Impact of Zinc Oxide Nanoparticles on Germination and Antioxidant System of Maize (*Zea mays* L.) Seedling under Cadmium Stress

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Abstract: This work aimed to investigate the impact of Zinc oxide nanoparticles (ZnO-NPs) on the stress induced by Cadmium (Cd) on grain germination and antioxidant system of Maize (*Zea mays* L.) seedling. Germination parameters **(**final germination percentage (FGP), mean daily germination (MDG), coefficient of velocity of germination (CVG), mean germination time MGT, germination index (GI), germination stress tolerance index (GSI), seedling vigor index (SVI) as well as dry matter stress tolerance index (DMSI)**)**, growth parameters (shoot and root fresh and dry weight), free radicals malondialdehyde (MDA) and reduced glutathione (GSH) concentrations, superoxide dismutase (SOD, EC 1.15.1.1), glutathione reductases (GR, EC 1.6.4.2), glutathione peroxidase (GPX, EC 1.11.1.9) and catalase (CAT, EC 1.11.1.6) activities were investigated. The results indicated that, addition of ZnO-NPs (500 mg L^{-1}) contribute a highly protection from Cd toxicity *via* decrease Cd concentration, MGT, MDA and promoting induction of MDG, CVG, GSI, SVI and DMSI, as well increasing GSH, SOD, GR, CAT and GPX activities.

Keywords: ZnO-NPs, Cd toxicity, Germination, Antioxidant system, Maize.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in all over the world included to Poaceae family. It is an important human nutrient, animal feed, biofuel and raw material for many industries. It is an important source of carbohydrate, protein, fixed oil, vitamin B, iron, and other minerals (Dwivedi *et al*., 2016)

The massive increasing of world populations may lead to increase contamination with heavy metal in arable land which led to massive pollution (Neilson and Rajakaruna, 2015). As a result, increased in crops production has taken a place worldwide but unfortunately, this action may lead to excess usage of undesirable agents excessive use of these components has tremendous impacts on various biochemical and physiological processes in plants such as, seed germination, plant growth and production. By repeated applications of fertilizers and irrigating crops with wastewater uses, that contain high levels of heavy metals, may have a potential impact on agricultural investments (Frost and Ketchum, 2000). Cd is one of the serious heavy metal that can cause much toxicity in plants and soil, and has easily taken up by crops (Sarkar, 2002). Cd has a direct effects on the roots than other parts of plants due to direct contact with this element, therefore, Cd inhibits growth, degrades chlorophyll, changes the composition of plant nutrients, inhibit essential elements, disturbs membrane functions, decrease enzymes activity, reduces minerals and water transport, change hormonal balance and induce the oxidative stress (Ferhad *et al.,* 2015).

Reactive oxygen species (ROS) including hydrogen peroxide, super oxide radical, hydroxyl radicals and singlet oxygen may be produced under Cd stress (Cho and Park, 2000). Consequently, these ROS products can cause oxidative damage to several essential macromolecules which leads to plant death (Molassiotis *et al.,* 2006). Indeed, the Cd toxicity may cause production of ROS, may inhibit antioxidant molecules and also lead to cellular damage or lipid peroxidation (Chien *et al.,* 2002). Therefore, Cd toxicity induces imbalance between production of ROS and antioxidant system in plants. MDA is polyunsaturated fatty acids produces as decomposition of bio membrane, which is the major indicator of oxidative injury (Demiral and Turkan, 2005). Plants can organized defense systems against oxidative stress, through enzymatic (SOD, GR, GPX and CAT) and non-enzymatic (GSH) antioxidation processes (Sbartai *et al.,* 2008). The primary defense is converts superoxide radical to hydrogen peroxide (H_2O_2) by SOD. Second step is reduced H_2O_2 to H_2O and O_2 by GPX and CAT in cytoplasm and other cellular compartments (Asada, 1999).

The nanotechnology applications have become fundamental emerged tools to eliminate the toxicity of heavy metal. It can be used as a photocatalytic agent for environmental pollutants. ZnO-NPs was applied in promoting seed germination, roots and stem growth and also increased plant yield as shown in Peanut seeds (Prasad *et al.,* 2012). The effects of nanoparticle induced antioxidant changes and their role in the alleviation of phytotoxicity in plant cells have been reported previously (Mousavi *et al.,* 2015) however, biochemical studies on the long-term effects of nanomaterial exposure to crop plants are limited. The modifications in cellular processes have achieved after application of nanoparticles, however, it enhance release of antioxidants enzymes, such as SOD, GPX, CAT and GR can assist plant cells in alleviating the oxidative stress (Priyanka and Venkatachalam, 2016).

Seeds germination is sensitive stage in the plant growth circle and it is the critical stage of plants to the alteration in surrounding environment. Thus, this stage is a best trend to study the toxicological mechanisms in

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plants by environmental contaminants (Sujing *et al.,* 2012).

In the present work, the effect of ZnO-NPs on the germination and antioxidant system of Maize (*Zea mays* L) seedling exposed to Cd was investigated. Germination and growth parameters, free radicals levels, antioxidant enzymes activity and antioxidant substances levels were evaluated.

MATERIAL AND METHODS

This work was conducted at the Biology Lab, Biological Science Department, Faculty of Science, University of Jeddah, KSA during year 2016. Grains of maize (*Zea mays* L.) cultivar Triple-hybrid 310 were obtained from the Field Crops Research Institute, Agriculture Research Center, Giza, Egypt.

ZnO-NPs 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 ZnO-NPs 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.

Seed preparation

Healthy and uniform size grains of maize were used in this study. The grains were sterilized with 2.5% NaOCl solution**,** then washed three times with deionized water. The grains (control and Cd groups) were immersed in water for 4 hours; the other seeds were divided into three parts, and immersed in ZnO-NPs at concentrations 250, 500 and 1000 mg L^{-1} for 4 hours.

Seed germination test

The seeds were placed in plastic pot (9 X 10 cm) and filled up with 0.3 kg of sand and peat-moss at 1:1 ratio then, different concentration of ZnO-NPs suspensions (ZnO-NPs treated groups), Cd solution (Cd groups) and deionized water for control group were added. All pots were placed in a growth room using complete randomize design. The treated solutions were added to the pots every 2 days.

Germination parameters

Grains become germinated when their coleoptile extension exhibits longer than 3 mm. The germinated grains were counted daily up to the end of germination (7 days) for determination of Final Germination Percentage (FGP) according to ISTA (1999), Mean Germination Time (MGT) according to Sadeghi *et al.* (2011), Germination Index (GI) according to AOSA (1983), Coefficient of Velocity of Germination (CVG) according to Scott *et al.* (1984) and Mean Daily Germination (MDG). Germination stress tolerance index (GSI), Dry matter stress tolerance index (DMSI), Seedling vigor index (SVI) were calculated according to Raskar and Laware (2013).

Growth parameters

Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW) were measured at the end of the experiment after 15 days.

Analysis of heavy metals

At the end of experiments, shoots and roots of seedling were washed totally with distilled water and the samples were oven dried at 78°C for three days. Dried samples were digested using a nitric (HNO_3)-sulfuric (H_2SO_4)-perchloric ($HClO_4$) acid mixture (4:1:8 v/v) (Jackson, 1973). The content of Zn and Cd was determined by the atomic absorption spectrophotometer (Thermo-electron, S Series GE 711838).

Plant preparation and Extraction

Shoot and root of seedling (0.5 g fresh weight of each) 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

SOD (EC 1.15.1.1), GPX (EC 1.11.1.9), GR (EC 1.6.4.2) and CAT (EC 1.11.1.6) activities in the plant tissues were determined using kits (Catalog nos. NWK-SOD01, NWK-GPX01, NWK-GR01 and NWK-CAT01 respectively) purchased from Northwest Life Science Specialties (NWLSS), Vancouver, Canada. Activity was assayed for SOD according to Nishikimi *et al.* (1972), GPX according to Paglia and Valentina (1967), GR according to Goldberg and Spooner (1983) and CAT according to Aebi (1984).

Antioxidant substances assay

GSH was determined in plant tissues according to Beutler *et al.* (1963) using kit that was supplied by Northwest Life Science Specialties (NWLSS), Vancouver, Canada, (Catalog no NWK-GSH01) following the manufacture instructions .

Free radical assay

MDA was analyzed by measuring the production of thiobarbituric acid reactive substances (TBARS) according to Ohkawa *et al*. (1979) using TBARS assay kit (Catalog no. 10009055, Cayman, USA).

Statistical Analysis

Results were expressed as mean ± SD (standard deviation). All data were subjected to one way completely randomize ANOVA. Least significant difference (LSD) test followed by Duncan's Multiple Range Test (Duncan 1955) at $p<0.05$ was used to compare mean. All data were analyzed by Costat statistical program.

RESULTS

Zinc and cadmium levels

Data in Table (1) indicated that Zn was detected in shoot and root of control seedling, whereas Cd was not detected in root of control and shoots of all treated seedling. Moreover, Cd levels increased in Cd treated seedling in concentration dependant as compared with control. The Cd levels decreased in groups treated with ZnO-NPs as compared with Cd treated seedling.

Germination and growth parameters

Results of germination parameters in Table (2) indicated that FGP, MDG, CVG, GI, GSI, SVI and DMSI decreased and MGT increased in treatment with Cd and/ or ZnO-NPs at 1000 mg L^{-1} compared to control. Whereas, FGP, MDG, CVG, GI, GSI, SVI increased and MGT decreased in treatment with Cd + ZnO-NPs 500 mg L^{-1} compared to Cd treatments.

Data of growth parameters in Table (3) indicate that RFW, RDW and SFW decreased in Cd and/ or ZnO-NPs 1000 mg L^{-1} treated comparing to control. In contrast, these parameters increased in treatments with Cd + ZnO-NPs 500 mg L^{-1} comparing to Cd treated seedling.

Effect of Cd and/or ZnO-NPs on MDA

Results in Table (4) reveled that MDA levels increased in Cd treated seedling (shoot and root) as compared with control. In contrast, MDA levels decreased in shoot and root of seedling treated with both Cd and ZnO-NPs as compared with that treated with Cd alone.

Effect of Cd and/or ZnO-NPs on antioxidant system

Results in Tables (5 and 6) indicated that SOD, GR, GPX and CAT activities as well GSH concentration decreased in shoot and root seedling by Cd treatments as compared with control. Whereas, ZnO-NPs increased the activity of antioxidant enzymes and GSH as compared with Cd treated seedling.

Table (1): Zinc and cadmium concentrations in shoot and root of *Zea mays* (µg g^{-1}) dry weight

Treatments (mg L^{-1})	Cd		Zn	
	Shoot	Root	Shoot	Root
Control	Nd	Nd	25.99 ± 0.97^{j}	30.63 ± 0.32^{i}
ZnONPs 250	Nd	Nd	65.11 ± 1.00^{h}	156.67 ± 2.03^{h}
ZnONPs 500	Nd	Nd	83.54 ± 1.59^{g}	594.13 ± 3.06^{f}
ZnONPs 1000	Nd	Nd	102.73 ± 2.52^{d}	1145.60 ± 4.58^{b}
Cd 25	Nd	14.67 ± 0.50^{c}	25.10 ± 1.01^{j}	30.58 ± 0.94^{i}
Cd 50	Nd	47.30 ± 1.41^{a}	25.83 ± 0.29^{j}	29.85 ± 0.22^{i}
ZnONPs 250 + Cd 25	Nd	12.32 ± 0.65^{d}	92.52 ± 0.65^{e}	656.84 ± 2.51^{d}
ZnONPs 250 + Cd 50	Nd	16.07 ± 1.65^{b}	57.79 ± 0.39^{i}	235.62 ± 0.57^{g}
ZnONPs 500 + Cd 25	Nd	3.83 ± 0.34^{f}	87.25 ± 1.30^{f}	728.84 ± 2.47^{c}
ZnONPs 500 + Cd 50	Nd	6.05 ± 0.53^{e}	118.56 ± 0.51^{c}	643.78 ± 0.50^{e}
ZnONPs 1000 +Cd 25	Nd	1.73 ± 0.25^{g}	151.43 ± 2.65^{a}	1183.74 ± 4.82^{a}
ZnONPs 1000 + Cd 50	Nd	3.62 ± 0.24^{f}	123.08 ± 0.68^{b}	1180.80 ± 2.65^{a}

Data are means ± SD of three independent experiments.
a , b,… or g indicated a significant difference at $p \leq 0.05$.
Nd; Not detected

Table (2): Effect of Cd and/or ZnO-NPs on germination parameters of *Zea mays* L.

Treatments (mg L^{-1})	*FGP*	*MDG*	*CVG*	*MGT*	*GI*	*GSI*	*SVI*	*DMSI*
Control	100.00 ± 0^{a}	17.78 ± 1.92^{ab}	42.82 ± 6.85^{a}	2.38 ± 0.38^{fg}	4.92 ± 0.52^{a}	100.00 ± 0.00^{b}	13.00 ± 0^{b}	100.00 ± 0^{b}
ZnONPs 250	100.00 ± 0^{a}	13.10 ± 1.03^{c}	25.37 ± 2.05^{ef}	3.96 ± 0.31^{ab}	3.02 ± 0.49^{cd}	80.54 ± 0.68^{f}	8.90 ± 0^{d}	68.46 ± 0^{d}
ZnONPs 500	100.00 ± 0^{a}	18.89 ± 1.92^{a}	44.54 ± 2.48^{a}	2.25 ± 0.13^{g}	4.82 ± 0.09^{a}	107.69 ± 1.00^{a}	13.93 ± 0^{a}	107.18 ± 0^{a}
ZnONPs 1000	95.83± 7.22^{b}	12.50 ± 0.00^{c}	25.06 ± 0.84^{ef}	3.99 ± 0.13^{ab}	2.66 ± 0.14d^{e}	76.81 ± 0.45^{g}	6.86 ± 0^{g}	55.13 ± 0^{g}
Cd 25	87.50 ± 0^{c}	10.94 ± 0.00^{c}	28.45 ± 1.80^{de}	3.52 ± 0.22^{c}	2.81 ± 0.08^{cde}	75.43 ± 0.45^{h}	5.72 ± 0^{j}	50.26 ± 0^{j}
Cd 50	75.00 ± 0^{d}	10.71 ± 0.00^{c}	23.10 ± 0.89^{f}	4.33 ± 0.17^{a}	1.49 ± 0.04^{f}	42.27 ± 0.65^{l}	3.55 ± 0^{l}	36.41 ± 0^{k}
ZnONPs 250 + Cd 25	100.00 ± 0^{a}	12.50 ± 0.00^{c}	27.65 ± 1.60^{def}	3.63 ± 0.22^{bc}	3.32 ± 0.32^{c}	69.32 ± 0.34^{i}	6.63 ± 0^{h}	51.03 ± 0^{i}
ZnONPs 250 + Cd 50	100.00 ± 0^{a}	15.87 ± 1.37^{b}	36.41 ± 1.66^{b}	2.75 ± 0.13^{ef}	4.03 ± 0.12^{b}	98.64 ± 0.51^{c}	7.70 ± 0^{f}	59.23 ± 0^{f}
ZnONPs 500 + Cd 25	100.00 ± 0^{a}	17.78 ± 1.92^{ab}	37.27 ± 4.46^{b}	2.71 ± 0.31^{ef}	4.03 ± 0.63^{b}	92.41 ± 0.35^{d}	8.33 ± 0^{e}	64.10 ± 0^{e}
ZnONPs 500 + Cd 50	100.00 ± 0^{a}	16.98 ± 2.87^{ab}	30.51 ± 2.50^{cd}	3.29 ± 0.26^{cd}	2.83 ± 0.37^{cde}	61.64 ± 0.32^{j}	9.57 ± 0^{c}	73.59 ± 0^{c}
ZnONPs 1000 +Cd 25	87.50 ± 0^{c}	17.50 ± 0.00^{ab}	33.38 ± 1.59^{bc}	3.00 ± 0.14^{de}	2.73 ± 0.29^{de}	88.54 ± 0.42^{e}	5.95 ± 0^{i}	52.31 ± 0^{h}
ZnONPs 1000 + Cd 50	87.50 ± 0^{c}	16.53 ± 1.68^{ab}	28.51 ± 2.33^{cde}	3.52 ± 0.30^{c}	2.39 ± 0.40^{e}	53.92 ± 0.50^{k}	3.70 ± 0^{k}	32.56 ± 0^{l}

Data are means ± SD of three independent experiments.
a , b,… or k indicated a significant difference at p ≤0.05.

Table (3): Effect of Cd and/or ZnO-NPs on growth parameters of *Zea mays* L.

Treatments (mg L^{-1})	*RFW*	*RDW*	*SFW*	*SDW*
Control	1.16 ± 0.24 ^{ab}	0.10 ± 0.02 ^{a}	0.28 ± 0.03 ^{bcde}	0.03 ± 0.00 ^{abc}
ZnONPs 250	1.02 ± 0.17 ^{abcd}	0.07 ± 0.01 ^{bc}	0.29 ± 0.01 ^{bcd}	0.03 ± 0.00 ^{a}
ZnONPs 500	1.23 ± 0.12 ^{a}	0.11 ± 0.03 ^{a}	0.31 ± 0.01 ^{bc}	0.03 ± 0.00 ^{ab}
ZnONPs 1000	0.71 ± 0.08 ^{efg}	0.05 ± 0.00 ^{cd}	0.28 ± 0.01 ^{bcd}	0.03 ± 0.00 ^{abc}
Cd 25	0.71 ± 0.05 ^{efg}	0.05 ± 0.00 ^{bcd}	0.20 ± 0.05 ^{fg}	0.02 ± 0.00 ^{e}
Cd 50	0.53 ± 0.05 ^{g}	0.03 ± 0.01 ^{de}	0.15 ± 0.05 ^{g}	0.02 ± 0.01 ^{e}
ZnONPs 250 + Cd 25	0.81 ± 0.21 ^{def}	0.05 ± 0.02 ^{bcd}	0.22 ± 0.04 ^{f}	0.02 ± 0.00 ^{de}
ZnONPs 250 + Cd 50	0.94 ± 0.14 ^{bcde}	0.05 ± 0.01 ^{bcd}	0.23 ± 0.03 ^{ef}	0.02 ± 0.00 ^{bcd}
ZnONPs 500 + Cd 25	1.04 ± 0.19 ^{abcd}	0.06 ± 0.01 ^{bc}	0.33 ± 0.02 ^{b}	0.03 ± 0.00 ^{a}
ZnONPs 500 + Cd 50	1.05 ± 0.09 ^{abc}	0.07 ± 0.01 ^{b}	0.39 ± 0.06 ^{a}	0.03 ± 0.00 ^{abc}
ZnONPs 1000 +Cd 25	0.82 ± 0.08 ^{cdef}	0.05 ± 0.00 ^{cd}	0.26 ± 0.02 ^{def}	0.02 ± 0.00 ^{bcd}
ZnONPs 1000 + Cd 50	0.65 ± 0.06 ^{fg}	0.02 ± 0.02 ^{e}	0.23 ± 0.03 ^{def}	0.02 ± 0.00 ^{cd}

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

Table (4): Effect of Cd and/or ZnO-NPs on MDA concentration in shoot and root of *Zea mays* L.

Treatments (mg L^{-1})	MDA (nmol gm^{-1})	
	Shoot	Root
Control	3.26 ± 0.06^{efg}	3.03 ± 0.04^{g}
ZnONPs 250	2.87 ± 0.15^{g}	2.81 ± 0.03^{j}
ZnONPs 500	3.14 ± 0.05^{fg}	2.88 ± 0.06^{i}
ZnONPs 1000	3.21 ± 0.17^{fg}	2.95 ± 0.02^{h}
Cd 25	6.47 ± 0.89^{b}	5.20 ± 0.02^{b}
Cd 50	8.23 ± 0.22^{a}	7.25 ± 0.02^{a}
ZnONPs 250 + Cd 25	4.83 ± 0.06^{c}	5.22 ± 0.01^{b}
ZnONPs 250 + Cd 50	6.42 ± 0.25^{b}	5.20 ± 0.02^{b}
ZnONPs 500 + Cd 25	5.14 ± 0.41^{c}	4.17 ± 0.02^{c}
ZnONPs 500 + Cd 50	4.15 ± 0.15^{d}	4.12 ± 0.03^{d}
ZnONPs 1000 +Cd 25	3.47 ± 0.06^{ef}	3.24 ± 0.02^{f}
ZnONPs 1000 + Cd 50	3.78 ± 0.21^{de}	3.70 ± 0.02^{e}

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

Table (5): Effect of Cd and/or ZnO-NPs on the antioxidant enzymes activity and antioxidant substances in shoot of *Zea mays* L.

Treatments (mg L^{-1})	SOD (U gm^{-1})	GR (U gm^{-1})	GPX (U gm^{-1})	CAT (U gm^{-1})	GSH (mg gm^{-1})
Control	132.33 ± 8.08^{ab}	89.10 ± 0.45^{ab}	12.38 ± 0.25^{a}	2.33 ± 0.96^{cd}	18.24 ± 0.25^{ab}
ZnONPs 250	133.67 ± 8.74^{ab}	89.45 ± 0.94^{ab}	12.67 ± 0.23^{a}	2.65 ± 0.39^{abc}	18.61 ± 0.56^{a}
ZnONPs 500	139.33 ± 1.53^{a}	92.16 ± 5.26^{a}	12.71 ± 0.61^{a}	2.90 ± 0.11^{ab}	18.74 ± 0.19^{a}
ZnONPs 1000	139.00 ± 1.00^{a}	91.62 ± 1.22^{a}	13.33 ± 0.21^{a}	3.10 ± 0.10^{a}	18.87 ± 0.39^{a}
Cd 25	112.67 ± 9.71^{e}	61.98 ± 3.08^{g}	8.45 ± 0.87^{b}	0.81 ± 0.01^{gh}	12.08 ± 0.61^{e}
Cd 50	101.00 ± 4.36^{f}	55.71 ± 5.73^{h}	6.07 ± 0.42^{c}	0.40 ± 0.06^{h}	9.14 ± 0.24^{f}
ZnONPs 250 + Cd 25	116.00 ± 2.00^{de}	68.13 ± 1.36^{ef}	9.18 ± 0.27^{b}	1.88 ± 0.05^{de}	13.91 ± 0.44^{d}
ZnONPs 250 + Cd 50	118.33 ± 6.51^{de}	65.36 ± 2.60^{fg}	6.26 ± 3.09^{c}	1.10 ± 0.46^{fg}	11.61 ± 0.22^{e}
ZnONPs 500 + Cd 25	127.33 ± 1.53^{bc}	71.00 ± 1.25^{e}	10.25 ± 0.40^{b}	2.29 ± 0.11^{cd}	14.04 ± 0.16^{d}
ZnONPs 500 + Cd 50	121.00 ± 2.00^{cde}	77.78 ± 1.47^{d}	8.87 ± 0.47^{b}	1.41 ± 0.06^{ef}	14.33 ± 0.72^{d}
ZnONPs 1000 +Cd 25	132.67 ± 1.53^{ab}	86.27 ± 0.97^{bc}	12.54 ± 0.17^{a}	2.67 ± 0.04^{abc}	17.86 ± 0.18^{b}
ZnONPs 1000 + Cd 50	123.00 ± 1.73^{cd}	82.46 ± 0.96^{c}	12.66 ± 1.97^{a}	2.40 ± 0.10^{bcd}	16.57 ± 0.63^{c}

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

Table (6): Effect of Cd and/or ZnO-NPs on antioxidant enzymes activity and antioxidant substances in root of *Zea mays* L.

Treatments (mg L^{-1})	SOD (U gm^{-1})	GR (U gm^{-1})	GPX (U gm^{-1})	CAT (U gm^{-1})	GSH (mg gm^{-1})
Control	121.33 ± 0.58^{c}	80.20 ± 2.10^{d}	4.85 ± 0.01^{c}	1.21 ± 0.02^{d}	14.28 ± 0.06^{c}
ZnONPs 250	123.33 ± 0.58^{b}	94.20 ± 0.01^{a}	4.89 ± 0.06^{c}	1.31 ± 0.02^{a}	14.53 ± 0.02^{a}
ZnONPs 500	126.33 ± 1.53^{a}	84.33 ± 0.02^{b}	4.97 ± 0.02^{b}	1.27 ± 0.01^{b}	13.89 ± 0.02^{d}
ZnONPs 1000	127.33 ± 0.58^{a}	82.12 ± 0.02^{c}	5.05 ± 0.03^{a}	1.24 ± 0.01^{c}	14.40 ± 0.02^{b}
Cd 25	100.00 ± 0.00^{g}	58.44 ± 0.02^{j}	2.94 ± 0.06^{i}	0.18 ± 0.01^{i}	7.65 ± 0.01^{j}
Cd 50	98.33 ± 0.58^{h}	50.41 ± 0.01^{k}	2.13 ± 0.03^{j}	0.10 ± 0.01^{j}	6.88 ± 0.01^{i}
ZnONPs 250 + Cd 25	114.33 ± 0.58^{e}	60.41 ± 0.02^{i}	4.41 ± 0.03^{e}	0.40 ± 0.01^{g}	8.74 ± 0.01^{i}
ZnONPs 250 + Cd 50	118.00 ± 1.00^{d}	58.30 ± 0.01^{j}	3.66 ± 0.05^{h}	0.23 ± 0.01^{h}	7.37 ± 0.01^{k}
ZnONPs 500 + Cd 25	121.00 ± 0.00^{c}	68.20 ± 0.02^{g}	4.16 ± 0.05^{f}	1.11 ± 0.01^{ef}	11.93 ± 0.01^{g}
ZnONPs 500 + Cd 50	112.00 ± 1.00^{f}	66.22 ± 0.03^{h}	3.93 ± 0.06^{g}	1.12 ± 0.00^{e}	10.95 ± 0.00^{h}
ZnONPs 1000 +Cd 25	120.33 ± 0.58^{c}	76.19 ± 0.02^{e}	4.90 ± 0.05^{c}	1.10 ± 0.00^{f}	12.43 ± 0.01^{e}
ZnONPs 1000 + Cd 50	118.33 ± 0.58^{d}	73.14 ± 0.01^{f}	4.61 ± 0.04^{d}	1.20 ± 0.00^{d}	12.31 ± 0.02^{f}

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

DISCUSSION

The present study was conducted to study the effect of ZnO-NPs on germination and anti-oxidative system of Maize (*Zea mays* L) exposed to Cd during seedling stage. Our results indicated that The Cd levels decreased in groups treated with ZnO-NPs as compared with Cd treated seedling. Moreover, the concentrations of Zn or Cd in shoot were less than in root in the same treated (Table 1), this may be attributed to the chemical and physical similarities between Zn and Cd which leads to interaction between them (Jaouhra *et al.,* 2011). The analogical results were reported in rice (Hassan *et al.,* 2005) and faba bean (Gowayed and Kadasa, 2015). In this study Zn was detected in shoot and root of control seedling, whereas Cd was not detected, this may be refer to the plants have very specific evolved and highly efficient mechanisms to absorb essential micronutrients from rhizosphere, even present at slight concentration (Tangahu *et al.,* 2011). Uptake of metal by plants depends on the bioavailability of the metal in the solution, which conversely depends on the reservation time of the metal, as well as the interaction with other substances and elements in the water. Furthermore, the bioavailability of some metals is restricted because of weak solubility in water and potent binding to soil particles (Clemens *et al.,* 2002). Cd ions uptake seem to be in competition with some nutrients like Fe, Ca, K, Mg, Mn, Cu and Zn for the same trans-membrane carrier (Rivetta *et al.,* 1997). In spite of the diverse mobility of metal ions in plants, generally the metal content in roots is greater than in the shoots (Ramos *et al.,* 2002).

In this study, results of most germination and growth parameters indicated that there are significantly inhibition effects for Cd and ZnO-NPs at 1000 mg L^{-1}. This may be associated with several disorders in the event chain of germination metabolism (Sujing *et al.,* 2012), as a result of Cd toxicity or level of NPs. Under various environmental conditions, first Cd enters to roots, and then they are likely to experience Cd damage first (Toppi and Gabrielli, 1999). Cd as serious metals have great mobility and the trace concentration is sufficient to produce its effective (Barceló and Poschenrieder, 1990). Heavy metal caused inhibition in root growth through its interference with cell division, including encouragement of abnormal mitosis and chromosomal aberrations (Talebi *et al.,* 2014). It might be due to the DNA synthesis inhibition in the cell cycle at S-phase or a blocking in the G2 phase (Sudhakar *et al.,* 2001). The present results are in agreement with the results obtained by Gowayed and Kadasa (2015) in faba bean. Cd was reduced the uptake and movement of water in the embryo axis and lowered the seedling development in *Suaeda salsa* seeds (Sujing *et al.,* 2012). Cd pollution decreased the root growth of cucumber (Chen, 1990), maize and pumpkin (Liu and Cui, 1991), wheat (Hong *et al.,* 1991) and garlic (Liu *et al.,* 2000).

In addition, many studies reported that Zn^{2+} appeared an inhibition effect on root growth (Talgar *et al.,* 2011). Pramod et al. (2011) revealed that the higher dose of ZnO-NPs suspension reduced root and shoot growth of gram and mung seedlings, which may be due to the levels of NPs. Prapatsorn *et al.* (2011) found that ZnO-NPs didn't affect rice grains germination but, in a higher concentration reduced the root length. Shu *et al.* (1997) found that the root vitality of *Stylosanthes guianensis* in mine tailings was reduced by heavy metals (Pb, Zn, Cu and Cd), and the absorption of inorganic nutrients was inhibited, which led to evident chlorosis, and significantly affected the growth. On the other hand, ZnO-NPs (500 mg L^{-1}) in combination with Cd significantly reduce the toxic effect by Cd. Jaouhra *et al.* (2011) reported that addition of Zn at low concentration reduced Cd uptake and led to highly protection to Tomato plants from Cd toxicity. Moreover, a significant increase in germination rate of durum wheat seeds was exposed to ZnO-NPs (Nadia *et al.,* 2016).

ROS has many of fundamental roles in regulating various genes expression, control several processes such as cell cycle, response to abiotic injury and systemic signaling. But, increasing generation of ROS from Cd toxicity led to oxidative stress through tumble antioxidant defense system of cells by inhibiting SOD, CAT, GPX and GR activities and GSH level (Cho and Seo, 2004). Numerous studies indicate that Cd toxicity, Zn deficiency and exposure to high Zn cause stimulation of lipid peroxidation and, in consequence, augmentation of ROS production, which may be the main reason for deterioration of different cellular functions (Mirta *et al.,* 2014)

Lipid peroxidation is initiate to produce MDA which as a sensitive diagnostic index of oxidative injury, resulting in disruption of metabolic function and loss of cellular integrity (Janero, 1990). The present results reported that MDA increased significantly in Cd treated seedling (Table 4). This result are in agreement with the results reported in the previous studies in barley (Wua *et al.,* 2003), green peas (Metwally *et al.,* 2004), chickpea (Kumari *et al.,* 2010), soybean (Hashem, 2014), and faba bean (Gowayed and Kadasa, 2016). In contrast with Cd data in Table (4) which indicated that MDA decreased in ZnO-NPs + Cd compared to Cd treated seedling. This is an indicator to protective effect of Zn-ONPs on oxidative stress induced by Cd. Where that Zn plays an major role in protection and stabilization of the biological membranes against alteration integrity and permeability of plasma membrane, peroxidative and oxidative stress (Aravind and Prasad, 2003), through preferably binds to the -SH groups of the membrane protein moiety, and conserve phospholipids and proteins from formation of disulfide and thiol oxidation (Sharma *et al.,* 1994).

In the present work SOD, GR, GPX and CAT activities and GSH concentration in shoot and roots of seedling were decreased in a plant samples treated with Cd in comparison to control (Tables 5 and 6). Cd toxicity induces various cell compartments to produce an excess of ROS that lead to inactivate SOD (Sandalio *et al.,* 2001), where SOD stimulates the disproportionation of O_2^- radicals (Vitoria *et al.,* 2001). Also, the inhibition in SOD (Serrano *et al.,* 2009) and CAT (Moussa, 2004) activities could be responsible for the overproduction of ROS, which would produce oxidative damages at macromolecules, being responsible for the Cd toxicity. In addition, Cd stimulated strong drooping in GR activity and therefore modulating the thiol level within germination process (Smiri *et al.,* 2011). Because of highly antioxidative properties of thiols, consequently they are able to counteract oxidative stress (Pichorner *et al.,* 1993). Moreover, Cd exposure led to inhibition in GSH level paralleled with a decrease of GR, then lowering substrate amount available for GPX (Vestena *et al.,* 2011). Also, inhibition of GSH stimulated by Cd has been mainly due to phytochelatin synthesis (Grill *et al.,* 1985). GPX was manifest not only by ability to reduced GSH but also other substrates like lipid hydroperoxides (Herbette *et al.,* 2002). This possibility and the isoenzymes diversity of GPX (Eshdat *et al.,* 1997), may partly explain the variation in responses of species to Cd stress (Vestena *et al.,* 2011). The inhibition of GR and GPX activities are indicator to reduced protection against oxidative injury (Schutzendubel and Polle, 2002a). Several studies reported that Cd-induced inhibition of GSH in many plant species (Smiri *et al.,* 2011). The decrease of CAT activity by Cd toxicity has been observed in faba bean (Gowayed and Kadasa, 2016) and poplar roots (Schutzendubel and Polle, 2002b).

In the present work SOD, GR, GPX and CAT activities as well the GSH concentration in shoot and root seedling were increased in samples treated with ZnO-NPs plus Cd in comparison to Cd alone (Tables 5 and 6). In contrast with Cd, Zn nutritional condition of plants impacts photo oxidative deterioration to chloroplasts, stimulated by ROS. Zn application promotes membrane-bound NADPH oxidase activity, which decreased ROS production (Cakmak and Marschner, 1988). Furthermore, it reduced process of photooxidation (Marschner and Cakmak, 1989), while SOD and CAT activities raise (Yu *et al.,* 1998). The connection between SOD and CAT actions eliminate efficiently hydrogen superoxide and peroxide, which led to plants protection against additional toxic hydroxyl radicals (Tavallali *et al.,* 2010). SOD protects 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). CAT removes H_2O_2 directly by breaking it down to H_2O and O_2 (Zhao, 2011). GPX detoxifies H_2O_2 to H_2O, using GSH directly as the reducing factor (Vestena *et al.,* 2011). GR deceased GSSG, consequently GSH regeneration made possible, closing the GPX cycle (Apel and Hirt, 2004). In tomato and cabbage ZnO-NPs induced SOD and CAT activities (Singh *et al.,* 2013) in Cucumber (Kim *et al.,* 2012) and in faba bean furthermore GPX, GR and GSH (Gowayed and Kadasa, 2016).

It could be concluded that, ZnO-NPs (500 mg L^{-1}) contribute as high protection for Maize germination as well seedling from Cd toxicity, by interfering with Cd uptake from soil and activation of the seedling antioxidant system that led to decrease in free radicals production.

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تأثير جريئات أكسيد الزنك متناهية الصغر على الإنبات ومضادات الأكسدة لبادرات الذرة الشامية المعرضة للإجهاد بالكادميوم

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يهدف هذه البحث إلى دراسة تأثير جزيئات أكسيد الزنك متناهية الصغر علي إنبات حبوب ومضادات الأكسدة لبادرات الذرة الشامية المعرضة للإجهاد بالكادميوم. لهذا الغرض تم قياس بعض صفات النمو (الوزن الغض والجاف للمجموع الجذري والخضري)، صفات الإنبات (النسبة النهائية للإنبات، متوسط الإنبات اليومي، معامل سرعة الإنبات، متوسط وقت الإنبات، معامل الإنبات، معامل الإنبات لتحمل الإجهاد، معامل قوة البادرات و معامل المادة الجافة لتحمل الإجهاد) وتقدير تركيز المالون دي الدهيد كمركب من مركبات الشقوق الحرة والجلوتاسيون المختزل كمركب من مركبات مضاد التأكسد غير الإنزيمية وكذلك نشاط بعض الأنزيمات المضادة للتأكسد مثل سوبر أكسيد ديس ميوتاز، جلوتاسيون ريدكتيز، جلوتاسيون بروكسيدز والكتاليز وذلك في كل من الجذور والمجموع الخضري للبادرات. أشارت النتائج إلى أن المعاملة بجزيئات أكسيد الزنك متناهية الصغر بتركيز ٥٠٠ مليجرام/لتر أدت إلى حماية عالية من سمية الكادميوم من خلال تقليص تركيز الكادميوم والمالون دي الدهيد وتقليل متوسط وقت الإنبات كما أدت إلى زيادة متوسط الإنبات اليومي، معامل سرعة الإنبات، معامل الإنبات لتحمل الإجهاد، معامل قوة البادرات و معامل المادة الجافة لتحمل الإجهاد، وبالتالي أدت أيضا إلى زيادة تركيز الجلوتاسيون المختزل ونشاط الأنزيمات المضادة للتأكسد سوبر أكسيد ديس ميوتاز، جلوتاسيون ريدكتيز، جلوتاسيون بروكسيدز والكتاليز. ومن نتائج البحث نستخلص أن المعاملة بجزيئات أكسيد الزنك متناهية الصغر تؤدي إلى حماية بادرات الذرة من التأثير السمي للكادميوم.