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Effect of exogenous application of α-tocopherol on antioxidant enzyme activities and lipoperoxidation content of faba bean (*Vicia faba* l.) Under cadimium stress

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Abstract

Field experiment we attempted to clarify the mechanism that might be involved in the ameliorative effects of α-tocopherol on faba bean plants grown under different Cd levels. The effect of α-tocopherol on MDA, glutathione contents, antioxidant enzyme activities and growth parameters.These were studied in plants grown in a nutrient solution supplemented with different concentrations of Cd (0 or 150µmol/l) and sprayed with α-tocopherol at concentrations of 0, 50 and 100 mg/l. Heavy metal stress caused an increase in malondialdehyde (MDA) content and the activity of certain enzymes, but glutathione were strongly depressed at relatively high Cd levels. Application of α-tocopherol caused a significant increase in ascorbate peroxidase (APX) and catalase (CAT) activity in the plant compared with untreated plants. Antioxidant activities are enhanced, helping to reduce oxidative damage and develop tolerance to heavy metal stress in α-tocopherol-treated bean plants. This α-tocopherol-induced increase in tolerance to heavy metal stress. The results show that treatment with α-tocopherol reduced the adverse effects of Cd stress on bean plants and may play a key role in stress tolerance.

Keywords : Antioxidant enzymes, cadmium, lipid peroxidation, cadimium and *Vicia faba*

Introduction

Agroecosystem contamination by cadmium (Cd), a persistent pollutant is a grave problem as it can remain in soils for a long time (Gall et al., 2015, Rashmi et al.,2020). Cadmium being highly soluble and readily available is promptly taken up by plants and introduced into food chains becoming toxic for all organisms including plants, animals and even humans (Dar et al., 2015 ; Naikoo et al., 2021). The heavy metals like cadmium (Cd) and other pollutants easily absorbed by soil and accumulated in different plant parts such as root, stem and leaf. Cd induces clastogenic and genotoxic disturbance in plants and inhibits root growth and cell division in different plants (Shikha and Singh, 2021 ; Fojtova and Kovarik, 2000). Available reports confirm that Cd induces oxidative burst via the generation of reactive oxygen species (ROS), such as H_2O_2, O_2.- radicals and OH radicals as well as disturbance to antioxidative systems (Romero-Puertas *et al.,* 2004).

The leguminous crop (Vicia faba L.) is one of the leguminous crops belonging to the family of leguminous Fabacea and is cultivated in various parts of the world, including Iraq. The nutritional importance of this winter crop is that green pods can be consumed directly after cooking and their fresh and dry seeds contain protein and considered food source for a number of countries in the world, especially Asian and low-income countries (Abdul Hafez 2011)

Vicia faba (broad bean) have been used widely to detect and evaluate toxicity of toxic chemicals (Iqbal, 2016., Leme and Marin-Morales, 2009, Iqbal and Bhatti, 2014, Iqbal and Bhatti, 2015, Qureshi et al., 2015). α-Tocopherol is a low molecular weight lipophilic membrane-located antioxidant. It protects cell membranes from oxidative damage (Taha et al., 2018) and polyunsaturated fatty acids from lipid peroxidation (Krieger-Liszkay and Trebst, 2006), and improves membrane stability and permeability. It helps also to provide an optimal environment for the photosynthetic machinery (Wise and Naylor, 1987). There was a positive correlation between α-Tocopherol and shoot/root growth in two grass species; tall fescue and creeping bentgrass (Zhang and Schmidt, 2000).

Tocopherols are believed to protect chloroplast membranes in plants from photo oxidation and help to provide an optimal environment for the photosynthetic machinery, their accumulations also occur as a response to variety of abiotic stress including high light, drought, salt and cold and may provide an additional line of protection from oxidative damage (Munné-Bosch and Algere, 2002). Therefore, this work was conducted to compare the effect of cadimium stress on

biochimicals parameters and antioxidant responses of faba bean (*V. faba* L.) in heavy metal tolerance and to examine whether exogenous application with vitamin E could mitigate the adverse effect of cadimium stress.

MATERIEL AND METHODS

Plant Material and Growth Conditions

Seeds of *Vicia faba.* L were germinated in the dark at 25°C for 7 days. After germination, plants were transferred to separated containers for hydroponics. Three-liter pots were used, containing ten plants each. Plants were grown in a controlled climate room at 24±2 °C and 50 % relative humidity, with a photoperiod of 16 h and a light intensity of 150µmol m^{-2} s^{-1}. The hydroponic medium consisted of Hoagland nutrient solution (Hoagland and Arnon, 1959). The medium was continuously aerated (100 ml min^{-1}) and replaced every 3 days. At three-leave stage, The 14-day-old *Vicia faba* seedlings were treated with two heavy metals (cadmium) added separately to the nutrient solution in the form of CdCl2, at a constant concentration of 150µmol/l combined with different concentrations of α-Tocopherol (0mg/1, 50mg/l, 100 mg/l). The seedlings were harvested after 14 days of treatment, and split into two parts : roots and stems.

Analytical methods

Lipidperoxidation measurement

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA, a product of lipid peroxidation) depending on the method of Heath and Packer (1968). Leaf samples (0.5 g) were homogenized in 3mL 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 10000×g for 10min. Then, the supernatant (1mL) was mixed with 4mL thiobarbituric acid (TBA) rea- gent (0.5% of TBA in 20% TCA), heated in a water bath at 95C for 30min and quickly cooled by transferring in ice bath. After that, MDA content was measured by observing the difference in absorbance at 532nm using an extinction coefficient of 155mM^{-1} cm^{-1} and expressed as nmol of $MDAg^{-1}$ FW.

Determination of membrane stability index

Membrane stability indices (MSI) were estimated, in 9 samples for each treatment, using duplicate 0.2 g samples of fully-expanded leaf tissue (Rady, 2016). One sample of each duplicate was placed in test-tube containing 10 ml of double-distilled water and heated at 40 °C in a water bath for 30 min. The electrical conductivity (C_1) of the solution was recorded using a conductivity bridge. The second sample was boiled at 100 °C for 10 min, and the conductivity was also measured (C_2). The formula: MSI (%) = $[1 - (C_1 \div C_2)] \times 100$ was applied for calculating the MSI.

Determination of glutathione

Glutathione is determined according to the method of Weck Berker and Cory (1988), which is based on measuring the absorbance of 2-nitro 5 mercapturic acid resulting from the reduction of 5-5 acid 'thiol-bis-2-nitrobenzoic (DTNB).

Enzymes extraction and activities determination

Leaves (0.5g, fresh weight) were homogenized in 50 Mm potassium phosphate buffer pH 7.6. Then, the homogenized samples were centrifuged at 12000×g for 20 min and the supernatant crude ex- tract was used for the estimation of both catalase (CAT) and ascorbate peroxidase (APX) activities (Loggini et al., 1999). CAT activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2 (Cakmak and Horst, 1991). The reaction mixture 3 ml contained 50mM phosphate buffer pH 7.2, 15 mM $H_2O_2$and 100 µl of crude enzyme extract. Enzyme was quantified using the extinction coefficient 39400 $M^{-1}cm^{-1}$. Ascorbate peroxidase (APX) activity was determined following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 ml of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H_2O_2 and 100 µl of crude enzyme extract (Nakano and Asada, 1981). Enzyme was quantified using the extinction coefficient 2800$M^{-1}cm^{-1}$.

Statistical analysis

Results were expressed as mean ± SD of three separate experiments. For each experiment, statistical analysis was performed using one-way ANOVA and the multiple comparison

method of Tukey, with the aid of R 3.4.3 computer program.

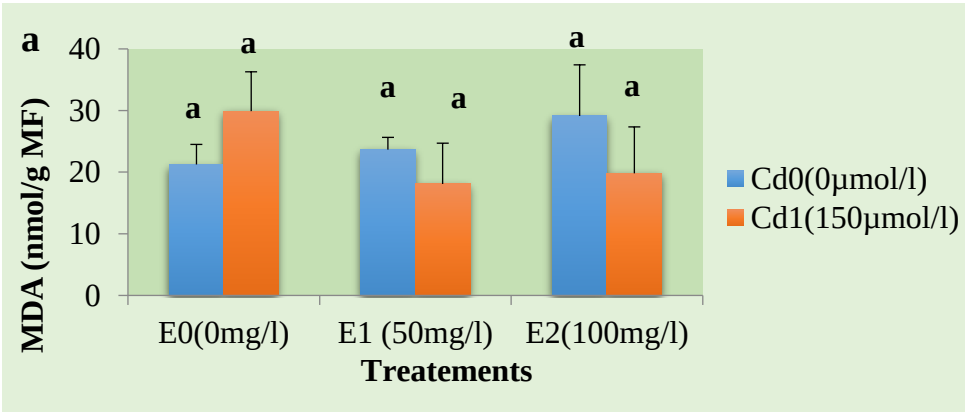
Results

The concentration of membrane lipoperoxides (MDA)

The level of malondialdehyde (MDA) resulting from the breakdown of polyunsaturated fatty acids in the plasma membrane. The accumulation of malondialdehyde was monitored in the roots and leaves of *Vicia faba*.

Figure 1a shows that at leaf level, the quantity of MDA did not show a significant difference between the groups (P=0.163). The accumulation of MDA varied according to the concentration of Cd and α-tocopherol, with Cd at 150µmol/l increasing the MDA content significantly compared with the control, whereas treatment with α-tocopherol (50mg/l, 100mg/l) reduced membrane lipoperoxides in the stressed groups.

At root level **(Figure 1b)**, there was a highly significant difference in MDA content between the six groups (P=0.004). The Cd concentration of 150 µmol/l was able to increase MDA production to a value higher than that of the aerial part, but treatment with α-tocopherol had an effective effect in reducing this metabolite in the roots. According to the statistical analysis, there was a significant difference between the group stressed by Cd alone and the groups treated with α-tocopherol under cadmium stress or not**.**



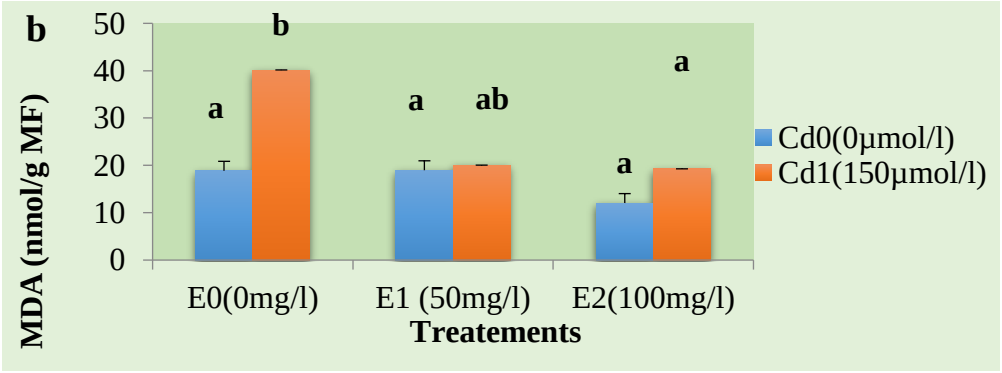


Figure 1 : Effects of α-tocopherol on malondialdehyde (MDA) levels in leaves (**a)** and roots (**b**) of bean seedlings under and without cadmium stress.

Glutathione (GSH)

Figure 2 shows the combined effect of Cd150µmol/l and α-tocopherol (50mg/l, 100mg/l) on glutathione content in the aerial and root parts of *vicia faba* seedlings. Our results show that the treatment does not induce a significant change in the quantity of GSH between the six groups in the two parts of the faba plant.

It was noted that the content of this metabolite was higher in the aerial part **(Figure 2a)** than in the root part (**Figure 2b).** It was observed that cadmium stress led to a sharp decrease in GSH compared with the control group, whereas the glutathione content increased with the increase in the concentration of α-tocopherol, regardless of whether or not the aerial and root parts were stressed.

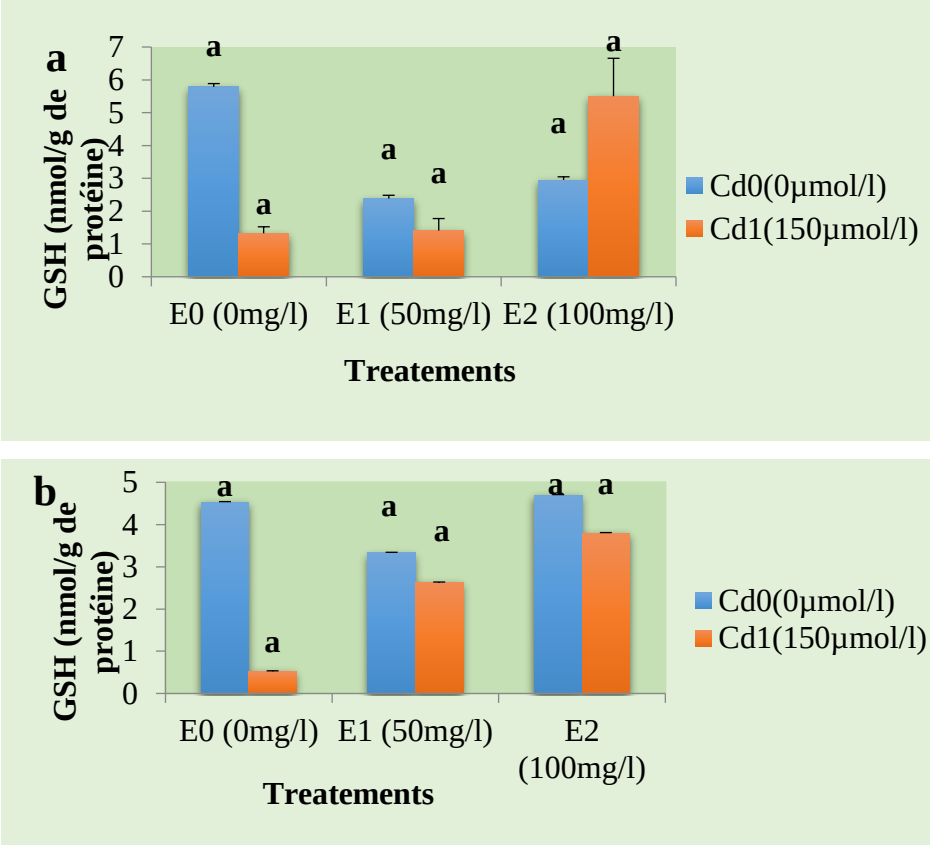


Figure 2 : Effects of α-tocopherol on glutathione content in the aerial part (**a**) and the root part (**b**) of bean seedlings subjected or not to cadmium stress.

The membrane stability index (MSI)

The results presented in **Figure 3** show no significant difference in the leaf membrane stability index between the six groups (P=0.881).

However, a downward trend in stability was recorded in the group stressed with 150 µmol/l CdCl2 in the absence of treatment, whereas the increase in MSI was accompanied by an increase in α-tocopherol concentrations (50mg/l and 100mg/l).

It was noted that in the groups exposed to stress, treatment with this vitamin improved the MSI to a certain threshold, depending on the concentration of α-tocopherol. Application of 50mg/l of this vitamin resulted in high membrane stability, which was reduced when the concentration was increased to 100mg/l.

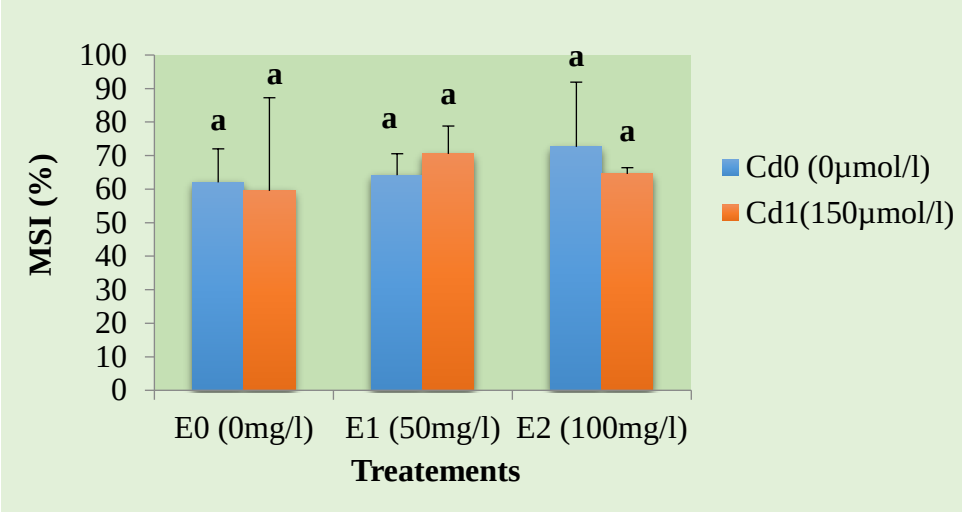


Figure 3 : Effects of α-tocopherol on the leaf membrane stability index in bean seedlings with and without cadmium stress.

Enzyme biomarkers

Catalase (CAT)

The results of the catalase activity are presented in **Figure 4**. In the aerial part **(Figure 4a)**, there was no significant difference between the groups (P=0.352). It can be seen that the presence of α- tocopherol at a concentration of 50mg/l in the group under cadmium stress led to a significant reduction in catalase compared with the group stressed by 150µmol/l Cd alone, whereas this activity was almost absent in the other groups.

In the root zone **(Figure 4b)**, there was a highly significant difference between the six groups (p = 0.00000761). We noted an increase in the activity of this enzyme in the Cd-stressed group

to a very high value compared with that of the aerial zone, whereas the exogenous application of α-tocopherol (50mg/l, 100mg/l) resulted in a highly significant decrease compared with the groups under or without cadmium stress**.**

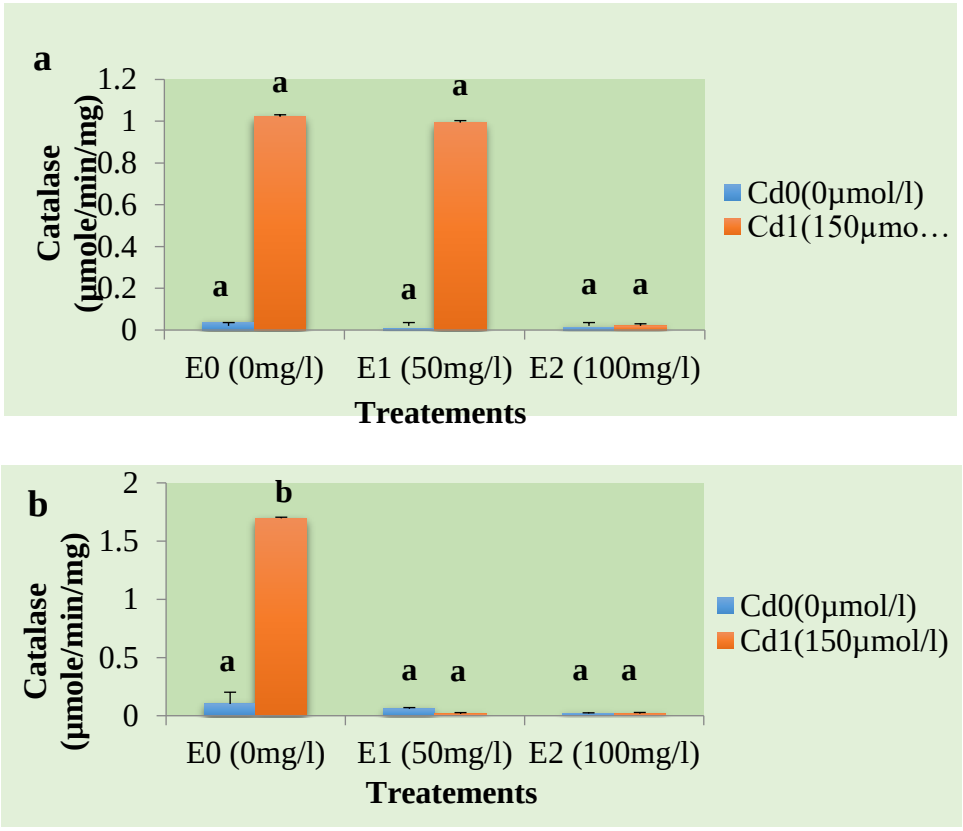


Figure 4 : Effects of α-tocopherol at different concentrations on catalase (CAT) activity in the aerial part (**a)** and the root part (**b)** of bean seedlings subjected or not to cadmium stress.

Ascorbate peroxidase (APX)

Figure 5 shows the effect of α-tocopherol at different concentrations on ascorbate peroxidase (APX) activity in both cadmium-stressed and non-cadmium-stressed parts of the bean.

In the aerial part **(Figure 5a)**, cadmium disrupted total APX activity. Our results show that there was a highly significant difference in the activity of this enzyme between the six groups (P= 0.000974), there was an increase in ascorbate peroxidase in the group stressed with Cd 150 µmol/l, while in the groups treated with Cd and α-tocopherol at different concentrations there was a highly significant decrease in the activity of this enzyme, while the groups treated only with α-tocopherol (50mg/l, 100mg/l) had lower APX activity than the control.

In the roots, ascorbate peroxidase activity is low compared with the aerial part, but from our results **(Figure 5b),** there is a highly significant difference between the six (06) groups (P= 0.00548). We can see that the group stressed by 150 µmol/l Cd has a very high APX activity compared with the control, while the other groups have a similar activity of this enzyme.

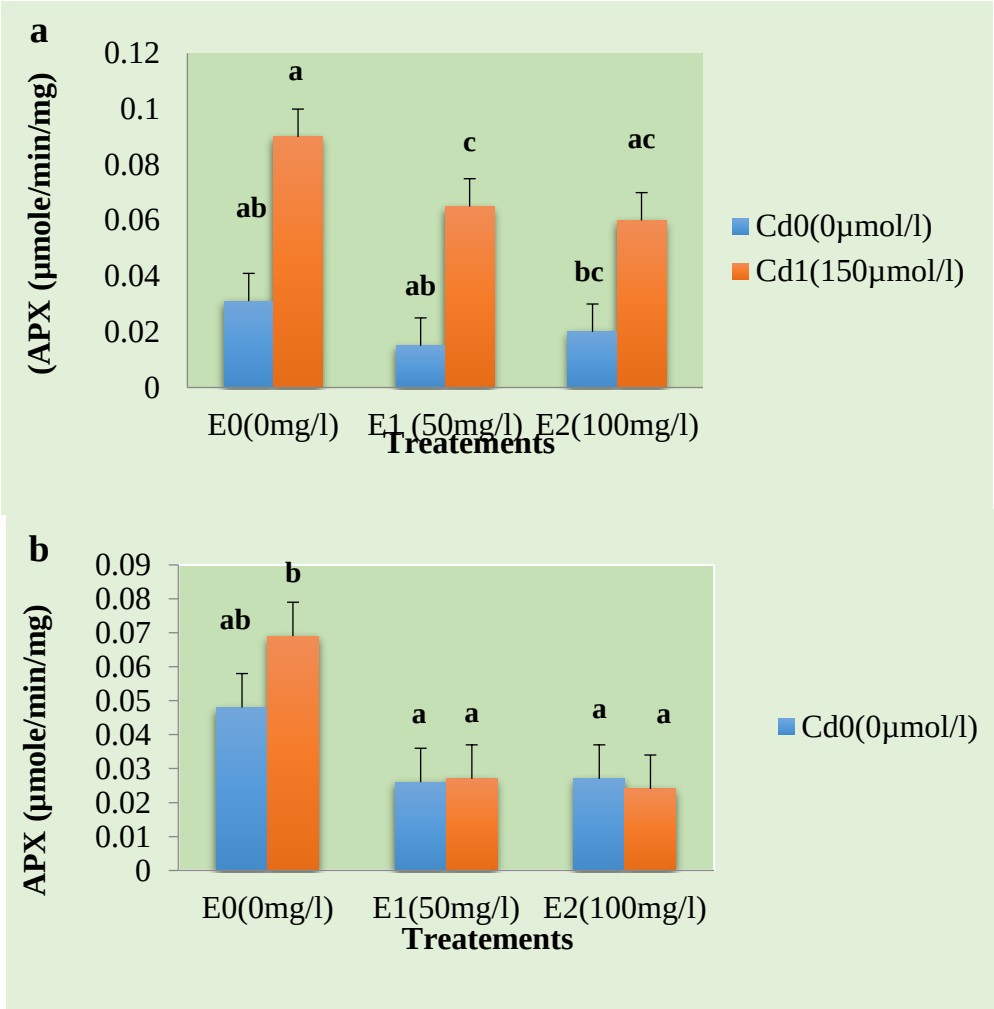


Figure 5 : Effects of α-tocopherol on ascorbate peroxidase activity in the aerial part (**a**) and the root part (**b)** of bean seedlings subjected or not to cadmium stress.

Discussion

The response of antioxidant enzymes to metal-induced oxidative stress helps to alleviate cellular damage by limiting ROS , such as catalase (CAT), which is located in peroxisomes and mitochondria, and ascorbate peroxidase (APX), which is located in chloroplasts and cytosol.

In our experiments, we found that Cd led to an increase in enzyme activity. Our work is in perfect agreement with the work of Souguir, (2009) who showed an activity of this enzyme in the roots. The study of Amin Mohamed et al, (2012) reported on the contrary a decrease in the activity of CAT in *brassica juncea* stressed by Cd.

We found an increase in APX in both parts of the bean in the presence of Cd this study is in agreement with the work of Benhamdi (2014) in *Arginessu* species and Mechakra (2014) in *Lygeum spartum L* species.

The increase in the activity of these enzymes is closely linked to the increase in the intracellular concentration of hydrogen peroxide, which is produced during oxidative stress. H2o2 is eliminated by CAT, POD and the enzymes of the ascorbate glutathione cycle (Kaya et al., 2020 ; Ashraf, 2009 ; Xu et al., 2011), in which APX catalyses the H2O2 reaction using ascorbate as a substrate (Iturbe-Ormaetxe et al., 2001 ; Anjum et al.,2016).

It was noted that APX activity is higher than that of CAT, indicating the high affinity of APX for H2O2 observed by Gill, whereas catalase does not require reducing power and has a high reaction rate but low affinity for H2O2 (Willekens et al., 1997 ; Mahaseth and Kuzminov, ,2017).

Furthermore, it was found that exogenous application of α-tocopherol (50mg/l, 100mg/l) during cadmium stress led to a decrease in the enzymatic activity of (CAT, APX) in both parts of the bean. In contrast, Sakr and El-Metwally (2009) and Khamees AL-Kareemawi and Muhmood (2019) recorded increases in antioxidant enzymes in response to the application of a-tocopherol to wheat against oxidative stress.

The reduction in catalase and APX activity is explained by the intervention of this vitamin in ameliorating the deleterious effects of ROS. Sadiq et al (2016) and El-Bassiouny et al (2015) found that α-tocopherol and nicotinamide suppressed lipid peroxidation and maintained the permeability of the plasma membrane. Protection against phytotoxic peroxidation in lipophilic environments can be achieved by antioxidants, such as α-tocopherol (vitamin E), which is thought to be the most effective radical-breaker. Consequently, many attempts have been made to reduce oxidative stress in plants by exogenous application of this vitamin (Hasanuzzaman and Fujita, 2014 ; Vendruscolo et al.,2019).

Some authors have demonstrated an increase in lipid peroxidation following exposure to heavy metals (Gill and Tuteja, 2010 ; Maria and Bebianno,2011). Heavy metals induce the production of ROS (such as $1O_2$, $O_2.^-$, OH.).

Root accumulation, which may indicate the development of oxidative stress in the roots of seedlings. The extent of lipoperoxidation seems to be related to the preferential accumulation of metal ions in this organ. TheROS formed and accumulated during Cd-induced stress will react with the polyunsaturated fatty acid to form lipid radicals and reactive aldehydes, leading to a drop in membrane fluidity and damage to membrane proteins (Gill and Tuteja, 2010). It

has been shown that the first site damaged by heavy metals is the cell membrane. This is due to an attack on its polyunsaturated fatty acids, the product of which is malondialdehyde (or MDA).

The high MDA content in aerial parts is due to the fact that, in addition to mitochondria and peroxisomes (the sites of free radical generation), they contain chloroplasts, which are often considered to be the main source of ROS in photosynthetic organisms (Foyer and Noctor, 2003 ; Edreva, 2005 ; Asada, 2006). Heavy metals generate ROS and cause chlorophyll degradation (Lichtenthaler and Miehé, 1997 ; Rau et al., 2007). This is due to the peroxidation of chloroplast membranes and the reduction of thyaloid membrane constituents (Shi et al., 2016).

Treatment with α -tocopherol resulted in a decrease in MDA content. This result is in agreement with that of Zhang et al (2017) who found that treatment with α -tocopherol significantly decreased MDA content. The presence of cadmium chloride in the nutrient solution causes a decrease in the membrane stability index (MSI). Our study is in agreement with that of Ouhaddach et al (2018), who reported that salt stress caused a decrease in MSI in *L. cheesmanii and L. peruvianum* species. Studies have shown that α-tocopherol reacts with the peroxy radicals formed in the bilayer to diffuse into the aqueous phase, trapping the cytotoxic H2O2 and reacting non-enzymatically with other ROS: singlet oxygen, superoxide radicals and hydroxyl radicals to stabilise membrane structures (Blokhina et al, 2003) by modulating membrane fluidity in a similar way to cholesterol and membrane permeability to small ions and molecules (Kim et al., 2008) and reducing the permeability of digalactosyldiacylglycerol vesicles to glucose and protons (Berglund et al., 1999).

Glutathione (GSH) is a non-enzymatic antioxidant that is very abundant in plants. It is a low molecular weight thiol. It is involved in defence against radicals, in the biosynthesis of phytochelatine (PC) and in resistance to metals by acting as direct chelator (Mendoza-Cozatl and Moreno-Sanchez, 2006),

In our experiments found that glutathione content decreased in both the aerial and root parts of plants in response to cadmium stress, whereas the work of Soughir (2009) shows an increase in this metabolite in the presence of Cd.

Reduced glutathione levels due to a strong defence capacity of other antioxidants (CAT, APX). The drop in GSH levels could also be attributed to increased use of GSH for the regeneration of ASA from DHA or for the synthesis of phytochelatins (Hossain et al., 2012 ; Chaudhary, K., Agarwal and Khan, 2018).

Conclusion

Cd leads to the development of defence systems in plants aimed at reacting to changes in their environment. These systems combat undesirable reactive species by preventing their accumulation, and detoxification involves both enzymatic and non-enzymatic mechanisms. Enzymatic defences involve ascorbate peroxidase and catalase. Non-enzymatic systems involve glutathione. Vitamin E treatment reduced cadmium toxicity. This is explained by the antioxidant power of vitamin E.

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References

Abdul Hafez,A.A. (2011). The importance of using vitamins to improve the growth and productivity of agricultural and horticultural crops. *Shams Agricultural Magazine17*(1) : 11-28.

Anjum, N. A., Sharma, P., Gill, S. S., Hasanuzzaman, M., Khan, E. A., Kachhap, K.,Tuteja, N. (2016). Catalase and ascorbate peroxidase—representative H 2 O 2-detoxifying heme enzymes in plants. *Environmental science and pollution research*, *23*, 19002-19029.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant physiology*, *141*(2), 391-396.

Ashraf, M. and Akram, N. A. (2009). Improving salinity tolerance of plants through conventional breeding and genetic engineering : an analytical comparison. *Biotechnology advances*, *27*(6), 744-752.

Benhamdi, A., Bentellis, A., Rached, O., Du Laing, G.,Mechakra, A. (2014). Effects of antimony and arsenic on antioxidant enzyme activities of two steppic plant species in an old antimony *mining area*. *Biological trace element research*, *158*, 96-104.

Berglund, A. H., Nilsson, R.,Liljenberg, C. (1999). Permeability of large unilamellar digalactosyldiacylglycerol vesicles for protons and glucose–influence of α-tocopherol, β-carotene, zeaxanthin and cholesterol. *Plant Physiology and Biochemistry*, *37*(3), 179-186.

Blokhina, O., Virolainen, E., Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress : a review. *Annals of botany*, *91*(2), 179-194.

Cakmak, I. and Horst, W. (1991) Effect of aluminium on lipid peroxidation, superoxide

dismutase,catalase and peroxidase activities in root tip of soybean (Glycine max). Plant Physiology. 83, 463-468.

Chaudhary, K., Agarwal, S., Khan, S. (2018). Role of phytochelatins (PCs), metallothioneins (MTs), and heavy metal ATPase (HMA) genes in heavy metal tolerance. *Mycoremediation and Environmental Sustainability : Volume 2*, 39-60.

Dar, T. A., Uddin, M., Khan, M. M. A., Hakeem, K. R., Jaleel, H. (2015). Jasmonates counter plant stress : a review. *Environmental and experimental Botany*, *115*, 49-57.

Edreva, A. (2005). Generation and scavenging of reactive oxygen species in chloroplasts : a submolecular approach. *Agriculture, Ecosystems & Environment*, *106*(2-3), 119-133.

El-Bassiouny, H. and Sadak, M. S. (2015). Impact of foliar application of ascorbic acid and α-tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. *Acta Biológica Colombiana*, *20*(2), 209-222.

Fojtová, M. and Kovařík, A. (2000). Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells. *Plant, Cell & Environment*, *23*(5), 531-537.

Foyer, C. H. and Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia plantarum*, *119*(3), 355-364.

Gall, H. L., Philippe, F., Domon, J. M., Gillet, F., Pelloux, J., Rayon, C. (2015). Cell wall metabolism in response to abiotic stress. *Plants*, *4*(1), 112-166.

Gill, S. S.and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930.

Hasanuzzaman, M., Nahar, K., Fujita, M. (2014). Role of tocopherol (vitamin E) in plants : abiotic stress tolerance and beyond. In *Emerging technologies and management of crop stress tolerance* (pp. 267-289). Academic Press.

Heath, R. L.and Packer, L. (1968). Photoperoxidation in isolated chloroplasts : II. Role of electron transfer. *Archives of biochemistry and biophysics*, *125*(3), 850-857.

Hoagland, D.R. and Arnon, D.J. (1959). The water- culture method of growing plants without soil. 3395 Calif. Agric. Exp. Stn. Circ 347, 26–29.

Hossain, M. A., Ngo, H. H., Guo, W. S., Setiadi, T. (2012). Adsorption and desorption of copper (II) ions onto garden grass. *Bioresource technology*, *121*, 386-395.

Iqbal, M. (2016). *Vicia faba* bioassay for environmental toxicity monitoring : a review. *Chemosphere*, *144*, 785-802.

Iqbal, M. and Bhatti, I. A. (2014). Re-utilization option of industrial wastewater treated by advanced oxidation process. *Pakistan Journal of Agriculture Sciences*, *51*(4), 1141-1147.

Iqbal, M. and Bhatti, I. A. (2015). Gamma radiation/H2O2 treatment of a nonylphenol ethoxylates : degradation, cytotoxicity, and mutagenicity evaluation. *Journal of hazardous materials*, *299*, 351-360.

Iqbal, M., Iqbal, N., Bhatti, I. A., Ahmad, N., Zahid, M. (2016). Response surface methodology application in optimization of cadmium adsorption by shoe waste: A good option of waste mitigation by waste. *Ecological engineering*, *88*, 265-275.

Iturbe-Ormaetxe, I., Matamoros, M. A., Rubio, M. C., Dalton, D. A., Becana, M. (2001). The antioxidants of legume nodule mitochondria. *Molecular Plant-Microbe Interactions*, *14*(10), 1189-1196.

Kaya, C., Ashraf, M., Alyemeni, M. N., Corpas, F. J., Ahmad, P. (2020). Salicylic acid-induced nitric oxide enhances arsenic toxicity tolerance in maize plants by upregulating the ascorbate-glutathione cycle and glyoxalase system. *Journal of Hazardous Materials*, *399*, 123020.

Khamees AL-Kareemawi, I. H. and Muhmood AL-Kazzaz, A. G. (2019). α-Tocopherol foliar application can alleviate the adverse effect of salinity stress on wheat plant, *Triticum aestivum* L. *Biochemical & Cellular Archives*, *19*(2).

Kim, C., Meskauskiene, R., Apel, K., Laloi, C. (2008). No single way to understand singlet oxygen signalling in plants. *EMBO reports*, *9*(5), 435-439.

Krieger-Liszkay, A. and Trebst, A. (2006). Tocopherol is the scavenger of singlet oxygen produced by the triplet states of chlorophyll in the PSII reaction centre. *Journal of experimental botany*, *57*(8), 1677-1684.

Leme, D. M. and Marin-Morales, M. A. (2009). Allium cepa test in environmental monitoring : a review on its application. *Mutation research/reviews in mutation research*, *682*(1), 71-81.

Lichtenthaler, H. K., Babani, F. (2004). Light adaptation and senescence of the *photosynthetic apparatus*. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. In *Chlorophyll a fluorescence : a signature of photosynthesis* (pp. 713-736). Dordrecht : Springer Netherlands.

Loggini, B., Scartazza, A., Brugnoli, E., NavariIzzo, F. (1999) Antioxidative defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiology. 119, 1091-1099

Mahaseth, T.and Kuzminov, A. (2017). Potentiation of hydrogen peroxide toxicity : From catalase inhibition to stable DNA-iron complexes. *Mutation Research/Reviews in Mutation Research*, *773*, 274-281.

Maria, V. L. and Bebianno, M. J. (2011). Antioxidant and lipid peroxidation responses in *Mytilus galloprovincialis* exposed to mixtures of benzo (a) pyrene and copper. *Comparative Biochemistry and Physiology Part C : Toxicology & Pharmacology*, *154*(1), 56-63.

Mendoza-Cozatl, D. G., Moreno-Sanchez, R. (2006). Control of glutathione and phytochelatin synthesis under cadmium stress. Pathway modeling for plants. *Journal of Theoretical Biology*, *238*(4), 919-936.

Mohamed, A. A., Castagna, A., Ranieri, A., di Toppi, L. S. (2012). Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. *Plant Physiology and Biochemistry*, *57*, 15-22.

Munné-Bosch, S. and Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Reviews in Plant Sciences*, *21*(1), 31-57.

Naikoo, M. I., Raghib, F., Dar, M. I., Khan, F. A., Hessini, K., Ahmad, P. (2021). Uptake, accumulation and elimination of cadmium in a soil-Faba bean (*Vicia faba)* -Aphid (*Aphis fabae*) -Ladybird (*Coccinella transversalis*) food chain. *Chemosphere*, *279*, 130522.

Ouhaddach, M., ElYacoubi, H., Douaik, A., Rochdi, A. (2018). Morpho-physiological and biochemical responses to salt stress in wheat (*Triticum aestivum L*.) at the heading stage. *Journal of Materials and Environmental Sciences*, *9*(6), 1899-1907.

Qureshi, A. S., Hussain, M. I., Ismail, S., Khan, Q. M. (2016). Evaluating heavy metal accumulation and potential health risks in vegetables irrigated with treated wastewater. *Chemosphere*, *163*, 54-61.

Rady, M. M., El-Yazal, M. A. S., Taie, H. A., Ahmed, S. M. (2016). Response of wheat growth and productivity to exogenous polyamines under lead stress. *Journal of crop science and biotechnology*, *19*, 363-371.

Rashmi, I., Roy, T., Kartika, K. S., Pal, R., Coumar, V., Kala, S., & Shinoji, K. C. (2020). Organic and inorganic fertilizer contaminants in agriculture : Impact on soil and water resources. *Contaminants in Agriculture : Sources, Impacts and Management*, 3-41.

Rau, S., Miersch, J., Neumann, D., Weber, E., & Krauss, G. J. (2007). Biochemical responses of the aquatic moss Fontinalis antipyretica to Cd, Cu, Pb and Zn determined by chlorophyll fluorescence and protein levels. *Environmental and Experimental Botany*, *59*(3), 299-306.

Romero-Puertas, M. C., Rodríguez-Serrano, M., Corpas, F. J., Gomez, M. D., Del Rio, L. A., & Sandalio, L. M. (2004). Cadmium-induced subcellular accumulation of O2·− and H2O2 in pea leaves. *Plant, Cell & Environment*, *27*(9), 1122-1134.

Sadiq, M. U. H. A. M. M. A. D., Akram, N. A., & Javed, M. T. (2016). Alpha-tocopherol alters endogenous oxidative defense system in mung bean plants under water-deficit conditions. *Pak. J. Bot*, *48*(6), 2177-2182.

Sakr, M. T., & El-Metwally, M. A. (2009). Alleviation of the harmful effects of soil salt stress on growth, yield and endogenous antioxidant content of wheat plant by application of antioxidants. *Pakistan Journal of Biological Sciences : PJBS*, *12*(8), 624-630.

Shi, J., Gao, L., Zuo, J., Wang, Q., Wang, Q., & Fan, L. (2016). Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation. *Postharvest Biology and Technology*, *116*, 98-104.

Shikha, D., & Singh, P. K. (2021). In situ phytoremediation of heavy metal–contaminated soil and groundwater : a green inventive approach. *Environmental Science and Pollution Research*, *28*, 4104-4124.

Souguir, D. (2009). *Modifications métaboliques, moléculaires et génotoxicité induites par le cadmium chez Vicia faba* (Doctoral dissertation, Université Blaise Pascal-Clermont-Ferrand II; Université d'Auvergne-Clermont-Ferrand I; Université du 7 Novembre, Carthage, Tunisie).

Taha, S. S., Mohamed, A. M., & Rady, M. M. (2018). Effect of exogenous α-Tocopherol on sweet pepper plants irrigated by diluted sea water. *Journal of Agricultural Studies*, *6*(1), 25-46

Vendruscolo, E. P., Rodrigues, A. H. A., Oliveira, P. R., Leitão, R. A., Campos, L. F. C., Seleguini, A., & De Lima, S. F. (2019). Exogenous application of vitamins in upland rice. *Revista de Agricultura Neotropical*, *6*(2), 1-6.

Weck-becker, G. and Cory, J.G. 1988. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer Lett. 40, 257-264.

Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., ... & Van Camp, W. (1997). Catalase is a sink for H2O2 and is indispensable for stress defence in C3 plants. *The EMBO journal*, *16*(16), 4806-4816.

Wise, R. R., & Naylor, A. W. (1987). Chilling-enhanced photooxidation : evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. *Plant Physiology*, *83*(2), 278-282.

Xu, X. Y., Fan, R., Zheng, R., Li, C. M., & Yu, D. Y. (2011). Proteomic analysis of seed germination under salt stress in soybeans. *Journal of Zhejiang University Science B*, *12*(7), 507-517.

Zhang, C. N., Zhang, J. L., Ren, H. T., Zhou, B. H., Wu, Q. J., & Sun, P. (2017). Effect of tributyltin on antioxidant ability and immune responses of zebrafish (*Danio rerio*). *Ecotoxicology and environmental safety*, *138*, 1-8.

Zhang, X., & Schmidt, R. E. (2000). Application of trinexapac-ethyl and propiconazole enhances superoxide dismutase and photochemical activity in creeping bentgrass (*Agrostis stoloniferous var. palustris*). *Journal of the American Society for Horticultural Science*, *125*(1), 47-51.