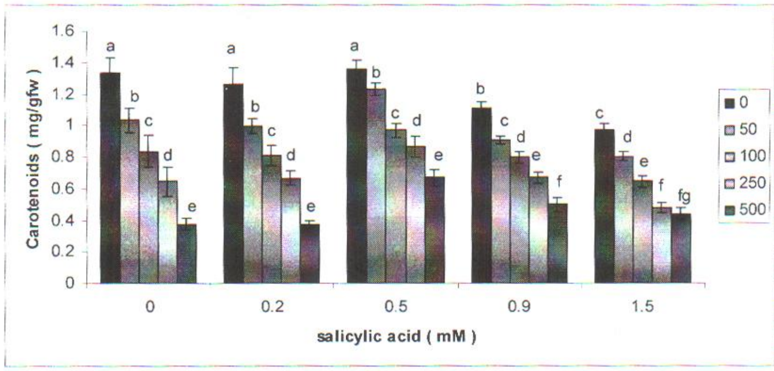


Figure 6. Mean variations of carotenoids content by changing of SA and Cd amounts

In addition, NADH production in vivo can be inhibited by Cd^{+2} through reducing

The rate of photosynthesis and respiration (Bazzaz & Govindjee, 1974). Furthermore, it might be possible that cadmium stimulates NADH oxidation (Bittle et al., 1974). There are many reports that show cadmium can distribute in the plant tissues (roots, shoots and fruits) and 70-80 percent of Cd remain in the roots and only a little amount of it transfer to the other organs(Nigam, R., Srivastava, M.N. 2001).

The results of this study indicate that the most accumulation of absorbed cadmium is in the plant's roots(figure 8).

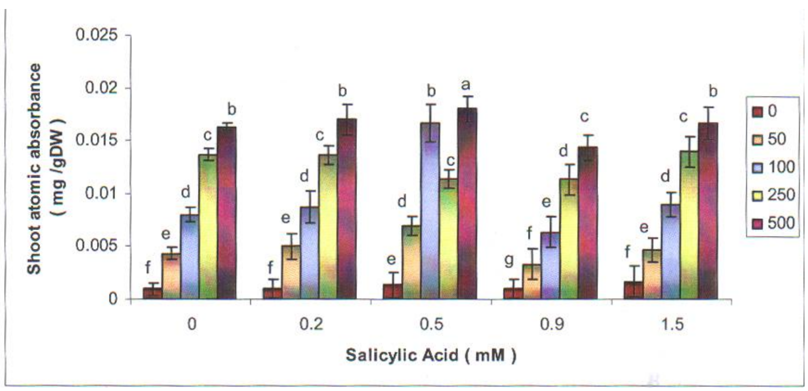
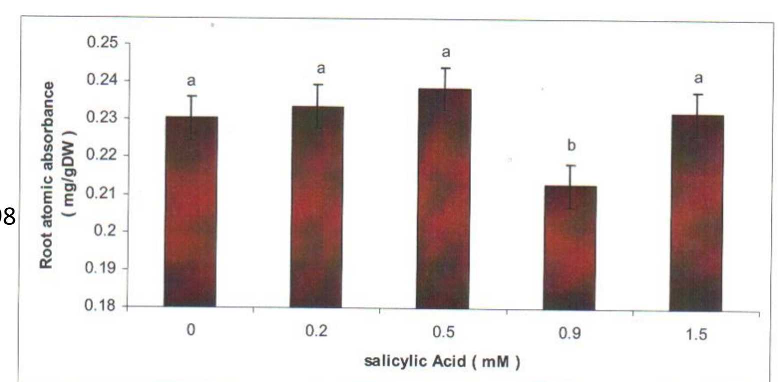


Figure 7. Mean variations of Cd absorption and accumulation in the plant's shoots by changing of SA and Cd amounts

Figure 8. Mean variations of Cd absorption and accumulation in the plant's roots by changing of SA amounts.



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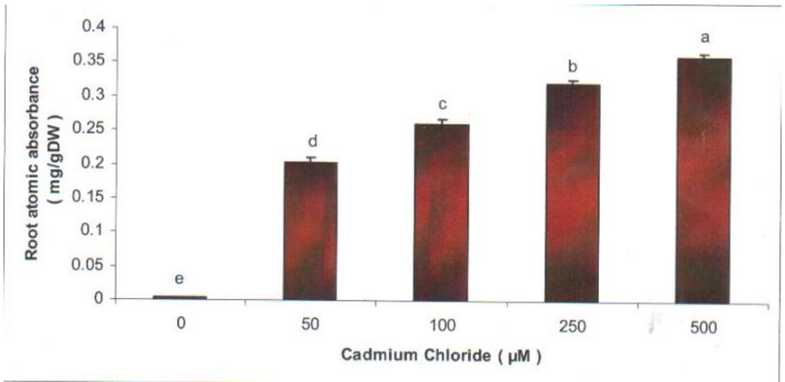

Figure 9. Mean variations of Cd absorption and accumulation in the plant's roots by changing of Cd amounts

Conclusion

Our study reveals some information about the interaction of Salicylic acid and Cadmium on the pigments and cadmium concentration in roots and shoots of *Mentha spicata*. On the whole, different responses occur to oxidative stress that induced by Cd in plants. Oxidative stress is one of the factors that effects on the growth and function of plants. In our investigation, SA can decrease the dangerous effects of Cd on *Mentha spicata*. In the concentrations of lower than SA=1.5mM and the different concentrations of Cd chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content increase because of SA application that indicates the modification and alleviation of stress by SA. On the other hand, the increasing of anthocyanines indicate the ability of *Mentha spicata* for inducing of response against the stresses.On the whole, further investigations are required to understand the interaction of SA and Cd in the plant metabolism.

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