THE EFFECTS OF GA_3 APPLICATION ON GROWTH, LIPID PEROXIDATION, ANTIOXIDANT ENZYMES ACTIVITIES, AND SUGARS LEVELS OF CADMIUM STRESSED TOMATO (*LYCOPERSICON ESCULENTUM* MILL. cv. CH) PLANTS

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In the present study, the interactive effects of different concentrations of Cd (0, 2.5, 5, 10 and 20 μM) and gibberellin A_3 (0, 5, and 10 μM) were examined on certain physiological parameters of tomato (*Lycopersicon esculentum* Mill. cv. CH) plants. The results showed that Cd treatments led to decrease in growth parameters, total chlorophylls, proteins and insoluble sugars contents, while, soluble sugars contents, lipid peroxidation, antioxidant enzymes activities and Cd accumulation revealed growing attitude. The combined application of GA_3 and Cd caused reduction of lipid peroxidation and improvement in growth, total chlorophylls, soluble proteins and insoluble sugars contents. In addition, soluble sugars contents, Cd accumulation and activities of antioxidant enzymes enhanced. These results suggest that GA_3 application alleviates adverse effects of Cd on growth and augments tolerance against Cd toxicity in tomato plants.

Key words: Antioxidant enzymes; cadmium; lipid peroxidation; tolerance; tomato.

Abbreviations: Cd – cadmium; GA_3 – gibberellin A_3; NAR – net assimilation rate; RGR – relative growth rate; RLGR – relative leaf growth rate; LWCA – leaf water content per unit area; SLA – specific leaf area; ROS – Reactive Oxygen Species; CAT – catalase; GPX – guaiacol peroxidase; APX – ascorbate peroxidase; MDA – Malondialdehyde.

INTRODUCTION

Cadmium is a non-essential metal, which naturally exists in low amounts in the environment. Particularly, at high concentrations, it adversely affects plant growth and development (Benavides *et al*. 2005). Cd as a toxic element for human and animals has become a major threat, especially in developed countries (Gratao

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et al. 2009). Since, plants have grown in contaminated soils uptake and collect Cd through metal transporters (Lopez-Millan *et al*. 2009). Furthermore, Cd accumulation usually occurs in the edible parts of plants without any visible symptoms, which led to entry of Cd into human food chain (Aibibu *et al*. 2010).

Some of Cd-induced physiological changes in plants contain oxidative stress, growth inhibition and interruption in plant's water balance and mineral nutrients homeostasis (Gill and Tuteja, 2010; Aibibu *et al*. 2010). The toxic impacts of Cd could be due to promoted production of reactive oxygen species (ROS) such as $O^{\bullet -2}$, H_2O_2 and $^{\bullet}OH^{-}$. To act against ROS invasion, plants are equipped with several enzymatic and non-enzymatic antioxidative mechanisms (Gill and Tuteja, 2010).

Gibberellins are phytohormones with pivotal roles in plants growth and development (Tuna *et al*. 2008). There are some reports that described gibberellins have the protective role in plant adaptation to abiotic stresses and detoxification of heavy metals (Siddiqui *et al*. 2011; Tuna et al. 2008; Maggio *et al*. 2010).

Therefore, the aim of this study was to examine the alleviation effects of GA_3 on growth, antioxidant enzymes, lipid peroxidation and sugars contents in Cd-stressed plants.

MATERIAL AND METHODS

Plant materials, growth conditions and stress treatments. Seeds of tomato (*Lycopersicon esculentum* Mill. cv. CH) were supplied by Falat company, Tehran, Iran. The seeds were sterilized with 1% sodium hypochlorite and washed several times with distilled water. They germinated in moist filter paper placed in sterilized petri dishes in darkness at 25 °C. Six-day-old seedlings were transferred to pots containing sterilized moist sands under light density nearly 60 μmol m^{-2} s^{-1}. Pots were transferred to a growth chamber (16 h light, 8 h dark and temperatures of 25±1°C in day and 18±1°C in night) and irrigated with half-strength and complete Hoagland's nutrient solution, respectively. 30-day-old plants were treated with different concentrations of $CdCl_2$ (0, 2.5, 5, 10 and 20 μM) and GA_3 (0, 5 and 10 μM). At last, 60-day-old plants were harvested for determination of growth and physiological parameters.

Growth assay. After harvesting, plants were divided into three fractions (leaves, stems and roots). Fresh weight and leaf area of each fraction were carefully determined. Dry weight of samples were measured after being dried at 105 °C for 24 h. Finally, growth parameters include NAR, RGR, RLGR, SLA and LWCA which were calculated by using the equations proposed by Watson (1952) and Evans and Hughes (1962).

Total chlorophyll. The total chlorophyll content was determined according to the method of Lichtenthaler (1987) and expressed as mg g^{-1} F.W.

Sugars contents. Soluble sugars of dried leaf (0.1 g) were extracted with 80% ethanol. The contents of soluble sugars were determined according to the method of Somogyi (1952) and Krishnaveni *et al.* (1984). The insoluble sugars contents were detected by using the remained residues after the soluble sugars extraction according to the method of Hellubus and Crarigi (1978). The contents of soluble and insoluble sugars were expressed as mg g^{-1} D.W.

Lipid peroxidation. Lipid peroxidation was detected according to the method of Heath and Packer (1968) by measuring malondialdehyde content and expressed as μmol g^{-1} F.W.

Enzymes assay. CAT activity was determined according to the method of Dazy *et al*. (2008) in reaction mixture contained 15 mM H_2O_2 and 50 mM potassium phosphate buffer (pH 7). The decomposition of H_2O_2 was measured by the decrease in absorbance at 240 nm for 3 min. The activity of GPX was determined by recording the increase of absorbance at 470 nm for 3 min, in response to tetraguaiacol formation (Dazy *et al.* 2008). The reaction mixture contained 25 mM potassium phosphate buffer (pH 6.8), 40 mM H_2O_2 and 20 mM guaiacol. APX activity was measured according to the method of Nakano and Asada (1981). The reaction mixture contained 250 mM potassium phosphate buffer (pH 7), 0.5 mM ascorbic acid, 1.2 mM H_2O_2, 0.1 mM EDTA and enzyme extract.

Cd content. The Cd content was determined according to the method of Wang *et al.* (1997). Dry weight of root (0.3 g) and leaf (0.5 g) were put in crucibles and placed in a furnace at 500 °C for 4 h to obtain the ash. The ashes were digested in 2.5 ml of 20% HCl for 30 min and then samples' volumes were reached to 5 ml. Finally, Cd content was measured by using ICP-OES (VISTA-PRO) apparatus.

Statistical analysis. Each treatment was replicated four times for statistical validity. The statistical analyses were performed through analysis of variance (two-way ANOVA) by using SPSS 17 and SAS 9 softwares and statistical significant was set at $p < 0.05$.

RESULTS AND DISCUSSION

The influence of Cd and GA_3 on growth parameters (NAR, RGR, RLGR, LWCA and SLA) is shown in Table 1. In the Cd-treated plants, the average amounts of growth parameters were significantly lower than that of control group. The growth inhibition induced by heavy metals in plants due to toxicity of heavy metals is usually accumulated in tissue (direct effects) or limitation of minerals and water uptake (indirect effects) (Nedjimi and Daoud, 2009; Aibibu *et al*. 2010). It suggests that growth inhibition occurred because of decrease in cell elongation rate which mainly happen by an irreversible damage of proton pumps responsible for the process and cross-linking of pectin in middle lamellae (Ahmad *et al.* 2011). The application of GA_3 led to moderate toxic effects of Cd on growth parameters.

GA_3 have promotive effects on cell division and cell enlargement and play a prominent role in the Cd detoxification via improvement of Ca^{2+} and other mineral nutrients uptake, antioxidant enzymes activities and decreasing lipid peroxidation (Maggio *et al.* 2010; Siddiqui *et al.* 2011).

Table 1

The effects of GA_3 application on the NAR, RGR, RLGR, LWCA and SLA of Cd stressed plants

$CdCl_2$ (μM)	GA_3 (μM)	NAR ($g\ m^{-2}\ d$)	RGR ($g\ kg^{-1}\ d^{-1}$)	RLGR ($cm^2\ m^{-2}\ d^{-1}$)	LWCA ($g\ (H_2O)\ m^{-2}$)	SLA ($m^2\ kg^{-1}$)
0	0	7.79 ± 0.14^{b}	78.61 ± 2.40^{cb}	1342.36 ± 26.49^{cb}	222.64 ± 4.98^{cb}	40.07 ± 0.84^{cb}
	5	8.26 ± 0.28^{a}	90.73 ± 2.58^{a}	1486.98 ± 21.98^{a}	239.84 ± 4.77^{a}	46.26 ± 1.44^{a}
	10	8.48 ± 0.10^{a}	91.70 ± 1.31^{a}	1488.70 ± 19.02^{a}	243.46 ± 4.00^{a}	45.19 ± 0.87^{a}
2.5	0	7.11 ± 0.15^{cde}	71.48 ± 1.97^{ed}	1278.23 ± 20.28^{cd}	203.66 ± 3.74^{gef}	38.05 ± 0.85^{cb}
	5	7.68 ± 0.09^{b}	80.77 ± 1.13^{b}	1379.37 ± 13.49^{b}	224.45 ± 3.25^{b}	41.28 ± 0.76^{b}
	10	7.73 ± 0.12^{b}	80.46 ± 0.72^{b}	1372.75 ± 14.99^{b}	217.82 ± 3.97^{cbd}	41.19 ± 1.16^{b}
5	0	6.64 ± 0.14^{fde}	63.71 ± 1.76^{gf}	1189.75 ± 20.28^{cd}	194.62 ± 3.12^{gh}	33.04 ± 0.94^{ed}
	5	7.10 ± 0.15^{cde}	70.21 ± 1.92^{ed}	1260.15 ± 19.32^{ed}	208.79 ± 1.88^{efd}	37.76 ± 1.08^{cb}
	10	7.35 ± 0.20^{cb}	73.49 ± 2.08^{cd}	1293.98 ± 18.44^{cd}	215.83 ± 5.75^{cebd}	39.19 ± 0.94^{cb}
10	0	6.60 ± 0.15^{fe}	61.00 ± 2.20^{gf}	1148.95 ± 28.49^{gf}	187.07 ± 5.03^{h}	31.44 ± 1.51^{e}
	5	6.82 ± 0.11^{fde}	66.73 ± 2.38^{ef}	1247.23 ± 38.07^{ed}	199.89 ± 5.06^{gfh}	36.08 ± 2.19^{cd}
	10	7.13 ± 0.15^{cd}	71.38 ± 2.25^{ed}	1263.00 ± 21.19^{ed}	210.48 ± 4.37^{cefd}	37.43 ± 1.08^{cb}
20	0	5.60 ± 0.23^{g}	46.84 ± 2.50^{h}	981.42 ± 27.78^{h}	165.29 ± 3.37^{i}	24.53 ± 0.30^{f}
	5	6.53 ± 0.12^{f}	58.26 ± 3.25^{g}	1108.25 ± 44.59^{g}	190.14 ± 2.84^{h}	29.44 ± 2.28^{e}
	10	6.57 ± 0.11^{f}	60.78 ± 1.89^{gf}	1148.16 ± 22.32^{gf}	191.41 ± 4.36^{gh}	31.17 ± 0.87^{e}

Data are the means of four replicates (Mean ± SEM) and different letters indicate significant differences at $P < 0.05$ level.

The results related to the effects of Cd and GA_3 on chlorophylls contents are presented in Table 2. In general, heavy metals toxicity led to decline in net photosynthesis and different photosynthetic pigments. It suggests that the decrease in chlorophylls content is the result of inhibition of some important enzymes involved in chlorophylls biosynthesis such as protochlorophyllide reductase and δ-aminolevulinic acid dehydratase. In addition, Mg ions in tetrapyrrole structure of chlorophyll replaced by Cd (Nedjimi and Daoud, 2009; Aibibu *et al*. 2010). The application of GA_3 helped plants to enhance tolerance against Cd toxicity. Similarly, an increase in the chlorophylls contents was reported with application of GA_3 in some of plants (Siddiqui *et al*. 2011; Maggio *et al*. 2010).

Effects of Cd and GA_3 on sugars contents have been displayed in Table 2. Cd treatments reduced insoluble sugars in comparison to control plants, while the contents of soluble sugars increased. Abiotic stresses like drought, cold and salinity led to major changes in carbohydrate metabolism (Devi *et al*. 2007). Verma and Dubey (2001) suggested that decline in the contents of insoluble sugars in Cd-treated plants may be due to increasing acid invertase and sucrose synthase activities. In addition, accumulation of soluble sugars may be attributed to their roles in regulation of internal osmotic (Verma and Dubey, 2001). The addition of

GA_3 increased both soluble and insoluble sugars contents. It is obvious that the relationship between photosynthesis and demand for carbohydrates was regulated by sucrose pathway. GA_3 has promotive effects on sucrose phloem transport and increases acid invertase activity in target tissues which were accompanied by increased hexose levels (Kozlowska *et al*. 2007).

Table 2

The effects of GA_3 application on the total chlorophylls, soluble sugars and insoluble sugars contents of Cd stressed plants

$CdCl_2$ (μM)	GA_3 (μM)	Total chlorophylls (mg g^{-1} F.W.)	Soluble sugars (mg g^{-1} D.W.)	Insoluble sugars (mg g^{-1} D.W.)
0	0	2.20 ± 0.067^{de}	102.76 ± 3.03^{h}	137.42 ± 2.16^{c}
	5	2.66 ± 0.047^{b}	146.83 ± 2.92^{a}	150.27 ± 2.40^{b}
	10	3.15 ± 0.087^{a}	140.42 ± 3.46^{ab}	168.35 ± 2.93^{a}
2.5	0	1.92 ± 0.063^{g}	109.17 ± 6.18^{h}	119.38 ± 3.13^{d}
	5	2.32 ± 0.043^{dc}	126.80 ± 2.40^{efcd}	134.85 ± 2.21^{c}
	10	2.36 ± 0.021^{c}	130.80 ± 2.26^{cd}	136.57 ± 1.98^{c}
5	0	1.54 ± 0.049^{h}	121.19 ± 1.85^{ef}	100.48 ± 4.08^{e}
	5	1.92 ± 0.049^{g}	125.99 ± 2.07^{efd}	108.22 ± 2.93^{e}
	10	2.13 ± 0.043^{fe}	132.41 ± 2.07^{bcd}	125.40 ± 3.81^{d}
10	0	1.52 ± 0.036^{h}	110.77 ± 1.53^{hg}	103.06 ± 2.16^{e}
	5	1.89 ± 0.046^{g}	177.98 ± 2.26^{fg}	124.54 ± 2.21^{d}
	10	2.03 ± 0.039^{fg}	121.19 ± 2.27^{ef}	125.40 ± 3.54^{d}
20	0	1.10 ± 0.054^{i}	126.80 ± 3.30^{efcd}	79.86 ± 3.29^{g}
	5	1.55 ± 0.038^{h}	135.61 ± 2.06^{bc}	91.89 ± 3.13^{f}
	10	1.48 ± 0.038^{h}	129.20 ± 2.09^{ecd}	85.88 ± 1.64^{gf}

Data are the means of four replicates (Mean ± SEM) and different letters indicate significant differences at $P < 0.05$ level.

Lipid peroxidation was increased in Cd-stressed plants. However, application of GA_3 assuaged the adverse effects of Cd on this attribute (Fig. 1). The peroxidation of polyunsaturated lipids in biological membranes is one of the main targets of oxidative stress in plant and animal cells. Heavy metal-induced oxidative stress leads to the formation of toxic free radicals such as $O^{\bullet -2}$, H^2O^2 and $^{\bullet}OH^{-}$ which induce lipid peroxidation (Gill and Tuteja, 2010). The observed decrease in MDA content by the application of GA_3 could be attributed to the positive roles of GA_3 on Ca^{2+} and other nutrients absorption which are involved in heavy metal detoxification (Khan *et al*. 2010; Maggio *et al*. 2010).

The activities of three enzymes involved in detoxification of oxidative stress were measured (Fig. 2). Toxic metals, such as Cd induces oxidative stress in plant cells because they are either directly or indirectly involved in ROS formation (Benavides *et al*. 2005). To act against ROS invasion, plants are equipped with several enzymatic and non-enzymatic antioxidative mechanisms (Gill and Tuteja, 2010). Therefore, increase the activities of antioxidant enzymes such as CAT, POX

and APX to diminish adverse impacts of oxidative stress in polluted soils. GPX is involved in lignin biosynthesis which can construct a physical barrier against Cd toxicity (Aibibu *et al*. 2010). Besides, APX is involved in H_2O_2 detoxification mechanisms via water-water and ASH-GSH cycles (Gill and Tuteja, 2010). It has been reported that plant hormones such as GA_3 had a crucial role in heavy metal detoxification through antioxidant enzymes activities augment and prevention from lipid peroxidation (Tuna *et al*. 2008; Maggio *et al*. 2010). Consequently, the enhancement of antioxidant enzyme system and the mechanisms that reduce ROS helped plants to enhance their tolerance against Cd stress (Tuna *et al*. 2008).

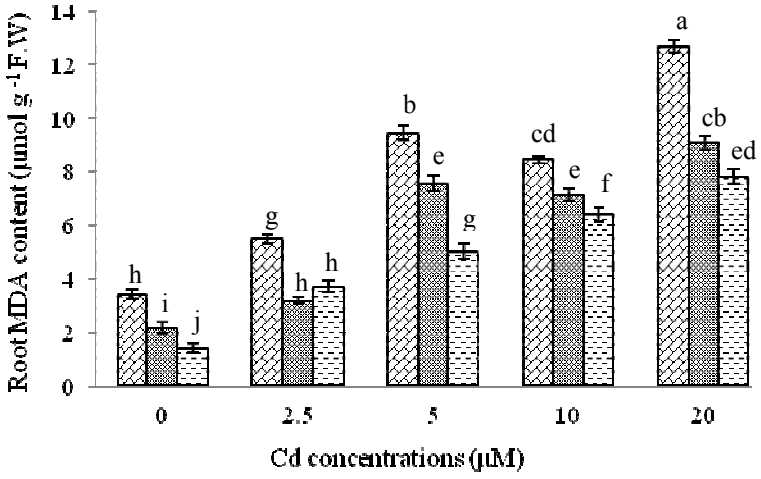

Fig. 1. The effects of GA_3 application on the MDA content of Cd stressed plants. Data are the means of four replicates (Mean ± SEM) and different letters indicate significant differences at $P < 0.05$ level.

The changes of Cd content in tomato plants exposed to Cd and GA_3 treatments are shown in Fig. 3. Although Cd is a non-essential element, plants can accumulate extra levels of it in different parts, especially in roots. The ability of different plants to uptake Cd is related to differences in the root capacity to secrete organic chelates such as citric acid and oxalic acid. These chelators had a promotive effect on Cd uptake and diminishing its toxicity by entrapment in the vacuoles (Lopez-Milan *et al*. 2009). Plant hormones have an important role in metal absorption and distribution mechanisms (Tuna *et al*. 2008). There are several studies which support chemical regulation of stomata and transpiration rate increase in plants exposed to GA_3. Therefore, the rate of water uptake and toxic metals, especially soluble metals, are increased by root system (Tassi *et al*. 2008).

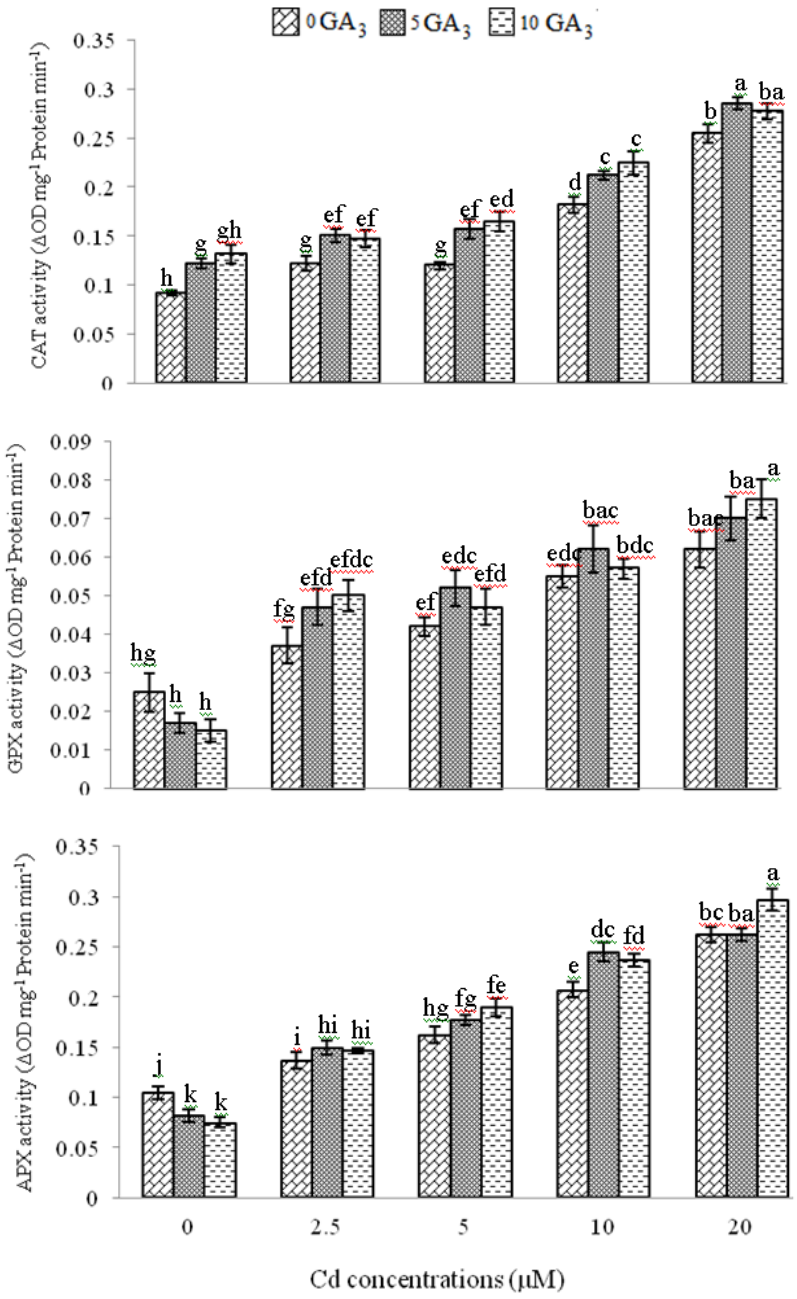

Fig. 2. The effects of GA_3 application on the GPX, CAT and APX activities of Cd stressed plants. Data are the means of four replicates (Mean ± SEM) and different letters indicate significant differences at $P < 0.05$ level.

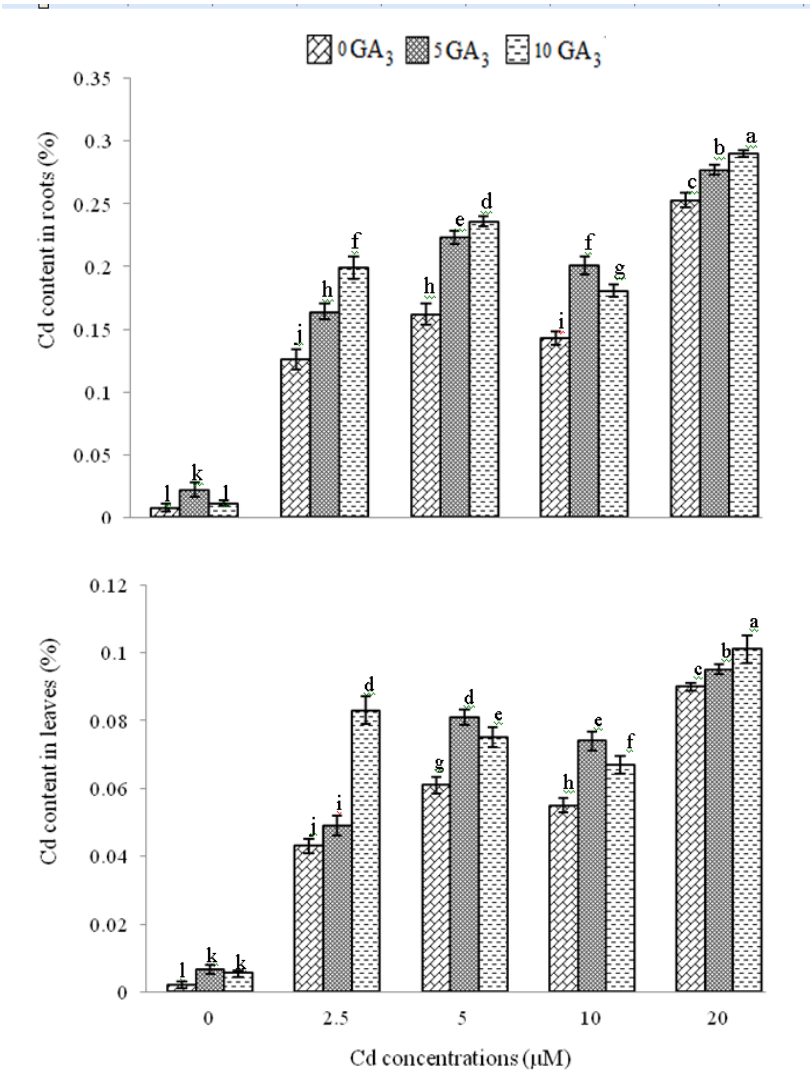

Fig. 3. The effects of GA_3 application on the Cd accumulation in roots and leaves of Cd stressed plants. Data are the means of four replicates (Mean ± SEM) and different letters indicate significant differences at $P < 0.05$ level.

CONCLUSION

Based on the results obtained, it can be deduced that GA_3 alleviates Cd toxicity to certain extent through declining lipid peroxidation and growth moderating. In addition, GA_3 increases plants tolerance against Cd-induced oxidative stress by enhancement of CAT, GPX and APX enzymes activities.

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