Effect of Zinc oxide nanoparticles on antioxidative system of Faba bean (*Vicia faba* L) seedling exposed to Cadmium

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Abstract: This work aimed to investigate the effect of Zinc oxide nanoparticles (ZnONPs) on oxidative stress induced by Cadmium (Cd) in Faba bean (*Vicia faba* L) seedling. The Malondialdehyde (MDA), Nitric oxide (NO) levels, Superoxide dismutase (SOD), Glutathione reductases (GR), Glutathione peroxidase (GPX) and Catalase (CAT) activity and Reduced glutathione (GSH) and Ascorbic acid (AsA) concentration in the shoot and root of seedling were investigated. The results indicated that, Cd increased the level of MDA and NO and induced a significant decrease of all antioxidant enzymes activities and antioxidant substances in comparison to control, while ZnONPs alone or with Cd treatments decreased the level of MDA and NO and induced a significant increase of all the antioxidant enzymes activities and antioxidant substances concentration comparison to Cd treated and/ or control. Subsequently adding ZnONPs to Cd treatments leads to alleviating the Cd toxicity.
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1. Introduction

In recent years, agriculture has become largely dependent on chemical fertilizer and wastewater irrigation and industry has added heavy metal to topsoil causing toxic effects on environmental system **(Nazar, *et al.*, 2012)**. Cd is considered as a main environmental injury to the agricultural system **(Toppi *et al.*, 1999)**. Cd accumulation in the plants may lead to many structural, physiological and biochemical changes **(Khan *et al.*, 2009)**. Cd accumulation led to disturbs the enzymes of photosynthesis, Calvin cycle, carbohydrate metabolism **(Shi *et al.*, 2010)** and alteration the antioxidant metabolism **(Khan *et al.*, 2009)**. Cd is stimulate oxidative stress through different indirect mechanisms because it doesn't participate directly in biological redox reactions **(Nazar, *et al.*, 2012)**. Moreover, it can either stimulate or inhibit many antioxidative enzymes activity before appear any toxic symptoms, depending on its concentration **(Aravind and Prasad, 2003; Smeets *et al.*, 2005)**. Once Cd enters to the plant, stimulates the NADPH oxidases activity, resulting to extracellular accumulation of reactive oxygen species (ROS) such as the superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) **(Jelonek *et al.*, 2012)**, lipid peroxidation such as MDA and oxidative burst **(Brahim *et al.*, 2010).** Also, it's accumulated other signaling molecule like NO as free radical **(Jelonek *et al.*, 2012)**. MDA is a general output of lipid peroxidation and a sensitive indicator of oxidative stress **(Janero, 1990)**. ROS are toxic products to cellular metabolites; its high accumulation can lead to deterioration of proteins, lipids and DNA **(Jaouhra *et al.*, 2011)**.

Consequently, plant cells needs to control increase accumulation of ROS by arranged the action of various antioxidant enzymes inclusive SOD, CAT and GR **(Foyer and Noctor, 2003)**. In the same time, with low molecular weight antioxidant metabolites substances such AsA and GSH. These enzymes supply cells with efficient mechanism for detoxifying ROS, during ascorbat-glutathione cycle. Also, the phospholipid hydroperoxide glutathione peroxidase (PHGPX) is considering a major candidate regulator to ROS-level which serves as a signaling enzyme and detoxifying. PHGPX, one of the glutathione peroxidase (GPX) family, is considered critical for safeguard membranes against oxidative stress **(Maiorino *et al.*, 1990).** In general activities of most anti-oxidant enzymes can increased, decreased or no change depending on many factors such as species, organ and age of the plant, type of metal, stress intensity and period of the treatment **(Gratão *et al.*, 2005).**

Zn is a significant component of numerous enzymes, which correlating with the proteins synthesis, metabolism of carbohydrate and phosphate, gene expression and regulation and safety of ribosome structural **(Singh *et al.*, 2013)**. It is an important molecule of key enzymes like Cu-Zn SOD **(Broadley, 2007)**. It plays a significant role within the cells in the defense system against ROS, therefore perform an

perfect protective factor against the oxidation of different vital cells component such as chlorophyll, lipid membrane and protein –SH groups **(Cakmak, 2000)**. ZnO is nearly insoluble in water but is used as fertilizer with zinc sulfate. Plants can absorb ZnO nanoparticles **(Zhao *et al.*, 2012)**, can synthesize ZnONPs **(Qu *et al.*, 2011)** and accumulate Zn.

Nanoscale particles are atomic or molecular aggregates with size 1–100 nm **(Ball 2002)**. Their physiochemical properties are modified due to huge surface to volume ratio in comparison to bulk materials **(Nel *et al.*, 2006)**. They are widely used in consumer products, pharmaceuticals, cosmetics, semiconductors, microelectronics and agriculture (**Colvin, 2003**). The production, use and disposal of nanoparticles result in accumulation in the environment. They have both positive **(Singh *et al.*, 2012)** and negative effects **(Lin and Xing 2007)** on organisms **(Yang *et al.*, 2006)**. Therefore, better understanding of behavior and impact of NPs on the health of plants/crops and hence on environment is highly demanding **(Colvin, 2003; Chow *et al.*, 2005; Owen and Depledge, 2005)**.

However, as a result of their unique advantages, some researchers have been performed on the toxicological effect of NPs on plants, yet research focusing on the investigation of the beneficial effects of NPs on plants still incomplete. NPs can prospect to improve the nano-pesticide fertilizers, herbicides and genes, which target specific cellular organelles to release their content in plants **(Siddiqui *et al.*, 2015).** The higher concentrations of ZnONPs have strong toxic effect **(Lin and Xing, 2007)**. Also, they caused oxidative stress **(Kim *et al.*, 2012),** which is an index for imbalance between ROS and antioxidant enzymes activities **(Singh *et al.*, 2013)**. The alteration in relationship is results in increased antioxidant activities defense system to cope with the oxidative stress **(Nel *et al.*, 2006; Kim *et al.*, 2012).**

Zn and Cd have different biological properties, there are many chemical and physical similarities. Cd and Zn association may be preventing the toxicity of Cd by Zn. The convergence of Cd and Zn in environment may lead to several interactions either synergistic or antagonistic in their uptake and accumulation in tissues **(Jaouhra *et al.*, 2011).** Therefore, many studies have been done to investigate the interaction between Cd-Zn on their uptake and accumulation in some plants species. However the impact of Cd-Zn interaction on antioxidant enzymes and ROS generation is still incomplete particularly in higher plants.

In the present work, the effect of ZnONPs on the antioxidant system of Faba bean (*Vicia faba* L) seedling exposed to Cd intoxication was investigated through monitoring of the free radicals levels, antioxidant enzymes activity and antioxidant substances levels.

2- Material and Methods:

This work was conducted in the Biology Lab, Biological Science Department, Faculty of Science, University of Jeddah, KSA during 2015. Seeds of Faba bean (*Vicia faba* L.) cultivar Giza 2 were obtained from the Field Crops Research Institute, Agriculture Research Center, Giza, Egypt.

ZnONPs was obtained in the form of dispersion from Sigma-Aldrich, Steinheim, Germany (CAS Number 1314-13-2) of concentration 50 wt.% in H_2O, average particle size (APS) was <35 nm. The particle size distribution (hydrodynamic diameter) was< 100 nm using dynamic light scattering (DLS) technique, pH 7±0.1 (for aqueous systems) and density 1.7±0.1 g mL^{-1} at 25 °C.

Preparation of test solutions:

Suspensions of ZnONPs in a concentration of 250, 500 and 1000 mg L^{-1} were daily prepared with deionized water and dispersed with a sonicator (JL-360, Shanghai, USA) for 20 min. 25 and 50 mg. L^{-1} Cd solution as $3CdSO_4.8H_2O$ were prepared with deionized water **(Gowayed and Kadasa, 2015)**.

Seed preparation:

252 healthy and uniform size seeds of Faba bean were used in this study. The seeds were Sterilizated using 2.5% NaOCl solution**,** then washed three times with deionized water. 63 seeds (control and Cd groups) were immersed in water for 4 hours; the other seeds were divided in to three parts, and immersed in ZnONPs at concentrations 250, 500 and 1000 μgL^{-1} for 4 hours **(Gowayed and Kadasa, 2015)**.

Seed germination test:

The seeds were placed in Petri dishes 90 mm (tri replicate) on filter paper then, 5 ml of ZnONPs suspensions (ZnONPs treated groups), Cd solution (Cd groups) and deionized water for control group were added. All dishes were placed in a growth room using complete randomize design. The treated solutions were added to the petri dishes as 7 ml of tat the second day, 10 ml at the fourth day and 10 ml at the sixth day **Gowayed and Kadasa, 2015)**.

Plant preparation and Extraction:

Shoot and root of seedling (0.5 g fresh weight of each) was frozen in liquid nitrogen until was used for preparation of the homogenate, samples were taken at 7^{th} day. The plant tissue was homogenized in an adequate amount of deionized water using sonicator (JL-360, Shanghai, USA). The homogenate

was centrifuged and the supernatant was preserved at -80 ^{0}C till be used for the biochemical analysis. protein concentration was determined by the method of **Bradford (1976).**

Antioxidant enzyme activity assay:

Plant tissue, SOD, GPX, GR and CAT activities were determined using the kits (Catalog nos. NWK-SOD01, NWK-GPX01, NWK-GR01 and NWK-CAT01) purchased from Northwest Life Science Specialties (NWLSS), Vancouver, Canada.

Antioxidant substances assay:

Plant tissue, GSH and ASA were determined using the kits that were supplied by Northwest Life Science Specialties (NWLSS), Vancouver, Canada, (Catalog no NWK-GSH01and NWK-Vit C01) following the manufacture instructions.

Free radical assay:

MDA was analyzed by measuring the production of thiobarbituric acid reactive substances (TBARS) using TBARS assay kit (Catalog no. 10009055, Cayman, USA). And NO was assayed using kit that supplied by Northwest Life Science Specialties (Catalog no NWK-NO 01).

Statistical Analysis:

Results were expressed as mean ± SD (standard deviation). All data were subjected one way completely randomize ANOVA analysis to calculate the least significant difference (LSD) at $p<0.05$ with Costat computer program.

3- Results:

a- Free radical substances:

Results in (Table 1) showed the effects of ZnONPs and/ or Cd on the concentration of MDA and NO as free radicals substances in the shoot and root of faba bean seedling. Our results indicated that ZnONPs treatment decreased the level of MDA and NO, while Cd increased them comparing to the control. Furthermore, the ZnONPs with Cd treatments lead to reduce the effect of Cd through decreasing the concentration of free radical substances.

Table 1: Effect of ZnONPs and/or Cd on free radical substances in shoot and root of faba bean seedling.

Treatments (mg. L^{-1})	MDA **(nmol/gm)**		NO **(nmol/gm)**	
	Shoot	Root	Shoot	Root
Control	7.067 ± 0.252^{fg}	5.060 ± 0.132^{i}	5.090 ± 0.061^{f}	4.367 ± 0.057^{h}
ZnONPs 250	7.087 ± 0.143^{fg}	4.973 ± 0.015^{j}	4.880 ± 0.050^{fg}	4.127 ± 0.021^{i}
ZnONPs 500	6.827 ± 0.222^{gh}	4.940 ± 0.020^{j}	4.683 ± 0.035^{g}	3.967 ± 0.068^{j}
ZnONPs 1000	6.470 ± 0.166^{h}	4.920 ± 0.000^{j}	3.187 ± 0.153^{h}	3.823 ± 0.025^{k}
Cd 25	9.090 ± 0.141^{b}	6.730 ± 0.036^{c}	9.160 ± 0.110^{a}	6.890 ± 0.010^{c}
Cd 50	11.073 ± 0.261^{a}	7.887 ± 0.015^{a}	9.470 ± 0.193^{a}	8.440 ± 0.010^{a}
ZnONPs 250 + Cd 25	8.153 ± 0.537^{c}	6.537 ± 0.032^{d}	6.443 ± 0.179^{e}	5.887 ± 0.015^{f}
ZnONPs 250 + Cd 50	9.480 ± 0.056^{b}	7.100 ± 0.010^{b}	7.327 ± 0.529^{d}	7.303 ± 0.015^{b}
ZnONPs 500 + Cd 25	8.093 ± 0.228^{cd}	6.117 ± 0.012^{f}	7.780 ± 0.303^{c}	5.303 ± 0.006^{g}
ZnONPs 500 + Cd 50	9.233 ± 0.252^{b}	6.403 ± 0.006^{e}	8.303 ± 0.176^{b}	6.670 ± 0.010^{d}
ZnONPs 1000 +Cd 25	7.380 ± 0.132^{ef}	5.430 ± 0.010^{h}	6.677 ± 0.045^{e}	5.867 ± 0.015^{f}
ZnONPs 1000 + Cd 50	7.740 ± 0.066^{de}	5.997 ± 0.015^{g}	7.063 ± 0.150^{d}	6.103 ± 0.006^{e}

Data are means ± SD of three independent experiments.
$^{a, b,\cdots}$ or ^{g} indicated a significant difference at $p \leq 0.05$.

b- Anti-oxidative enzymes activities:

The effects of ZnONPs and/ or Cd on the Anti-oxidative enzymes activities in shoot and root were shown in (Tables 2 and 3). Cd induced a significant decrease in SOD, GR, CAT and GPX activity in comparison to control. On the contrary ZnONPs treatments induced a significant increase in the anti-oxidative enzymes activity in comparison to control. Whereas the ZnONPs with Cd increased SOD, GR, CAT and GPX activity in comparison to Cd alone.

c- Anti-oxidative substances:

The effects of ZnONPs and/ or Cd on the anti-oxidant substances in shoot and root were shown in (Table 4). Our results reported that the GSH and AsA levels were decreased by in seedling exposed to Cd. On the contrary ZnONPs treatments induced an increase in GSH levl in comparison to control. ZnONPs with Cd increased the level of GSH and AsA comparing to Cd treated seedling.

Table 2: Effect of ZnONPs and/or Cd on the anti-oxidative enzymes activities **(U/gm)** in shoot of faba bean seedling.

Treatments (mg. L^{-1})	SOD	GR	GPX	CAT
Control	101.300 ± 1.572^{b}	24.667 ± 0.153^{b}	4.100 ± 0.149^{c}	1.607 ± 0.015^{a}
ZnONPs 250	104.110 ± 0.840^{ab}	25.337 ± 1.082^{b}	3.973 ± 0.050^{c}	1.650 ± 0.017^{a}
ZnONPs 500	105.300 ± 1.127^{a}	28.740 ± 1.109^{a}	4.953 ± 0.035^{b}	1.637 ± 0.021^{a}
ZnONPs 1000	106.770 ± 2.458^{a}	24.100 ± 4.000^{bc}	5.673 ± 0.212^{a}	1.633 ± 0.035^{a}
Cd 25	78.330 ± 0.764^{f}	19.500 ± 0.600^{f}	1.227 ± 0.101^{h}	0.660 ± 0.017^{f}
Cd 50	60.670 ± 1.650^{i}	15.880 ± 0.288^{g}	0.790 ± 0.026^{i}	0.473 ± 0.040^{g}
ZnONPs 250 + Cd 25	$86.100 \pm 2.848d^{e}$	21.633 ± 0.416^{def}	1.877 ± 0.021^{f}	0.807 ± 0.032^{e}
ZnONPs 250 + Cd 50	70.900 ± 1.572^{h}	22.033 ± 0.862^{cde}	1.917 ± 0.035^{f}	0.810 ± 0.026^{e}
ZnONPs 500 + Cd 25	83.700 ± 2.261^{e}	23.633 ± 0.379^{bcd}	1.997 ± 0.083^{f}	0.817 ± 0.015^{e}
ZnONPs 500 + Cd 50	74.670 ± 2.367^{g}	20.133 ± 0.651^{ef}	1.677 ± 0.040^{g}	0.930 ± 0.046^{d}
ZnONPs 1000 +Cd 25	96.430 ± 3.508^{c}	24.033 ± 0.116^{bc}	3.187 ± 0.021^{d}	1.487 ± 0.031^{b}
ZnONPs 1000 + Cd 50	88.200 ± 1.411^{d}	23.367 ± 0.252^{dcd}	2.933 ± 0.055^{e}	1.283 ± 0.035^{c}

Data are means ± SD of three independent experiments.
$^{a, b,\cdots}$ or ^{g} indicated a significant difference at $p \leq 0.05$.

Table 3: Effect of ZnONPs and/or Cd on Anti-oxidative enzymes activities **(U/gm)** in root of faba bean seedling.

Treatments (mg. L^{-1})	SOD	GR	GPX	CAT
Control	90.367 ± 2.401^{a}	19.100 ± 0.625^{c}	2.580 ± 0.062^{d}	1.240 ± 0.020^{c}
ZnONPs 250	88.720 ± 0.010^{b}	19.893 ± 0.012^{b}	2.713 ± 0.025^{c}	$1.260 \pm 0.026b^{c}$
ZnONPs 500	89.493 ± 0.021^{ab}	20.100 ± 0.010^{b}	2.883 ± 0.006^{b}	1.277 ± 0.006^{ab}
ZnONPs 1000	90.200 ± 0.036^{a}	20.603 ± 0.025^{a}	2.990 ± 0.010^{a}	1.300 ± 0.010^{a}
Cd 25	67.793 ± 0.021^{h}	14.200 ± 0.020^{g}	1.870 ± 0.010^{h}	0.733 ± 0.035^{h}
Cd 50	55.590 ± 0.017^{j}	10.393 ± 0.021^{i}	1.017 ± 0.015^{i}	0.383 ± 0.006^{i}
ZnONPs 250 + Cd 25	70.073 ± 0.021^{g}	16.487 ± 0.012^{f}	2.197 ± 0.015^{f}	0.837 ± 0.006^{g}
ZnONPs 250 + Cd 50	60.870 ± 0.017^{i}	13.793 ± 0.021^{h}	2.077 ± 0.015^{g}	0.747 ± 0.021^{h}
ZnONPs 500 + Cd 25	79.493 ± 0.021^{e}	17.800 ± 0.020^{d}	2.370 ± 0.017^{e}	1.033 ± 0.012^{e}
ZnONPs 500 + Cd 50	73.407 ± 0.031^{f}	16.800 ± 0.010^{e}	2.223 ± 0.015^{f}	0.990 ± 0.010^{f}
ZnONPs 1000 +Cd 25	86.707 ± 0.012^{c}	18.903 ± 0.006^{c}	2.587 ± 0.015^{d}	1.180 ± 0.010^{d}
ZnONPs 1000 + Cd 50	83.703 ± 0.025^{d}	17.497 ± 0.006^{d}	2.387 ± 0.012^{e}	1.057 ± 0.015^{e}

Data are means ± SD of three independent experiments.
$^{a, b,\cdots}$ or ^{g} indicated a significant difference at $p \leq 0.05$.

Table 4: Effect of ZnONPs and/or Cd on antioxidant substances in the shoot and root of faba bean seedling.

Treatments (mg. L^{-1})	GSH **(mg/gm)**		AsA **(µg/gm)**	
	Shoot	Root	Shoot	Root
Control	23.323 ± 0.490^{bc}	18.200 ± 0.400^{d}	0.834 ± 0.010^{a}	0.424 ± 0.005^{a}
ZnONPs 250	23.267 ± 0.681^{bc}	19.027 ± 0.038^{c}	0.838 ± 0.003^{a}	0.422 ± 0.004^{a}
ZnONPs 500	23.390 ± 0.789^{b}	19.703 ± 0.015^{b}	0.837 ± 0.002^{a}	0.425 ± 0.001^{a}
ZnONPs 1000	25.467 ± 0.808^{a}	20.100 ± 0.010^{a}	0.844 ± 0.006^{a}	0.430 ± 0.010^{a}
Cd 25	12.343 ± 0.389^{g}	12.110 ± 0.020^{j}	0.467 ± 0.013^{f}	0.213 ± 0.012^{g}
Cd 50	8.400 ± 0.265^{h}	7.397 ± 0.015^{k}	0.294 ± 0.007^{h}	0.122 ± 0.004^{i}
ZnONPs 250 + Cd 25	18.073 ± 0.172^{e}	15.000 ± 0.010^{g}	0.535 ± 0.008^{d}	0.280 ± 0.010^{e}
ZnONPs 250 + Cd 50	14.477 ± 1.020^{f}	13.203 ± 0.015^{i}	0.398 ± 0.018^{g}	0.187 ± 0.006^{h}
ZnONPs 500 + Cd 25	16.827 ± 1.351^{e}	15.890 ± 0.010^{f}	0.694 ± 0.006^{c}	0.327 ± 0.003^{c}
ZnONPs 500 + Cd 50	17.607 ± 1.472^{e}	13.830 ± 0.017^{h}	0.500 ± 0.020^{e}	0.269 ± 0.001^{f}
ZnONPs 1000 +Cd 25	21.960 ± 0.728^{c}	16.407 ± 0.012^{e}	0.798 ± 0.018^{b}	0.398 ± 0.002^{b}
ZnONPs 1000 + Cd 50	20.120 ± 0.301^{d}	14.893 ± 0.006^{g}	0.714 ± 0.024^{c}	0.314 ± 0.006^{d}

Data are means ± SD of three independent experiments.
$^{a, b,\cdots}$ or ^{g} indicated a significant difference at $p \leq 0.05$.

4- Discussion:

Cd is a non-redox heavy metal incapable to implement single electron transfer reactions, and doesn't produce ROS but stimulate oxidative stress through interfering with the antioxidant defense system **(Puertas *et al.*, 2007)**. lipids one a key components of cell membranes, they are sensitive to oxidation processes generating lipid peroxides is an indicator for increase production of toxic oxygen species **(Srivastava *et al.*, 2011)**. Lipid peroxidation is an important symptom of heavy metal toxicity **(Cakmak and Horst, 1991)**. MDA content is considered to be an indicator of oxidative damage **(Dhindsa and Matowe 1981)**. Our results reported that MDA increased in Cd treated seedling (Table 1). Once Cd enters to the plant it can induce MDA generation, this may be enplaned by the induction of lipid proxidation by Cd **(Ohkawa *et al.*, 1979)**. This result at the same line as reported by **Kumari *et al.*, (2010)** that Cd encourage oxidative injury in chickpea leaves. The increased of lipid peroxidation level and H_2O_2 concentration in tissue of soybean leaves exposed to Cd indicate that the metal caused oxidative damage to plants **(Hashem, 2014).**

NO is signaling molecule within plant cells that participate in many processes of growth and development, in addition to the regulation of many responses to abiotic and biotic stress agent. The mode of action signaling of NO at the molecular level includes modification of protein by binding to heme or iron-sulfur centers, critical Cys residues and Tyr residue nitration through peroxynitrite ($ONOO^-$) formation **(Jelonek and Wieczorek, 2011)**. Our results reported that Cd increase the concentration of NO (Table 1). When Cd enters to the plant it can accumulated other signaling molecule like nitric oxide (NO) as free radical **(Jelonek *et al.*, 2012)**. Many of evidence suggests that Cd stress increases generation of NO in plants **(Chmielowska *et al.*, 2014)**, **Bartha *et al.*, (2005) and Kopyra *et al.*, (2006)** in roots seedling of pea and cell suspensions of soybean, respectively, after treated by Cd in short-term.

Our study investigated the protective effect of ZnONPs on oxidative stress induced by Cd. The results showed that MDA and NO decreased in ZnONPs treated seedling (Table 1). It is known that Zn has the ability to stabilize and protect the biomembranes against peroxidative and oxidative stress, integrity of plasma membrane loss and also change the permeability of plasma membrane **(Bettger and. O'Dell, 1981)**. Zn may have a function in alteration of free radicals and their related processes via antioxidant characteristic **(Zago and Oteiza, 2001).** Moreover, Zn preferably binds to the groups -SH of the membrane protein moiety, and conserve proteins and phospholipids from formation of disulfide and thiol oxidation **(Chvapil, 1973),** through binding to a site nigh to the group of sulfhydryl directly, or by radical modifications which leads to obvious enzymes stability, proteins and lipid membrane construction **(Sharma *et al.*, 1994).** These results consistent with the results obtained by **Hassan *et al.*, (2005)** who revealed that supplementation of Zn to the growth medium has radically mitigate the toxicity of Cd by reducing MDA content in two rice cultivars grown in nutrient culture solution.

The equilibrium among the stable-state levels of various ROS are decided by the reaction between producing various ROS and scavenging ROS mechanisms **(Asada and Takahashi, 1987; Asada, 1999; Polle, 2001).** The enzymatic and non-enzymatic anti-oxidant overcome ROS. The enzymatic antioxidant included SOD, CAT, GPX and GR **(Bowler *et al.*, 1992; Asada, 1999; Mittler, 2002),** and non-enzymatic anti-oxidant as AsA and GSH **(Noctor and Foyer, 1998).** The level of ROC in cells can be increased by reduce the antioxidants enzymes interested in their detoxification, such as SOD, GR, POX or CAT **(Sandalio *et al.*, 2001).** In the present work the enzymatic antioxidants SOD, GR, GPX and CAT and non enzymatic antioxidant as AsA and GSH in shoot and root seedling were decreased in a group treated with Cd in comparison to control (Tables 2, 3 & 4).

SOD is protect the cells against ROS, which converting O_2^- to H_2O_2 and O_2, then GPX and CAT and thereafter detoxifying H_2O_2 **(Vestena *et al.*, 2011).** Cd toxicity induces various cell compartments to produced an excess of ROS that lead to inactivate SOD **(Sandalio *et al.*, 2001)**, where SOD stimulate the disproportionation of O_2^- radicals **(Vitoria *et al.*, 2001)**. Also, the inhibition in SOD activity could be responsible for the overproduction of ROS, which would produce oxidative damages at macromolecules, being responsible for the Cd toxicity **(Serrano *et al.*, 2009).** The inhibition of SOD activity by Cd has been showed in *Phaseolus vulgaris* (**Somashekaraiah *et al.*, 1992),** *Helianthus annus* **(Gallego *et al.*, 1996),** in the leaves of corn and wheat **(Vitoria *et al.*, 2001),** and in *Allium sativum* **(Zang *et al.*, 2005).**

CAT eliminate H_2O_2 by breaking it down directly to form water and oxygen **(Zhao, 2011).** It is mainly existent in mitochondria and peroxisomes, which oftentimes reduced dependent exposure to high concentrations of Cd **(Fornazier *et al.*, 2002).** The reduction may be also associated with decay caused by induced peroxisomal proteases or may be due to enzyme photoinactivation **(Hameed *et al.*, 2011).** The inhibition of CAT by Cd was also associated with ROS accumulation **(Moussa, 2005).** The decrease of

CAT activity by Cd toxicity has been observed in *Phaseolus vulgaris* **(Somashekaraiah *et al*., 1992; Chaoui *et al*., 1997),** *Phaseolus aureus* **(Shaw, 1995),** *Helianthus annuus* **(Gallego *et al*., 1996),** and *Secale cereale* **(Streb *et al*., 1993).**

GPX detoxifies H_2O_2 to H_2O, uses GSH directly as the reducing factor **(Vestena *et al.,* 2011)**. The regeneration of GSH is made possible by the decrease GSSG by GR, closing the GPX cycle **(Apel and Hirt, 2004)**. Our results observed reductions in the GR and GPX activity. The observed inhibition of GR in plants treated by Cd was paralleled with a decrease in GSH concentration, lowering the amount of substrate available for GPX **(Vestena *et al.,* 2011).** GPX appears to be capable of using reduced substrates other than GSH, including lipid hydroperoxides, as suggested by **Herbette *et al.* (2002).** This possibility and the great variety of GPX isoenzymes **(Eshdat *et al.,* 1997)** may explain, at least in part, the difference in species responses to Cd that was observed by **Vestena *et al.,* 2011**. The reductions in GR and GPX activity are indicative of a limited protection against oxidative stress **(Schutzendubel and Polle, 2002).** The decrease of GSH by Cd has been observed in sunflower plants *(***Gallego *et al.,* 1996 and Groppa *et al.,* 2001)**.

AsA is a first as well as second antioxidant found in plants and has diverse physiological roles. **Anjum *et al.,* (2008)** found that applying Cd to Rapeseed (*Brassica campestris* L.) at all growth stage significantly decreased the AsA and GSH contents in the leaves. The reduction in AsA and GSH was recorded depended on exposure time and dose of Cd. The modulation in the non-enzymatic antioxidants substances as AsA and GSH are well documented in Cd motivate oxidative stress **(Foyer, 1993)**

The data showed that antioxidant enzymes and antioxidant substances significant increased in ZnONPs treated seedling (Tables 2, 3 & 4). SOD activity significantly increased in seedling either treated in ZnONPs alone or with Cd. This increased in activity may be due to great synthesis of SOD. Zn is capable to participate in the Cu/Zn SOD structure isozyme, thus its treated increased SOD activity (**Asadi *et al.,* 2012**). Our results are in accordance with the date reported by **Aravind and Prasad (2005), Cherif *et al., (*2011), Hajiboland and Beiramzadeh (2008) and Tavallali *et al.,* (2010).**

GPX, GR and CAT activity increased in seedling treated with ZnONPs either alone or with Cd (Tables 2 &3). Treatments of Zn with Cd prompt a high increased of GPX, GR and CAT activity especially at 1000 μgL^{-1} ZnONPs, as indicator to antioxidant enzymes efficiency in existence of ZnONPs than other treated without ZnONPs. Possibly, Zn is required indirectly for rise enzymes activity contributory in detoxification of ROS such as GR, GPX and CAT **(Cakmak, 2000).** Zn may be antagonizes Cd toxicity by preserve the antioxidant enzymes levels and the efficient activity of ROS scavenging within the cells **(Jaouhra *et al.,* 2011).** Zn has been observed in many systems to antagonize the catalytic properties of the redox-active transition iron and copper with regard to their capability to support conversion H_2O_2 and O_2^- to OH^- **(Powell, 2000). Aravind *et al.* (2009)** observed that addition Zn to Cd stimulate reduction NADPH oxidation and consequently O_2^- radical then barring the induction ROS formation. **Jaouhra (2011)** conclude that addition low concentration of Zn contribute a highly preserve to *Solanum lycopersicum* from Cd toxicity via decrease Cd uptake, lipid peroxidation and promoting antioxidant enzymes activities of ROS scavenging. **Singh *et al.* (2013) and Kim *et al.*, (2012)** reported that ZnONPs caused significant increase in SOD and CAT activities in (*Brassica oleracea*, *Brassica oleracea* and *Lycopersicon esculentum* L.) and *Cucumis sativus* respectively.

Gowayed and Kadasa (2015) reported that increase ZnONPs decreased Cd levels in the shoot and root of the faba bean. Zn decreased Cd uptake and as indicator to the high competition between Cd and Zn for the same membrane- carriers in bread and durum wheat **(Hart *et al.,* 2002)** and *Ceratophyllum demersum* **(Aravind and Prasad, 2003).**

Conclusion:

ZnONPs posses and antioxidant activity in the Faba been seedling exposed to Cd intoxication, through decreasing the MDA and NO, and inducing of SOD, GR, CAT, GPX activity, GSH and AsA levels.).

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