Malays. Appl. Biol. (2012) 41(2): 25–30

INTERACTION EFFECT OF CADMIUM AND SALICYLIC ACID ON PROLINE AND ANTIOXIDANT ENZYME ACTIVITY IN SOYBEAN

MAREFAT, E., SHABANI, L.* and AMOOAGHAIE, R.

Department of Biology, Faculty of Science, Shahrekord University, Shahrekord, I.R.IRAN
**Email: lshabani@gmail.com, shabani-l@sci.sku.ac.ir*
Tel: +981 3814424405; fax: +981 3814424419

ABSTRACT

Possible mediatory role of salicylic acid in protecting soybean (*Glycine max* L.) plantlets against cadmium toxicity was investigated. Changes in growth, proline and soluble carbohydrates contents, lipid peroxidation and activities of H_2O_2 scavenging enzymes in salicylic acid (0.5 mM) treated plantlets of soybean in Hoagland's solution containing 200 µM $CdCl_2$ were monitored. Results showed that fresh and dry weight of shoots and roots were reduced while proline and soluble carbohydrates contents and lipid peroxidation in leaves of soybean treated with cadmium were significantly increased compared to controls. Guaiacol peroxidase (1.06±0.05, 1.65±0.22), ascorbate peroxidase (42.16±1.09, 57.2±1.68) and catalase (1729.66±20.13, 4345±230.5) activities were significantly enhanced for (-Cd-SA) and (+Cd-SA) treatments respectively. Lipid peroxidation and proline content were lowered by salicylic acid treatment, while soluble carbohydrates were increased. The activities of guaiacol peroxidase, ascorbate peroxidase and catalase were also lesser in leaves of salicylic acid-treated plants. Salicylic acid alleviated cadmium toxicity not only at the level of antioxidant defense, but also by affecting other mechanisms of detoxification.

Key words: H_2O_2 scavenging enzymes, Lipid Peroxidation, Salicylic acid, Soluble Carbohydrates

INTRODUCTION

Heavy metals are important pollutants. Certain heavy metals such as zinc (Zn), nickel (Ni) and copper (Cu) are constituent parts of important enzymes and pigments; they are essential elements and their toxicity appears only in concentrations higher than the physiological requirement of plants. Other heavy metals such as tin (Sn), lead (Pb) and cadmium (Cd), are non-essential metals, and become toxic for plants in low concentrations. Concentrations of both essential and non-essential heavy metals are controlled by plants (Babula *et al*., 2008). Cd is one of the non-essential heavy metals, and an environmental pollutant and because of high mobility in low concentrations, enters the food chain easily. Like other heavy metals, Cd reduces growth in aerial and underground parts of plants, including reduction in size and biomass, leaf necrosis, leaf epinastic, browning of the root tip and swelling of the root cells (Guillermo *et al*., 2007). Cd forms complex with proteins, substitutes essential metal ions to plasma membrane, and makes changes in anti-oxidative system, induces free radicals formation and reactive oxygen species (ROS) and thus the oxidative stress is increased by Cd. One of the prominent effects of these ROS is the peroxidation of lipids and loss of membrane integrity (Guillermo *et al*., 2007; Guo *et al*., 2007).

Salicylic acid (SA) is an endogenous signaling molecule which is involved in specific responses to biotic and abiotic stresses, including heavy metals toxicity (El-Tayeb *et al*., 2006). Salicylic acid causes both adjustment of reactive oxygen species and antioxidants, and induction of gene expression (Qinghua & Zhujun, 2008; Murtaza *et al*., 2010). Salicylic acid signaling pathway is associated with increased hydrogen peroxide, therefore, pretreatment with SA may increase oxidative levels caused by stresses like heavy metals. In other words, SA may play an important role in regulation of induced-oxidant levels caused by biotic and abiotic stresses, leading to higher tolerance of plants to the stress (Guo *et al*., 2007; Wu *et al*., 2008). Although the mechanisms of cytoplasmic toxicity were similar in all organisms,

* To whom correspondence should be addressed.

different plant species and varieties showed a wide range of plasticity in Cd tolerance, from high degrees of sensitivity (most plants) to certain hyperaccumulating phenotypes of tolerant higher plant (McGrath *et al*., 2001).

Soybean (*Glycine max* L.) is an important crop, containing useful proteins, oligosaccharides, edible fiber, isoflavones, and minerals (Mateos-Aparicio *et al*., 2008). It was reported as a relatively tolerant plant to Cd; some cultivars could grow in 500 mM Cd (Keramat *et al*., 2009). The toxic effects of Cd on soybean growth have been studied; but little is known on how to reduce damages caused by toxicity of Cd in soybean. In this study, we investigated the role of SA against Cd-induced oxidative stress in soybean plants.

MATERIALS AND METHODS

1.1. Plant growth conditions

Seeds of hybrid cultivar (Williams×Colombus), commonly cultivated in Iran, were obtained from Agricultural Researches Center, Shahrekord, Iran. Effect of various concentrations (25, 50, 100, 200, 400 µM) of $CdCl_2$ on germination and post-germination growth of plantlets were examined. Treatment of seedling with 200 µM $CdCl_2$ resulted in 50% inhibition in growth (data not shown). This concentration was used in the subsequent steps of study.

Seeds were sterilized using 10% sodium hypochlorite for 5 min and washed with distilled water several times. Half of the seeds were then presoaked in 0.5 mM SA, the other half in distilled water for 6 h. The seeds were then transferred to plastic pots containing perlite. Plants were grown in a growth chamber at a day/night cycle of 13h/11h, at 28ºC/18ºC. Relative humidity was 50% and light intensity was 7000 lux. Pots were irrigated with distilled water for 3-4 days. Half of the pots (12 pots) containing 200 µM $CdCl_2$ were irrigated with Hoagland's solution (Hoagland & Arnon, 1950) and the other half (12 pots) were irrigated with Hoagland's solution once every two days. Both groups of potted plants were harvested after three weeks. The fresh weights of the shoots and roots of plantlets were measured immediately. Root and shoot samples were dried at 75ºC for 2 days before dry weight was determined.

Biochemical parameters

Leaf proline content was measured according to the method described by Bates *et al*. (1973). About 0.3 g of leaf tissues from the control, or treated plants were homogenized by the addition of 1 ml of 3% sulphosalicylic acid solution in an ice bath. Homogenates were centrifuged at 18000×g for 5 min at 4ºC and absorbance of the pink-red upper phase was recorded at 520 nm, against a toluene blank. A standard curve for proline in the range 0.01–1.5 mM was constructed to determine the proline concentration in each sample.

Soluble carbohydrates of leaves were estimated by Anthrone reagent according to the method described by False (1951). Fresh plant sample (0.05g) was then homogenized well in 2.5 ml of 80% ethanol using a mortar. The extract was placed in a warm bath at 90ºC for 60 min before it was filtered and the ethanol was allowed to evaporate. Pellet was dissolved in 2.5 ml double distilled water, then 200 µl of each sample was poured in a test tube and 5 ml anthron reagent was added to each test tube. After mixing, the samples were placed in the warm bath at 90ºC for 17 min, then cooled on ice. Absorbance readings were taken at 625 nm wavelength with a JENWAY spectrophotometer (JENWAY 6300).

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, according to the method described by Heath & Packer (1968). Tissue sample (0.5 g) was homogenized in 10 ml of 0.1% trichloroacetic acid (TCA). Homogenate was centrifuged at 15000×g for 10 min. Supernatant was collected and mixed with 4.0 ml of 0.5% thiobarbituric acid (TBA). The mixture was heated at 95ºC for 30 min and then quickly cooled using an ice bath. After centrifugation at 10000×g for 10 min, the absorbance of the supernatant was reading using spectrophotometer (JENWAY 6300) at 532 nm. The value for nonspecific absorbance at 600 nm was subtracted. The MDA content was calculated using its absorbance coefficient of 155 $mmol^{-1}$ cm^{-1} and expressed as nmol (MDA) g^{-1} fresh weight.

Catalase assay

Fresh shoot (0.1 g) was homogenized in 1.5 ml extraction buffer of 50 mM PBS, pH 7.8, 4% polyvinylpyrroidone (PVP) in an ice bath using a pre-chilled mortar and pestle. After centrifuging at 10000×g for 20 min at 4ºC, the supernatant was used for analysis of CAT, GPX and APX activities. CAT activity was determined using the method described by Aebi (1983). The sample was added to a reaction solution containing 10 mM H_2O_2 in PBS buffer. CAT activity was measured by monitoring the decrease in absorbance at 240 nm and using an absorbance coefficient for H_2O_2 of 0.039 mM^{-1} cm^{-1}. One unit of CAT gives a H_2O_2 decomposition rate of 1 ìmol min^{-1} at 25ºC.

Guaiacol peroxidase assay

Peroxidase activity in shoot extracts was assayed using the method described by Lin & Kao (2000). The assay mixture consisted of 9 mM guaiacol and

19 mM H_2O_2 in 50 mM phosphate buffer, pH 7 at 30ºC. Reaction product was measured at 470 nm. The extinction coefficient was 26.6 mmol/cm. A unit of guaiacol peroxidase enzyme activity was defined as enzyme content which causes the formation of 1 µM tetraguaiacol in one min.

Ascorbate peroxidase assay

Shoot extract was prepared as described in preparation for catalase assay. The activity of APX was measured using a procedure modified from that described previously by Nakano & Asada (1981). The reaction mixture consisted of 625 µl of extraction buffer (pH 7.8), 175 µl of 0.5 mM ascorbic acid, 50 µg bovine serum albumin, and 100 µl shoot extract. The reaction was started by adding 50 µl of 250 mM H_2O_2. Decrease in absorbance at 290 nm was measured as an indication of H_2O_2 dependent oxidation of ascorbic acid. The enzyme activity was calculated using an absorbance coefficient of 2.6mM^{-1} cm^{-1} for ascorbic acid. One unit of enzyme activity was defined as oxidized amount of ascorbic acid at a rate of 1 µmol min^{-1} at 25ºC.

Statistical analysis

Significance of differences was tested at $p \leq 0.05$ using one way ANOVA, and Duncan's Multiple Range test. Data were analyzed using SPSS (V.16.0) software package. All the experiments were repeated at least three times and data presented are means of three separate experiments ± SD.

RESULTS

Plant growth parameters

Effects of Cd or salicylic acid on fresh and dry weights of root and shoot, separately, and effect of combined Cd and salicylic acid on fresh and dry weights of root and shoot were analyzed using ANOVA. Cd significantly decreased roots and shoots fresh weight by 50% and 41.2% and roots and shoots dry weight by 31% and 45% respectively, compared to control plants. Root and shoot fresh weight and root dry weight compared to control plants were not significantly affected by salicylic acid, while shoots dry weight were significantly increased up to 10% (Table 1). However, fresh weight of roots and shoots were significantly increased 40% and 31%, and root and shoot dry weight 21% and 30% respectively by salicylic acid in Cd treated plants compared to control plants (Table 1).

Biochemical parameters

The oxidative damage brought about by Cd stress in soybean, was investigated by measuring lipid peroxidation in leaves. MDA contents indicate

Table 1. Effects of Cd and SA on growth parameters of soybean plants

Treatments	DW (g)		FW (g)	
	Root	Shoots	Root	Shoots
-Cd-SA	$0.68^{a} \pm 0.012$	$4.10^{a} \pm 0.13$	$0.16^{a} \pm 0.004$	$0.96^{b} \pm 0.01$
-Cd+SA	$0.66^{a} \pm 0.019$	$3.88^{a} \pm 0.26$	$0.17^{a} \pm 0.002$	$1.06^{a} \pm 0.07$
+Cd-SA	$0.34^{c} \pm 0.011$	$2.41^{c} \pm 0.06$	$0.11^{c} \pm 0.008$	$0.52^{d} \pm 0.04$
+Cd+SA	$0.57^{b} \pm 0.017$	$3.52^{b} \pm 0.07$	$0.14^{b} \pm 0.003$	$0.75^{c} \pm 0.05$

The data of root and shoots dry and fresh weight are means ± S.D. from three experiments. Within a column, mean values followed by different letters (a, b, c, d) are significantly different (DMRT, $p \leq 0.05$).

Table 2. Effects of Cd and SA on lipid peroxidation, proline and soluble carbohydrates contents in soybean shoot

Biochemical parameters	-Cd		+Cd	
	-SA	+SA	-SA	+SA
Lipid peroxidation (nmol MDA/g FW)	$442.11^{c} \pm 20.33$	$441.93^{c} \pm 12.87$	$793.5^{a} \pm 11.23$	$560.83^{b} \pm 18.1$
Proline (µmol/g FW)	$2.32^{b} \pm 0.25$	$1.13^{c} \pm 0.04$	$4.16^{a} \pm 0.2$	$2.63^{b} \pm 0.08$
Soluble carbohydrates (mg/g FW)	$7.65^{c} \pm 0.31$	$7.92^{c} \pm 0.44$	$9.76^{b} \pm 0.22$	$12.98^{a} \pm 0.14$

The data are means ± S.D. from three experiments. Mean values followed by different letters (a, b, c, d) are significantly different ($P \leq 0.05$).

lipid peroxidation and increased by about 79% upon Cd exposure in leaves of the SA-free controls, but by less than 26% in soybean seedlings exposed to SA and therefore decreased Cd toxicity (Table 2). Analysis of variance showed that the effects of Cd and salicylic acid on proline content was significant at $P \leq 0.01$ but the interaction effect of Cd and salicylic acid had not significant effect on proline content. Proline content increased by about 79% upon Cd exposure, and the Cd response was decreased by 13% after the SA pretreatment (Table 2). Concentrations of the soluble carbohydrates increased upon Cd exposure in both the control and the SA treatments. The SA-induced increase in soluble carbohydrates contents was insignificant in the control plants (Table 2).

Antioxidant enzyme activities

Cd significantly increased the activities of H_2O_2 scavenging enzymes, namely GPX, APX and CAT by 55%, 36% and 151% respectively, compared to the control plants. These increases were significantly lower when SA treatment was applied. The amounts of increased activities of GPX, APX and CAT due to Cd treatment were lowered by SA treatment in Cd-treated plants up to 7%, 18% and 35% respectively. SA treatment also decreased the activities of GPX, APX and CAT in control (-Cd) plants, up to 29%, 27% and 15% respectively (Table 3).

DISCUSSION

The results of this study depressive effects of Cd on soybean growth, have also been reported by other researchers (Guillermo *et al*., 2007; Keramat *et al*., 2009; Mahmoodabadi *et al*., 2009). Negative effects of Cd on growth of mustard (Ahmad *et al*., 2010), corn (Krantev *et al*., 2008) and pea (Sandalio *et al*., 2001), have already been reported. Induced-growth reduction by Cd is probably related to inhibition of normal cell division by Cd linkage to sulfhydryl groups of compounds that are involved in regulation of plant cell division (Choudhary *et al*., 2006). Decrease of dry weight in plants treated with Cd could be due to decrease in photosynthesis process, which has been reported by Krantev *et al*. (2008). Results of this study showed that salicylic acid pre-treatment for 6 h increased root and shoot fresh and dry weight in treated-plantlets with Cd. A similar study by Guo *et al*. (2007) reported that pre-treatment of rice seeds for 6 h in salicylic acid increased root fresh weight in plants treated with Cd. Choudhury & Panda (2004) also showed that salicylic acid pre-treatment for 16 h increased growth and dry weight of rice roots, while Cd severely inhibited root growth in absence of salicylic acid.

Increase in leaf proline and soluble carbohydrate content, in Cd stress condition was observed in this study. It has been shown by other researches that Cd toxicity induced proline accumulation in rice (Choudhury & Panda, 2004), *Arachis hypogaea* (Dinakar *et al*., 2009) and wheat (Amani, 2008). Application of SA decreased proline contents in control and Cd-treated plants, indicating relative decrease of cadmium stress after SA treatment. Contrary to the results of this study, El-Tayeb *et al*. (2006) reported that salicylic acid induced proline accumulation in three organs of sunflower plants compared to controls. They also found that salicylic acid decreased proline accumulation in the stem, but induced proline accumulation in the roots under Cu stress. Inverse relationship between biomass (dry weight) and proline and soluble carbohydrates accumulation under Cd stress is reported, which indicates that those osmolytes might be produced to be consumed in plant growth (Maggio *et al*., 2002). Accumulation of proline causes decreased cell division or delayed growth (Maggio *et al*., 2002).

Salicylic acid increased carbohydrate content in Cd-treated plants in our study (Table 2). In a similar report, El-Tayeb *et al*. (2006) showed that salicylic acid increased soluble, non soluble and total soluble sugars contents in stems and leaves of plants treated with Cu. It seems that SA affects carbohydrate metabolism in soybean. As carbohydrates protect and stabilize biomembranes, they would decrease

Table 3. Effects of Cd and SA on antioxidative enzymes (Catalase (CAT), Ascorbate peroxidase (APX)) and Guaiacol peroxidase (GPX) in soybean shoot

Antioxidative enzymes (μml H_2O_2/g FW min)	-Cd		+Cd	
	-SA	+SA	-SA	+SA
Catalase (CAT)	$1729.66^{c} \pm 20.13$	$1460^{d} \pm 36.04$	$4345^{a} \pm 230.5$	$2348.66^{b} \pm 156.92$
Ascorbate peroxidase (APX)	$42.16^{b} \pm 1.09$	$30.5^{d} \pm 0.96$	$57.2^{a} \pm 1.68$	$34.46^{c} \pm 0.65$
Guaiacol peroxidase (GPX)	$1.06^{b} \pm 0.05$	$0.75^{c} \pm 0.08$	$1.65^{a} \pm 0.22$	$1.13^{b} \pm 0.12$

The data are means ± S.D. from three experiments

MDA production, proline content and antioxidant enzyme activities. Lower levels of lipid peroxidation were observed in SA-treated plants, suggesting an enhanced capacity for protection from oxidative damage by Cd stress in these plants.

It has been reported that SA reduced Cd toxicity on plant growth and development due to induction of antioxidant responses (Rao *et al*., 1997). Our results showed that activities of CAT, GPX and APX antioxidant enzymes in the leaves of soybean plantlets under Cd stress were decreased when subjected to salicylic acid treatment. The question was then raised whether decreased Cd toxicity by salicylic acid, was due to induction of antioxidant defense. Metwally *et al*. (2003) reported that levels of transcripts responsible for ABC transporters where elevated in response to salicylic acid treatment in *Arabidopsis thaliana*. Members of ABC transporter family facilitated vacuolar sequestration of heavy metals. They stated that it was likely that the formation of stable acid complexes of Cd-salicylic decreased Cd toxicity after pretreatment with salicylic acid (Metwally *et al*., 2003). Patterns of antioxidant enzymes activities in the presence of Cd, revealed that the oxidative stress was low in plants treated with SA. Therefore, SA-induced decrease in Cd toxicity, was not due to induction of antioxidant defense system. SA might have played an important role in induction of ABC transporters in leaves of soybean. However, further experiments are to be conducted to support this claim.

The general conclusion is that, salicylic acid could act as a pro-oxidant which increased the hydrogen peroxide as a defense signaling molecule, probably via decreasing activities of hydrogen peroxide scavenging enzymes and decreasing the proline (scavenger of antioxidants and free radicals), leading to plant tolerance to Cd. Salicylic acid acted as an antioxidant directly by decreasing OH and superoxide radicals which means decrease in oxidative stress, MDA level and lipid peroxidation, and fixing the membrane stability, or indirectly by increasing the carbohydrates content.

REFERENCES

Aebi, H. 1984. Catalase *in vitro*. *Methods in Enzymology*, **105:** 121-126.

Ahmad, P., Nabi, G. & Ashraf, M. 2010. Cadmium-induced oxidative damage in Mustard [*Brassica juncea* (L.) Czern. and Coss.] plants can be alleviated by salicylic acid. *South African Journal of Botany*, **77:** 36-44.

Amani, A.L. 2008. Cadmium induced changes in pigment content, ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *Triticum aestivum* seedlings. *Australian Journal of Basic and Applied Sciences*, **2:** 57-62.

Babula, P., Adam, V., Opatrilova, R., Zehnalek, J., Havel, L. & Kizek, R. 2008. Uncommon heavy metals, metalloids and their plant toxicity: a review. *Environmental Chemistry Letters*, **6:** 189-213.

Bates, L., Waldren, R. & Teare, I. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39:** 205-207.

Choudhary, M., Jetley, U.K., Khan, M.A., Zutshi, S. & Fatma, T. 2006. Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. *Ecotoxicology and Environmental Safety*, **66:** 204-209.

Choudhury, S. & Panda S.K. 2004. Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza Sativa* L. roots. *Bulgarian Journal of Plant Physiology*, **30:** 95-110.

Dinakar, N., Nagajyothi, P.C., Suresh, S., Damodharam, T. & Suresh, C. 2009. Cadmium induced changes on proline, antioxidant enzymes, nitrate and nitrite reductases in *Arachis hypogaea* L. *Journal of Environmental Biology*, **30:** 289-294.

El-Tayeb, M.A., El-Enany, A.E. & Ahmed, N.L. 2006. Salicylic acid-induced adaptive response to copper stress in sunflower (*Helianthus annuus* L.). *Plant Growth Regulation*, **50:** 191-199.

Guillermo, O., Noriega, K.B., Balestrasse, A.B. & Tomaro, M.L. 2007. Cadmium induced oxidative stress in soybean plants also by the accumulation of d-aminolevulinic acid. *BioMetals*, **20:** 841-851.

Guo, B., Liang, Y.C., Zhu, Y.G. & Zhao, F.J. 2007. Role of salicylic acid in alleviating oxidative damage in rice roots (*Oryza sativa* L.) subjected to cadmium stress. *Environmental Pollution*, **147:** 743-749.

Heath, R.L. & Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125:** 189-198.

Hoagland, D.R. & Arnon, D.I. 1950. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station, **347**(2nd edit).

Keramat, B., Manouchehri Kalantari, K. & Arvin, M.J. 2009. Effects of methyl jasmonate in regulating cadmium induced oxidative stress in soybean plant (*Glycine max* L.). *African Journal of Microbiology Research,* **3:** 240-244.

Krantev, A., Yordanova, R., Janda, T., Szalai, G. & Popova, L. 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. *Journal of Plant Physiology*, **165:** 920-931.

Lin, C.C. & Kao, C.H. 2000. Effect of NaCl stress on H_2O_2 metabolism in rice leaves. *Plant Growth Regulation*, **30:** 151-155.

Maggio, A., Miyazaki, S., Veronese, P., Fujita, T., Ibeas, J.I., Damsz, B., Narasimhan, M.L., Hasegawa, P.M., Joly, R.J. & Bressan, R.A. 2002. Dose proline accumulation plays an active role in stress-induced growth reduction. *The Plant Journal*, **31:** 699-712.

Mahmoodabadi, M.R., Ronaghi, A., Khayyat, M. & Hadarbadi, G. 2009. Effects of zeolite and cadmium on growth and chemical composition of soybean (*Glycine max* L.). *Tropical and Subtropical Agroecosystems*, **10:** 515-521.

Mateos-Aparicio, L., Redondo, C.A., Villanueva-Suarez, M.J. & Zapata-Revilla, M.A. 2008. Soybean, a promising health source. *Nutrition in Hospitals*, **23:** 305-312.

McGrath, S., Lombi, E. & Zhao, F.J. 2001. What's new about cadmium hyperaccumulation? *New Phytologist,* **149:** 2-3.

Metwally, A., Finkemeier, I., Georgi, M. & Dietz, K.J. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiology*, **132:** 272-281.

Murtaza, G., Asghar, R. & Abdul, M. 2010. Changes in specific activity of ascorbate peroxidase during seed development of pea (*Pisum sativum* L.) treated with salicylic acid. *African Journal of Biotechnology*, **9:** 5333-5337.

Nakano, Y. & Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, **22:** 867-80.

Qinghua, S.H. & Zhujun, Z. 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. *Environmental and Experimental Botany*, **63:** 317-326.

Rao, M.V., Paliyath, G., Ormord, D.P. & Watkins, C.B. 1997. Influence of salicylic acid on H_2O_2 production, oxidative stress and H_2O_2 metabolizing enzymes. *Plant Physiology*, **115:** 137-149.

Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero-Puertas, M.C. & Del-Rio, L.A. 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, **52:** 2115-2226.

Wu, H.S., Raza, W., Fan, J.Q., Sun, Y.G., Bao, W., Liu, D.Y., Huang, Q.W., Mao, Z.S., Shen, Q.R. & Miao, W.G. 2008. Antibiotic effect of exogenously applied salicylic acid on *in vitro* soilborne pathogen, *Fusarium oxysporum* f. sp. niveum. *Chemosphere*, **74:** 45-50.