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EFFECT OF COMPOST ON THE ANTIOXIDANT DEFENSE SYSTEMS OF CUCUMBER (*Cucumis sativus* L.) AGAINST CADMIUM TOXICITY

[15]

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ABSTRACT

The effects of compost on plant growth, photosynthetic pigments, activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and glutathione reductase (GR), as well as concentrations of ascorbate (ASC) and glutathione (GSH) were investigated in cucumber (*Cucumis sativus* L.) plants treated with cadmium (Cd^{2+}). Four different treatments were applied: control without any treatment (T1), compost at 10% rate by weight (T2), cadmium (Cd^{2+}) at 100 µM (T3) and combined treatment of compost + Cd^{2+} (T4). The leaves showed apparent symptoms of Cd^{2+} toxicity, i.e. chlorosis, and the plant growth, as well as chlorophylls and carotenoids were significantly inhibited in Cd^{2+}-treated plants. In addition, compared with the control, 100 µM Cd^{2+} considerably increased H_2O_2 concentration and lipid peroxidation indicated by accumulation of thiobarbituric acid reactive substances (TBARS), pointing out to cellular oxidative stress. The addition of 10% compost to the sandy soil decreased the contents of TBARS and the production of H_2O_2, but increased the levels of photosynthetic pigments, and improved the plant growth. These results suggest that compost protects plants from oxidative damage, and this protection is performed via significant increase in the activities of the antioxidant enzymes namely SOD, APX, DHAR, GR, as well as the levels of antioxidants, ASC and GSH. The present results coincided with the conclusion that compost components, i.e. macro- and micronutrients, organic matters, humic substances, hormone-like substances, biotic agents, emission of carbon dioxide and nitric oxide and many others, are effectively contributed in the physiological metabolism to counteract oxidative stress induced by Cd^{2+} contaminated soil.

Abbreviations: APX, ascorbate peroxidase; ASC, reduced ascorbate; Cd^{2+}, cadmium; Co, compost; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; EDTA, ethylene diamine tetraacetic acid; GR, glutathione reductase ; GSH, reduced gultathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances

INTRODUCTION

As one of the most toxic environmental pollutants (**Zhang *et al* 2008**), cadmium (Cd^{2+}) has a strong influence on metabolic activities of crop plants by inducing a number of physiological changes, such as growth inhibition, changes in water and ion metabolism, photosynthesis inhibition, enzyme activity changes, and free radical formation (**Ekmekci *et al* 2008**). Cd^{2+} induces severe oxidative stress by producing excessive ROS, causing severe damage to membrane systems, cell organelles and DNA (**Geissle *et al* 2009**), although they act as signaling molecules that mediate many key physiological processes (**Gechev *et al* 2006**). MDA is a major product of membrane lipid peroxidation, and its level is an indicator of the extent of oxidative damage (**Zhang *et al* 2010**).

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To scavenge ROS and alleviate their deleterious effects, plants have developed protective enzymatic and non-enzymatic mechanisms. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), whereas non-enzymatic antioxidants include glutathione and ascorbate (**Apel and Hirt, 2004**). These defense mechanisms against oxidative damage have been explicitly observed in plants subjected to Cd^{2+} stress (**Ekmekci *et al* 2008**).

A major challenge in agricultural practice and research today is how to cope with environmental stress including heavy metal stress in an economical and an environmentally sustainable approach. Compost derived from agricultural wastes can improve crop tolerance and increase plant growth by providing better soil structure, supply of nutrients, and by building up antagonistic micro-organisms (**Stevenson, 1994**; **Heather *et al* 2006**; **Pilon-Smits *et al* 2009**). Compost can also effectively remedy heavy metal contaminated soil by transforming heavy metals from soluble and exchangeable fractions to fraction associated with organic matter, carbonates fraction, and residual fraction, which are unavailable to plants (**Li *et al* 2008**). Therefore, addition of compost to soils influences a wide array of plant agronomic and physiological characteristics and is, therefore, a crucial component for any sustainability of agriculture system (**Brady and Weil, 2000**). Consequently, we hypothesize that compost addition to agricultural soils could result in improving protection capacity of plants under stressful heavy metal conditions. To date, little information is available in relation to plant metabolic and physiological changes under heavy metal stress by compost application (**Gandolfi *et al* 2010**; **Han-Song *et al* 2010**).

The aim of this study was to address the effects of compost derived from cow manure, chicken manure and wheat straw on protection of cucumber (*Cucumis sativus* L. cv. Sweet Crunch) plants, as a model of vegetable crop, against Cd^{2+} toxicity by measuring plant growth, photosynthetic pigments, lipid peroxidation, and some enzymatic and non-enzymatic antioxidants.

MATERIALS AND METHODS

Plant material and treatments

Sandy soil was collected from the soil layer 0-25 cm depth at Faculty of Agriculture Farm, Suez Canal University, Ismailia, Egypt. The compost produced from composting a mixture of cow manure, chicken manure and wheat straw at a ratio of 3:1:1 (v/v) was used in this study. Chemical characteristics of sandy soil and compost after 160 days of composting were stated elsewhere (**Tartoura, 2009**).

Cucumber (*Cucumis sativus* L. cv. Sweet Crunch) seeds were planted in plastic pots (3 seeds pot^{-1}) containing a sandy soil amended with the compost at rate of 100 g Kg^{-1} soil and grown along with control that received no compost under natural conditions. The environmental conditions were as follows: a 12-h photoperiod, temperature of 25/20°C day/night, and a relative humidity of 65/70%. The pre-planting irrigation was applied 15 days before planting. After germination, seedlings were watered at intervals of 48 h. For Cd^{2+} stress treatment, the cucumber plants at 21-old- days were irrigated with the solution at 100 µM $CdSO_4$ and incubated for 14 days. The experimental design consisting of: a control [sandy soil and no added compost (T1)] and three treatments [sandy soil amended with the compost (T2), sandy soil treated with Cd^{2+} (T3), sandy soil amended with compost and treated with Cd^{2+} (T4)] were arranged in a randomized, complete block design, each with three replicates of 21 plants. Data are the mean values of three independent experiments. During the growing period, the water content of the soil in all pots was maintained near 80% of the field capacity. The youngest fully developed leaves were taken after 5 and 10 days for biochemical assays.

Plant growth measurement

After 14 days of treatment, shoot height and root length were measured. The plants were harvested, divided into shoots and roots, dried at 70°C to constant weight and weighed.

Determination of chlorophyll and carotenoid content

Chlorophyll and carotenoids were extracted by homogenizing ca 0.2 g fresh leaves in chilled ethanol solution (19:1 ethanol: water (v/v)). Chlorophyll and carotenoid contents in supernatants were spectrophotometrically determined at 665, 649 and 470 nm; the calculation method followed **Lichtenthaler and Wellburn (1983)**.

Determination of Cd^{2+} contents

The concentration of Cd^{2+} in dried leaves was determined by atomic absorption spectrophotometer as described by **Sandalio *et al* (2001)**.

Determination of H_2O_2 and lipid peroxidation

H_2O_2 was measured by monitoring the absorbance of the titanium-peroxide complex at 408 nm as described by **Nag *et al* (2000)**. Absorbance values were calibrated to a standard curve generated using known concentrations of H_2O_2. Lipid peroxidation was determined via thiobarbituric acid-reacting substances according to the method of **Sudhakar *et al* (2001)**. Absorbance of the supernatant was read at 532 and 600 nm. After subtracting the non-specific absorbance at 600 nm, the TBARS concentration was determined by its extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of antioxidant enzyme activities

Soluble proteins in plant leaves were extracted with 50 mM ice cold potassium phosphate buffer (pH 7.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 1mM ethylene diaminetetraacetic acid (EDTA). The activities of SOD, APX, and GR were determined as described by **Jiang and Zhang (2001)**. DHAR activity was determined according to the method described by **Nakano and Asada (1981)**. Protein content was estimated according to **Bradford (1976)** using bovine serum albumin as a standard.

Determination of ascorbate and glutathione concentrations

One gram of fresh leaves were ground in liquid nitrogen and extracted with 5 ml of 6.5% ice-cold metaphosphoric acid containing 1 mM EDTA, centrifuged at 15,000g for15 min, and the supernatant was used for the assay. Total ascorbate and reduced ascorbate (ASC) were estimated according to **Law *et al* (1983)**. Total ascorbate was determined by incubating samples with 1 mM DTT (final concentration). A standard curve for ASC was used.

Non-protein thiols were extracted by homogenizing 0.3 g leaf fragments in 2 ml of 5% sulphosalycilic acid (pH 2), containing 1 g polyvinylpyrrolidone (PVP). After centrifugation at 10,000g for 20 min at 4°C, the supernatants were used for the analysis. Total glutathione (GSH plus GSSG) was determined by the recycling method according to **Anderson (1985)**, using GSH as standard by measuring the absorbance increment at 412 nm, with glutathione reductase, DTNB and NADPH. To quantify GSSG, 2-vynilpyridine was added to the extract.

Statistical analysis

Data were tested by analysis of variance (ANOVA) using the CoStat system for Windows, version 6.311 (CoHort software, Berkeley, CA 94701). All data presented are the mean values. The measurement was done with three replicates. Statistical assays were carried out by ANOVA test and means were compared by the least significant difference (LSD) test. Comparisons with p values <0.05 were considered significantly different.

RESULTS

Cd^{2+} toxicity symptom, growth and pigments

Figure (1) shows that Cd^{2+} treatment induced cholorosis of cucumber leaves and application of compost inhibited the development of Cd^{2+} toxicity symptoms. Application of compost promoted the growth of both shoot and root. Cd^{2+} treatment significantly inhibited the plant growth parameters; however, this inhibition was significantly alleviated by compost application. Cd^{2+} accumulation was noted in the Cd^{2+} -treated plants, and compost treatment notably decreased the accumulation of leaf Cd^{2+} in compost-treated plants, as shown in **Table (1)**.

The Cd^{2+} symptom was in accordance with change of levels of photosynthetic pigments. Cd^{2+} treatment also greatly decreased the levels of Chl a, Chl b, total Chl and carotenoid in cucumber leaves after both 5 and 10 days of treatment; however compost application significantly increased the levels of such pigments, as shown in **Figure (2A–D)**. Unlike the change of pigment contents, Cd^{2+} treatment did not show statistically significant effects on Chl a/Chl b at 5 days and significantly increased carotenoid/ chlorophyll; while addition of compost significantly increased carotenoid/ chlorophyll under Cd^{2+} stress **(Fig. 2E and F)**.

H_2O_2 and lipid peroxidation concentrations

Changes in H_2O_2 concentration in response to compost application after subjecting cucumber plants to Cd^{2+} were evaluated. Cd^{2+} treatment significantly increased H_2O_2 level in cucumber leaves, especially at 10 days after treatment, relative to the control. However, compost application significantly decreased leaf H_2O_2 level in compost-treated and compost + Cd^{2+}-treated plants, as shown in **Fig. (3A)**.

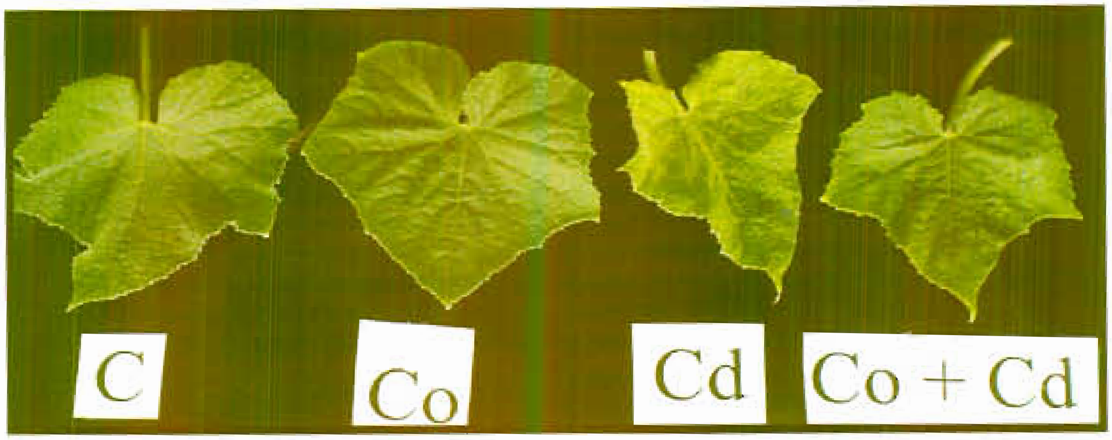

Fig. 1. Effects of compost addition on the phenotype of *Cucumis sativus* leaves treated with Cd^{2+}

Table 1. Effect of compost on shoot height, root length, shoot and root DW, total DW, and leaf Cd^{2+} content of 14 day treatment cucumber plants grown in sandy soils ± Cd^{2+}. (T1), control; (T2) compost; (T3), Cd^{2+} treatment; and (T4) compost + Cd^{2+} treatment

Treatments	Shoot height (cm)	Root length (cm)	Shoot DM (g plant^{-1})	Root DM (g plant^{-1})	Total DM (g plant^{-1})	Cd content (µg g^{-1} DW)
T1	59.64^{b}	80.65^{b}	4.94^{b}	0.552^{b}	5.57^{b}	n.d.
T2	70.08^{a}	89.71^{a}	5.77^{a}	0.625^{a}	6.30^{a}	n.d.
T3	44.22^{d}	49.95^{d}	3.45^{d}	0.396^{d}	3.89^{d}	117.2^{a}
T4	51.95^{c}	58.88^{c}	4.27^{c}	0.495^{c}	4.81^{c}	69.8^{b}

Data show the means of three replicates from three independent experiments. Within a column, mean values followed by small different letters are significantly different ($p < 0.05$).

TBARS were measured as an index of lipid peroxidation and its level is an indicator of the extent of oxidative damage. Changes in TBARS levels were similar to those of H_2O_2. Cd^{2+} treatment significantly increased leaf TBARS level, whereas compost treatment significantly reduced the accumulation of TBARS **(Fig. 3B)**. Thus, it is evident that application of compost considerably decreased the levels of H_2O_2 and TBARS in leaves of compost-treated plants, as compared with untreated ones.

Antioxidant enzymatic activities

Changes in the activities of antioxidant enzymes (SOD, APX, DHAR, and GR) in response to compost application after subjecting cucumber plants to Cd^{2+} were evaluated. As shown in **Fig. (4A)**, compost application increased SOD activity in the leaves of compost and compost plus Cd^{2+}-treated plants. Cd^{2+} treatment significantly increased SOD activity and the highest SOD activity being at in the combined treatment. **Figure (4B)** shows that Cd^{2+} treatment considerably promoted APX activity in the leaves, and the effect was more obvious with the increasing time after treatment. Compost addition significantly increased APX activity. The highest APX activity was noted at 5 and 10 days of the Co + Cd^{2+} -treated plants. As shown in **Fig. (4C)**, Cd^{2+} treatment significantly increased DHAR activity in the Cd^{2+}-treated plants. However, compost application reduced DHAR activity in compost-treated plants, but significantly increased leaf DHAR activity in the cucumber grown under Cd^{2+} stress. **Figure (4D)** shows that treatment with Cd^{2+} significantly increased GR activity in the leaves. Compost addition to sandy soil increased GR activity in the cucumber leaves for 5 and 10 days, with the highest GR activity being at 10 days of compost-treated plants grown under Cd^{2+}.

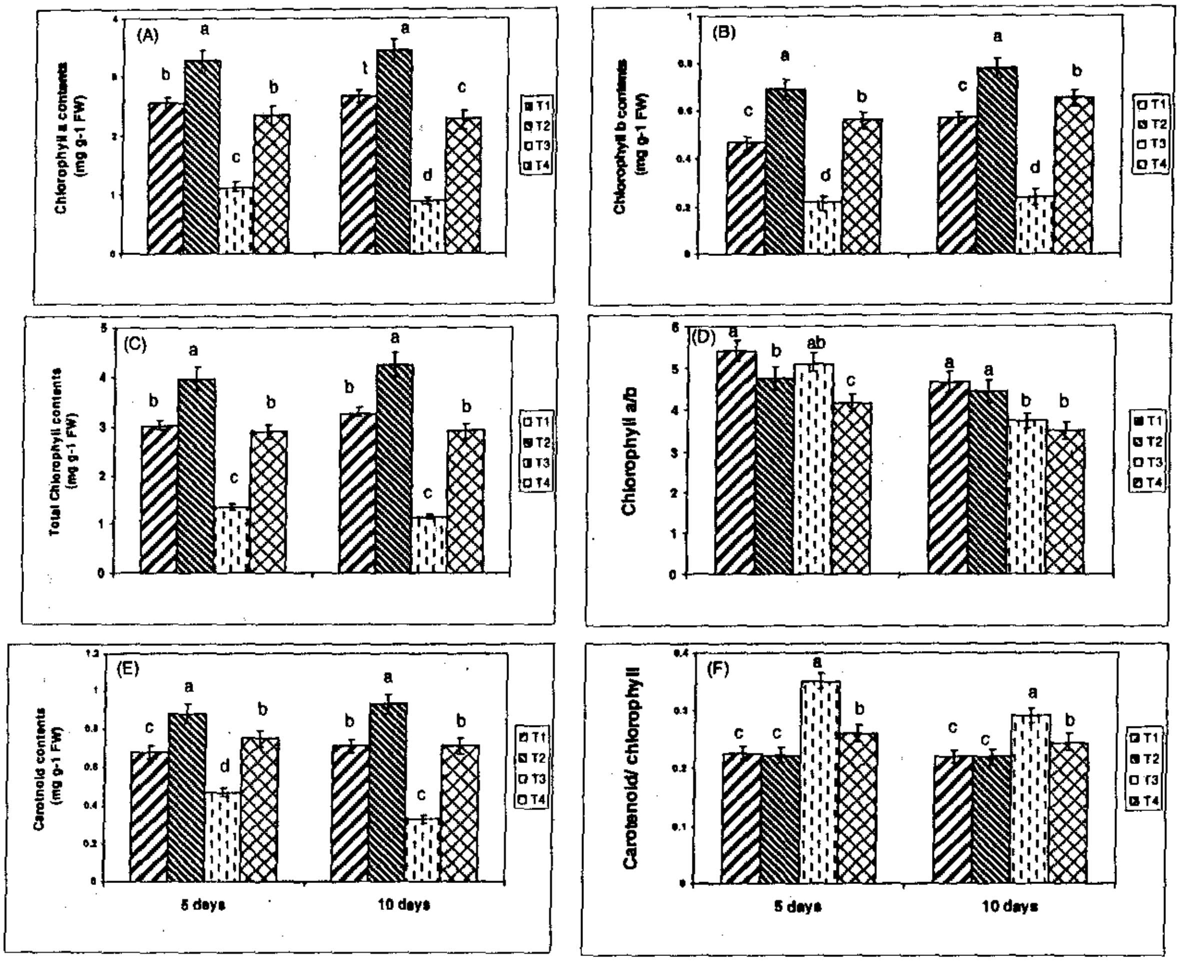

Fig. 2. Effects of compost on chlorophyll a contents (A), chlorophyll b contents (B), carotenoid contents (C), total chlorophyll contents (D), chlorophyll a/b (E) and carotenoid/ chlorophyll (F) in the leaves of *Cucumis sativus* plants grown in sandy soils ± Cd^{2+}. (T1) control, (T2) compost (Co), (T3) Cd^{2+} treatment, and (T4) Co + Cd^{2+} treatment. Data are mean values ± SD. Within the same treatment day, mean values followed by small different letters are significantly different ($P < 0.05$).

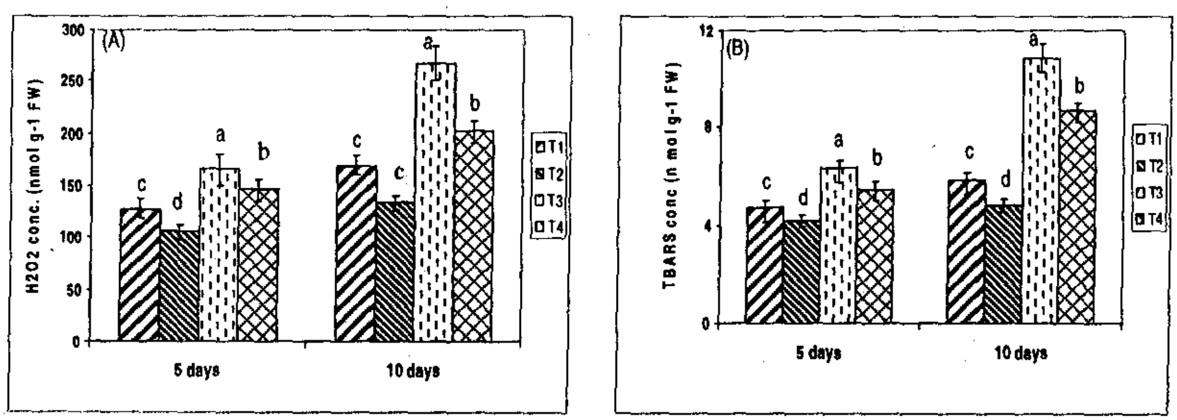

Fig. 3. Effects of compost on H_2O_2 (A) and TBARS (B) in the leaves of *Cucumis sativus* plants grown in sandy soils ± Cd^{2+}. Rest of legend is the same as Fig. 2.

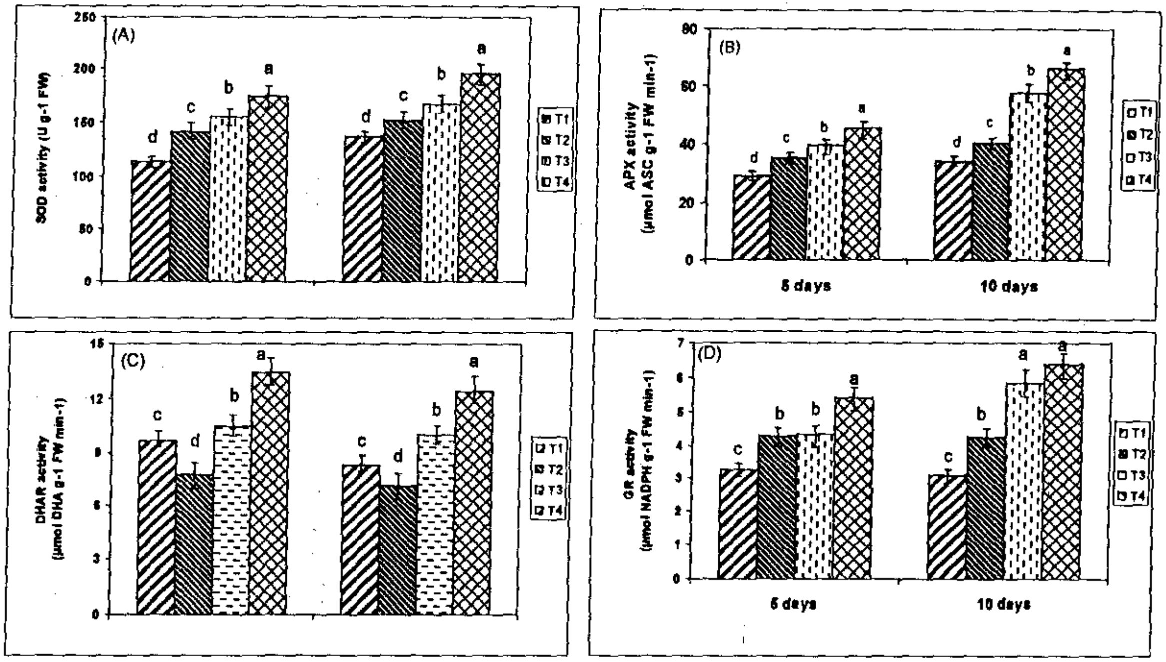

Fig. 4. Effects of compost on activities of SOD (A), APX (B), DHAR (C), and GR (D) in the leaves of *Cucumis sativus* plants grown in sandy soil ± Cd^{2+}. Rest of legend is the same as Fig. 2.

Ascorbate and glutathione concentrations

Fig. (5) shows that Cd^{2+} treatment significantly decreased the levels of ASC, DHA and total ascorbate in the leaves. Compost application increased the levels of ASC and total ascorbate at 5 and 10 days of the cucumber treatment under Cd^{2+} stress. The ratio of ASC/ DHA was significantly reduced by Cd^{2+} stress at 10 days of the treatment; however, this reduction was alleviated by compost addition.

Fig. (6) shows that Cd^{2+} stress treatment considerably decreased the concentrations of GSH and total glutathione. Compost application significantly increased GSH level of compost-treated plants subjected to Cd^{2+} stress. Cd^{2+} stress increased GSSG level when compost was not added. The ratio of GSH/ GSSG was significantly reduced by Cd^{2+} stress; however, this reduction was alleviated by compost addition.

DISCUSSION

Cd^{2+} toxicity can induce complex physiological and biochemical changes in plants. The most obvious symptoms include leaf chlorosis, inhibition of plant growth and chlorophyll biosynthesis (**Das *et al* 1997 and Ekmekci *et al* 2008**). Recently, there is some evidence indicating that compost has a great number of beneficial effects on plant growth under environmental stress conditions, including heavy metal (**Ciecko *et al* 2001**; **Chang-Chien *et al* 2006**; **Tartoura, 2009**; **Antolín *et al* 2010 and Han-Song *et al* 2010**). In the present study, Cd^{2+} treatment of cucumber plants induced leaf chlorosis, reduced plant growth parameters and significantly decreased the levels of photosynthetic pigments, which was positively correlated with the visual symptom of chlorosis. Inhibition of cucumber growth was significantly alleviated by compost application. Similar Cd^{2+} inhibitory effects have been obtained in various plant species (**Pál *et al* 2006**; **Zhang *et al* 2008**; **López-Millán *et al* 2009 and Feng *et al* 2010**). The strong inhibition of chlorophylls and carotenoids contents under Cd^{2+} stress **Fig. (2)** suggests that Cd^{2+} may cause oxidative stress in the leaves of cucumber. However, application of compost reversed the chlorosis and increased the levels of chlorophyll and carotenoids at the same level of Cd^{2+} toxicity. It is known that the decrease of chlorophyll level inhibits plant ability to absorb and utilize light energy, and consequently leads to reduced photosynthesis. Carotenoids are considered to be antioxidants against free radicals since they can remove oxygen from the excited chlorophyll–oxygen complex (**Young, 1991**). Two possible mechanisms of Cd^{2+} toxicity on photosynthesis have been proposed to explain

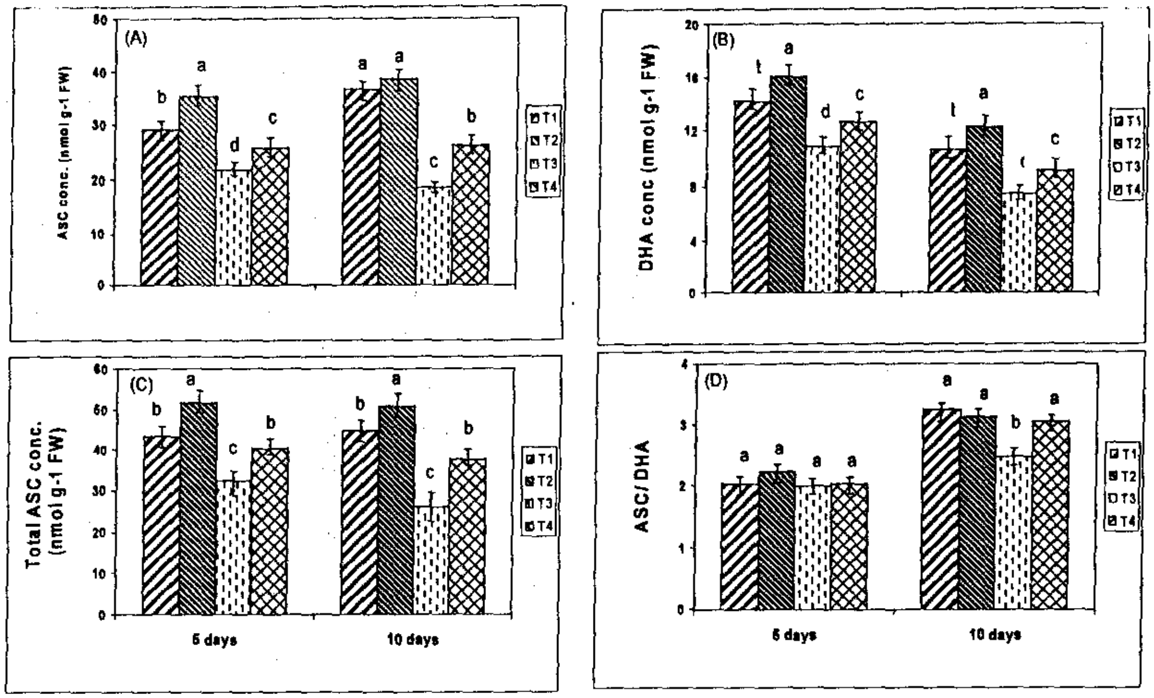

Fig. 5. Effects of compost on the levels of ASC (A), DHA (B), total ASC (C) and ASC/ DHA ratio (D) in the leaves of *Cucumis sativus* plants grown in sandy soil ± Cd^{2+}. Rest of legend is the same as Fig. 2.

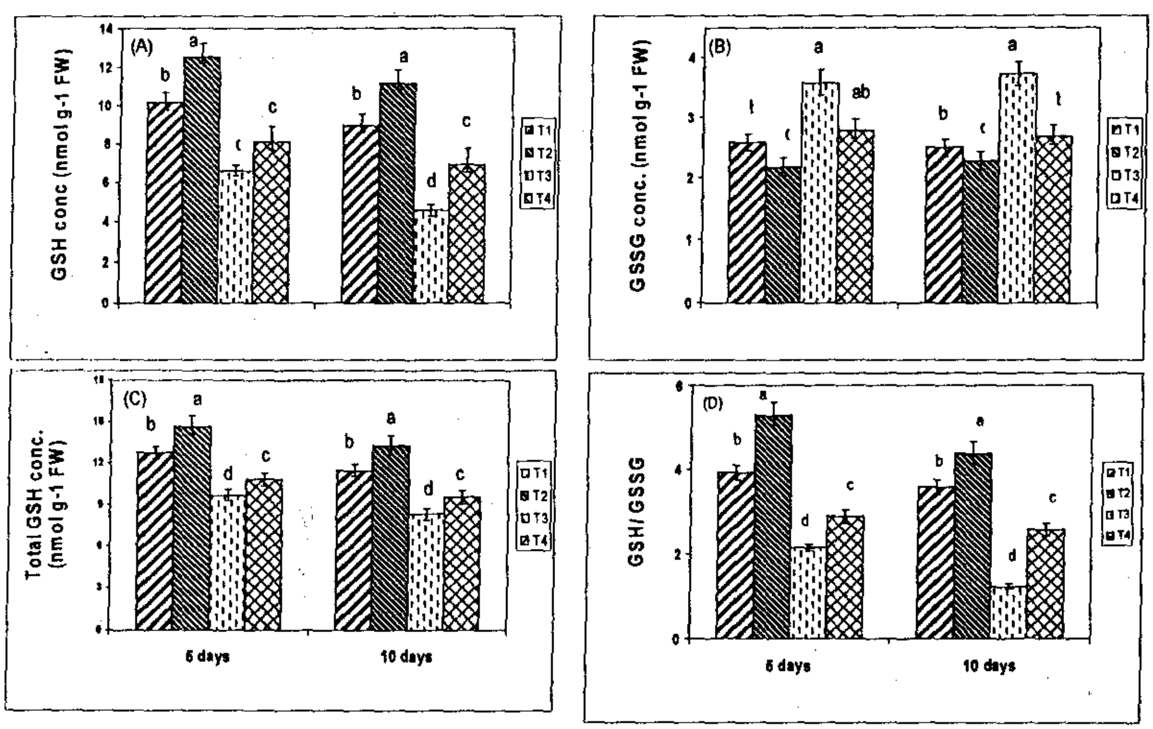

Fig. 6. Effects of compost on the levels of GSH (A), GSSG (B), total glutathione (C) and GSH/ GSSG ratio (D) in the leaves of *Cucumis sativus* plants grown in sandy soil ± Cd^{2+}. Rest of legend is the same as Fig. 2.

the decrease in chlorophyll pigments. Cd^{2+} can affect both chlorophyll biosynthesis by inhibiting protochlorophyllide reductase and the photosynthetic electron transport by inhibiting the water-splitting enzyme located at the oxidizing site of photosystem II, as reported by **Van Assche and Clijsters (1990)**. Cd^{2+} treatment also caused an accumulation of Cd^{2+} in cucumber leaves and application of compost decreased the leaf Cd^{2+} content. The lower levels of Cd^{2+} in cucumber leaves may be explained by the fact that compost acts as an immobilizing agent of heavy metals that makes Cd^{2+} less soluble and consequently decreased Cd^{2+} uptake of cucumber plants, as also reported by **Bolan & Duraisamy (2003)**; **Han-Song *et al* (2010)**, and **Satoa *et al* (2010)**. It may be concluded that compost alleviated the inhibitory effect of Cd^{2+} on growth parameters and photosynthetic pigments of cucumber plants.

Oxidative stress is a well-documented effect of metal toxicity in plants, including Cd^{2+} stress. In the present study, Cd^{2+} at 100 µM caused an enhanced level of TBARS in cucumber leaves, which is an index of lipid peroxidation and, therefore, of oxidative stress. Similar to TBARS, considerable leaf H_2O_2 formation was noted in the Cd^{2+}-treated cucumber. However, under the same level of Cd^{2+} level, application of compost decreased the levels of H_2O_2 and TBARS, indicating that compost alleviates Cd^{2+} induced oxidative damage.

Hydrogen peroxide could be efficiently eliminated by non-enzymatic and enzymatic defense systems (**Ekmekci *et al* 2008**). Superoxide dismuatase and ascorbate–glutathione cycle enzymes play important roles in removing H_2O_2 under oxidative stress conditions. SOD constitutes the first line of defense against ROS, which is crucial for the removal of $O_2^{\cdot-}$ in the compartments where $O_2^{\cdot-}$ radicals formed (**Shao *et al* 2008**). In the present investigation, a significant enhancement in SOD activity was noted in cucumber plants when exposed to Cd^{2+} stress, suggesting an important role in removing $O_2^{\cdot-}$ induced by Cd^{2+}. Application of compost further increased SOD activity in compost-treated plants subjected to Cd^{2+}-contaminated soil, and therefore increasing the $O_2^{\cdot-}$ anion scavenging activity. The decomposition of $O_2^{\cdot-}$ radical is accompanied by production of the toxic metabolite H_2O_2.

Enzymes of ASC and GSH cycle play a crucial role in removing ROS and maintaining the cellular redox status (**Drazkiewicz *et al* 2003 and Liu *et al* 2007**). In this cycle, APX mainly plays an important role in removing H_2O_2 and DHAR and GR can provide substrate for APX by catalyzing reactions. In this study, activities of APX, DHAR and GR were increased in cucumber plants when subjected to Cd^{2+} stress, suggesting their active role in constant detoxification of H_2O_2 under Cd^{2+} toxicity. DHAR and GR could keep higher substrate for APX by increasing their activities. Application of compost further increased activities of APX, DHAR and GR in compost-treated plants under Cd^{2+} stress, indicating that compost effectively improves Cd^{2+} tolerance in cucumber to oxidative stress via increasing the efficiency of ASC and GSH cycle. Our data suggest compost application resulting in improvement of Cd^{2+} tolerance, which associated with increasing the activities of antioxidant enzymes.

Additionally, ASC and GSH are two important non-enzymatic antioxidants, which also scavenge ROS under oxidative conditions and in turn protect the cellular membranes from lipid and protein oxidation (**Foyer, 2003**). In this study, Levels of ASC and GSH significantly decreased in cucumber leaves treated with Cd^{2+}. The decline of ASC level reduced the availability of substrate for APX, resulting in elevation of H_2O_2 accumulation. GSH depletion could cause oxidative stress, as also reported by **De Vos *et al* (1992)**. According to **Metwally *et al* (2005)**, more Cd^{2+}-sensitive pea genotypes had lower root GSH concentrations, whereas less sensitive genotypes had higher root GSH concentrations in response to Cd^{2+} treatment. GSH concentrations were also higher in Cd^{2+}-tolerant genotypes than in Cd^{2+}-sensitive genotypes of *Silene vulgaris* (**De Knecht *et al* 1994**). Enhanced levels of such antioxidants in Cd^{2+}-treated cucumber plants grown in compost amended soil indicate their active involvement in detoxification of ROS directly as well as through the ASC-GSH cycle, as also reported by **Asada and Takahashi (1987)**. Indeed, one mechanism of increasing tolerance to Cd^{2+} toxicity may be the formation of non-enzymatic antioxidants. The protective roles of ASC and GSH in plant protection against heavy metal stresses have previously been reported by **Xiang *et al* (2001) and Kwon *et al* (2003)**. In addition, **Zhao *et al* (2005)** showed that wheat plants treated with L-galactono-1, 4-lactone (GalL), the biosynthetic precursor of ASC were more Cd^{2+} tolerant. The metabolite Monodehydroascorbate, as a result of APX catalysis and certain non-enzymatic reactions, is unstable and highly reactive, and it disproportionates spontaneously to DHA that is reduced by DHAR using GSH as an electron donor. Compost-treated plants had higher leaf DHAR activity than that in untreated ones under Cd^{2+} toxicity. In this respect, **Kwon *et al* (2003)** reported that transgenic tobacco plants expressing a human DHAR gene properly worked for the protection against oxidative stress in plants. Elevated DHAR activity in cucumber leaves grown in Cd^{2+}-contaminated soil could increase the conversion of DHA to ASC. However, level of ASC decreased in Cd^{2+}-treated plants may be due to increasing oxidation of ASC and prevention of ASC formation under Cd^{2+} toxicity. In agreement with the present results, **Romero-Puertas *et al* (2007)** indicated that ASC content was decreased under Cd^{2+} stress. On the other hand, the decrease in DHA level led to a decrease in the substrate of DHA to ASC catalyzed by DHAR, resulting in a lower ASC/DHA ratio. Addition of compost increased ASC level at Cd^{2+} stress, which probably attributed to increased DHAR activity or lower oxidative stress caused by compost application. GSH is oxidized to GSSG during the conversion of DHA to ASC, which depends on GR to reform GSH by oxidizing NAD(P)H to $NAD(P)^+$. Present results showed that there is a gradual shift to more oxidized cellular redox status as reflected by the decrease in the ratios of ASC/ DHA and GSH/ GSSG. In fact, it is well established that not only ASC and GSH concentrations, but also the ASC/ DHA and GSH/GSSG ratios are important to maintain cellular redox status (**May *et al* 1998**; **Smirnoff and Wheeler, 2000**). Present data indicate that high ASC/ DHA and GSH/GSSG ratios could be maintained by compost application in the cucumber plants exposed to Cd^{2+} treatment.

Based on the foregoing results, the increase in antioxidant compounds and related enzyme activities under heavy-metal stress can help plants to better survive under Cd^{+2} stress conditions . We showed that compost increased enzymatic and non-enzymatic antioxidants. This evidence suggested that compost alleviates Cd^{+2} induced oxidative damage by increasing the expression of genes related to production of antioxidant molecules and antioxidant enzymes activities. In agreement with our data, a correlation between the intracellular antioxidant capability to reduce ROS and heavy metal-stress tolerance has been demonstrated in a number of plant species (**Freeman *et al* 2004**; **Halliwell, 2006**; **Song *et al* 2009**). The functional roles of compost in protecting cucumber plants against Cd^{2+} toxicity are mainly attributed to the beneficial effects of compost application on the physicochemical properties of soil and its biology. Compost components could synergistically act in affecting numerous physiological and biochemical functions of plants, including water and mineral uptake and transport, photosynthesis, enzyme activation and osmotic potential. In addition, compost represents a good source of all macro- and micronutrients, organic matters, hormone-like substances, biotic agents, carbon dioxide, nitric oxide and many others, which play important roles in stimulating metabolic processes, promoting growth, and increasing the synthesis and accumulation of more metabolites in plant tissues, as also reported by **Akiyama *et al* (2003)**, **Heather *et al* (2006)**, **Geissler *et al* (2009)**, **Pilon-Smits *et al* (2009)**, and **Jia *et al* (2010)**. Apart from nutrients and organic matters, composted organic matters contain a substantial amount of humic substances (**Delana *et al* 1990**), which affect plant growth and development. In addition, Cd^{+2} may be tightly bound to insoluble organic matter such as large humic acid molecules and humin, thereby increasing the capacity of soil to adsorb Cd^{2+} (**Kaschl *et al* 2002**). Further, humic substances contain a variety of functional groups, including COOH, phenolic OH, enolic OH, alcoholic OH, quinone, hydroxyquinone, lactone, and ether (**Stevenson, 1994**). According to **Xia** and **Rayson (2002)**, the binding of Cd^{2+} to various organic materials was associated with carboxylate functionalities. The kinetic experiment showed the evidence that the Cd^{2+} binding occurs via an ion exchange as well as by electrostatic interaction between carboxylate groups and Cd^{2+} (**Choi and Yun, 2006**). The esterification of carboxyl groups and hydrolysis of ester groups in the native biomass provided strong evidence that the carboxyl functionality is the main group responsible for binding Cd^{2+} (**Sawalha *et al* 2007**).

CONCLUSIONS

The results of the present work suggest that compost application resulting in reducing the Cd^{+2} uptakes due to changing of its available forms to some unavailable forms. Compost addition also resulted in efficient antioxidant machinery systems allowed the compost-treated plants to cope better with Cd^{+2} oxidative stress.

The results of the present work also suggest that Cd^{2+} treatment can increase the accumulation of H_2O_2, induce lipid peroxidation, and chlorophyll degradation in the cucumber plants. However, compost application protects plants from Cd^{2+}-induced oxidative stress damage and increased Cd^{2+} tolerance in cucumber plants. This protection is achieved via increased the capacity of the antioxidant machinery system that allowed the cucumber plant to cope effectively better with heavy metal stress. All together, the obtained results suggest that compost application could be advantageous against Cd^{2+} toxicity and may confer plant tolerance to soil contaminated with Cd^{2+}.

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حوليـــات العلــوم الزراعية
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تأثير الكمبوست على نظم مضادات الأكسدة الانزيمية لنباتات الخيار ضد سمية الكادميوم

[١٥]

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الموجـــز

توثر العناصر الثقيلة بما فيها الكـادميوم تـأثيرا سلبيا على نمو وتطور وانتاجية النباتـات المختلفـة. أجرى هذا البحث بهدف دراسة تأثير الكمبوست على النمو، صـبغات التمثيـل الـضوئى المتمثلـة فـى الكلوروفيلات والكاروتينويدات، أنـشطة مـضادات الأكـسدة الانزيميـة المتمثلـة فـى انزيمـات superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR) بالاضافة الى مـستويات مـضادات الأكـسدة غيرالانزيمية المتمثلة فى الأسـكوربيت ascorbate، الجلوتاثيون glutathione فى نباتات الخيار المعاملـة بالكادميوم بتركيز ١٠٠ ميكرومولار. تـم اسـتخدام أربعة معاملات هى كـالآتى: (١) مقارنـة دون أى معاملات، (٢) كمبوست بمعدل اضافة للتربة بمعـدل ١٠% وزنا لكل وزن، (٣) كبريتات كادميوم بتركيز ١٠٠ ميكرمولار، (٤) كمبوست + كادميوم. أظهرت نتائج تلك الدراسة أن النباتات المعاملـة بالكـادميوم احتوت أوراقها الحديثة التكوين على اصفرار، تثبيط كل من نمو نباتات الخيـار، بنـاء الكلـوروفيلات، الكاروتينويدات فيها مقارنة بالنباتات عير الكونترول. وقد احتوت النباتات المعاملة بالكادميوم على زيـادة معنوية فـى محتـوى الأوراق مـن فـوق أكـسيد الأيدروجين، نواتج أكسدة الليبيدات المتمثلة فى تراكم الـ thiobarbituric acid reactive substances ويـدل ذلك على أنها فى مرحلة اجهاد تأكسدى نـاتج عـن تعرضها لسمية الكادميوم. من ناحيـة أخـرى فقـد احتوت النباتات المعاملة بالكمبوست على مـستويات منخفضة من كل من الشق الحر المتمثل فـى فـوق أكسيد الأيدروجين، نواتج أكسدة لبيبدات الأغشية فى حين زاد محتواها من صبغات التمثيل الضوئى وقـد انعكس ذلك واضحا فى تحسين نموها. تقترح نتـائج تلك الدراسة أن استخدام الكمبوست قد ادى الى حماية نباتات الخيار من تلف الاجهاد التأكسدى الناشئ مـن التأثير السام لعنصر الكادميوم. تعزى تلك الحماية الى الزيادة المعنوية فى أنشطة مضادات الأكسدة الانزيمية وغير الانزيمية السابق الاشارة اليها التى أدت أدوارا فسيولوجية هامة فى تخفيض مستويات فـوق أكـسيد الأيدروجين وبالتالى عدم حـدوث أكـسدة لليبيـدات الأغشية البلازمية واحتفاظها بحيويتها. يـستنتج مـن تلك الدراسة أن مكونات الكمبوسـت المتمثلـة فـى المغذيات الكبرى والصغرى، المادة العضوية، المواد الدبالية، وأشباه الهرمونات النباتية، الكائنـات الحيـة الدقيقة المفيدة لنمو وتطور النباتات، انبعـاث ثـانى أكسيد الكربون، أكسيد النيتريك قد شاركت بشكل فعال فى عمليات التحول الغـذائى ذات العلاقـة بتخفيـف الاجهاد التأكسدى الناتج عن التأثير السام للكادميوم.

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