

# Influence of Zinc Oxide Nanoparticles on Cadmium Toxicity on Germination of Faba Bean (*Vicia faba* L.)

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**Abstract:** This work aimed to study the influence of zinc oxide nanoparticles (ZnONPs) on Cadmium (Cd) toxicity on germination of Faba bean (*Vicia faba* L.). The results indicated that, the levels of Zn and Cd significantly increased in shoot and root in a correlation with their dose. The levels of shoot and root Cd decreased in plants treated with the increasing of ZnONPs dose. ZnONPs and/ or Cd increased the shoot length, shoot fresh weight and shoot dry weight and whereas decreased root length, root length /shoot length and root dry weight in comparison with the control. ZnONPs and/ or Cd decreased the Mean Daily Germination MDG, Germination Index GI, Coefficient of Velocity of Germination CVG, Promptness index PI, Germination stress tolerance index GSI, Seedling vigor index SVI and Root length stress tolerance index RLSI whereas increased Mean Germination Time MGT in comparison with control. ZnONPs at 250 mg L<sup>-1</sup> with Cd 50 mg L<sup>-1</sup> showed the highest decrease in MDG, GI, CVG, PI and GSI whereas MGT recorded highest values. The highest decreased in SVI was shown in ZnONPs at 500 mg L<sup>-1</sup> with Cd 25 mg L<sup>-1</sup>. Meanwhile, the highest decreased in RLSI was shown in ZnONPs at 1000 mg L<sup>-1</sup> with Cd 50 mg L<sup>-1</sup>. It can be concluded that, ZnONPs decreased Cd level in the seedling of Faba bean, as well as germination parameters whereas increased some growth parameters.

**Keywords:** ZnONPs, Cd toxicity, Faba bean, Germination.

## INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important seed crops in the world. It is an important protein-rich food that provides a wide number of populations in the developing countries as a source of cheap protein, thus partly recovering the large deficiency in animal protein sources (Safaa and Leur 2011).

Zinc (Zn) is representative the second most plentiful transition metal in organisms after iron (Auld 2001). Zn is a fundamental micronutrient for plants, animals and humans. Generally, Zn absorbed as cation (Zn<sup>2+</sup>) in plants, which works as the metal component or as a functional structure or a regulatory co-factor of many enzymes (Prasad *et al.*, 2012).

Cd is a highly toxic heavy metal which induces oxidative stress in plants (Hasan *et al.*, 2009). It is mainly entered to the ecosystem through mining and smelting of Zn, industry and the application of phosphate fertilizers (Waisberg *et al.*, 2003). Cd ions are readily taken up by the roots (Fargašová, 2004) and accumulated in different parts of the plant which cause various phytotoxic symptoms such as inhibition in root, shoot growth (Eshghi *et al.*, 2010) by inhibiting cell division and the growth of cells or both of them (Pal *et al.*, 2006). Moreover, it's change in morphological, physiological and biochemical characteristics in plants (Benavides *et al.*, 2005; Talebi *et al.*, 2014).

Although, Zn and Cd have different biological properties, there are many chemical and physical similarities. The association of Cd and Zn may be preventing the toxicity of Cd by Zn. The convergence of Cd and Zn in environment may lead to several synergistic and antagonistic interactions in their uptake and tissue content (Jaouhra *et al.*, 2011).

Nanotechnology is an evolutionary science and has introduced many novel applications in the many fields of science as biotechnology and agricultural industries.

Nanoparticles (NPs) are molecular aggregates or atomic with size between 1 and 100 nm (Ball 2002; Roco 2003), that can sharply change their physical-chemical advantages compared to macro-molecules (Nel *et al.*, 2006; Hediati, 2012). They have powerful advantages as a result of unique physical and chemical characteristics and huge surface area relative to the size, which give them the possibility to improve the life quality and contribute competitiveness in industry field (Homa and Aghili, 2014). However, as a result of their unique advantages, some researches have been done 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). Despite the much information available on the toxic effect of NPs in plant system, few studies have been conducted on mechanisms, by which NPs exert their effect on plant growth and development. In many studies, increasing evidence suggests that ZnONPs increase plant growth and development Siddiqui *et al.*, 2015, peanut (Prasad *et al.*, 2012), soybean (Sedghi *et al.*, 2013), wheat (Ramesh *et al.*, 2014) and onion (Raskar and Laware, 2014).

Seeds germination and seedling roots are sensitive stages in the plant growth circle and it is the critical stage of plants to the alteration in surrounding environment (Liu *et al.*, 2011; Sfaxi-Bousbih *et al.*, 2010). Thus, this stage is a best trend to study the toxicological mechanisms in plants by environmental contaminants (Sujing *et al.*, 2012).

There are controversial reports about the effect of NPs on the growth and germination of plants (Mahajan *et al.*, 2011). Therefore, the present work aimed to study the influence of ZnONPs on Cd toxicity on germination parameters of Faba bean (*Vicia faba* L.).

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## MATERIALS AND METHODS

The present investigation was conducted under controlled condition in the Biology Lab, Biological Science Department, Faculty of Science, University of Jeddah, KAU during August 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<sub>2</sub>O, 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<sup>-1</sup> at 25°C.

Preparation of test solutions: Suspensions of ZnONPs in a concentration of 250, 500 and 1000 mg L<sup>-1</sup> were daily prepared with deionized water and dispersed with a sonicator (JL-360, Shanghai, USA) for 20 min. 25 and 50 mg. L<sup>-1</sup> Cd solution as 3CdSO<sub>4</sub>.8H<sub>2</sub>O were prepared with deionized water.

Seed preparation: 252 healthy and uniform size seeds of Faba bean were used in this study. The seeds were sterilized using 2.5% NaOCl solution according to Lin and Kao (1996), 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 mgL<sup>-1</sup> for 4 hours.

Seed germination test: the seeds were placed in Petri dishes 90 mm (tri replicate) on filter paper then, add 5 ml of ZnONPs suspensions (ZnONPs treated groups), Cd solution (Cd treated groups) and deionized water for control group. All dishes were incubated in a growth room (25±2°C, 65% relative humidity and dark for 72 h then 14/10-h light/dark cycle) using complete randomize design. After that 7 ml of treated solution add at second day, 10 ml at fourth day and 10 ml at sixth day for each Petri dish.

Germination parameters: Seeds becomes germinated when their radical extension exhibits longer than 3 mm (Prapatsorn *et al.*, 2011). The germinated seeds were counted daily up to end the germination (4 days) for determination of Final Germination Percentage (FGP), Mean Germination Time (MGT), Germination Index (GI), Coefficient of Velocity of Germination (CVG) and Mean Daily Germination (MDG) was calculated according to Gowayed and Almaghrabi (2013).

Promptness index (PI), Germination stress tolerance index (GSI), Root length stress tolerance index (RLSI), Vigor index (SVI) calculated according to Raskar and Laware (2013) using following formulae:

- $PI = nd2 (1.0) + nd4 (0.75) + nd6 (0.5) + nd8 (0.25)$
- Where n is the no. of seed germinated at day d
- $GSI = PI \text{ of treated seeds} / PI \text{ of control seeds} \times 100.$
- $SVI = Germination\% \times Seedling \text{ length (cm)}.$
- $RLSI = \text{Root length of treated seedling} / \text{Root length of control seedling} \times 100.$

Growth parameters: The growth parameters shoot length (SL), root length (RL), 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 8 days.

Analysis of heavy metals: At the end of exterminate shoots and roots of seedling were washed totally with distilled water and the samples oven dried at 78°C for three days. Dried samples were digested using a nitric (HNO<sub>3</sub>)-sulfuric (H<sub>2</sub>SO<sub>4</sub>)-perchloric (HClO<sub>4</sub>) 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).

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.

## RESULTS AND DISCUSSION

### a- Zinc and Cadmium determination:

Germination and early seedling development assay has been regarded as a basic experiment for evaluating the toxic effect of any metal or chemical type of plants (Ahsan *et al.*, 2007; Wang and Zhou, 2005). The toxicity of metal is an important evidence to evaluate germination and plant growth. Seedling growth is considered as an indicator of mental stress on the plant's ability to survive. Under heavy metal stress, the processes of germination, like embryo growth, will be depressed (Ahsan *et al.*, 2007; Talebi *et al.*, 2014). The chemical similarity for Cd and Zn in their association in ecosystem can lead to interaction between them (Jaouhra *et al.*, 2011). In this study, concentrations of Zn and Cd were detected in Faba bean seedling (Table 1). Zn is detected in shoot and root of control plants, whereas Cd not detected. Also, we observed that the levels of heavy metals in the tested plants increased significantly with the increased concentration of ZnONPs application or Cd. It indicates that the ZnONPs are uptake and transported to the seedling due to increase in Zn content at conformable ZnONPs treatment. Moreover, increasing concentration of ZnONPs decrease concentration of Cd and the reverse is also observed. In general the content of the metal is multiplied in the root than shoot (Sresty and Rao, 1999; Homa and Aghili, 2014) in the same treatment. Hassen *et al.* (2005) found analogical results in them study in rice plant and reported that the increase level of Zn in culture medium leads to decrease in Cd absorption and the increasing content in roots with positive correlated in its content in shoot. The correlation between Cd-Zn uptake may be is due to them competition (Hart *et al.*, 2002; Jaouhra *et al.*, 2011).

### b- Growth parameters:

Data in (Table 2) indicated that ZnONPs and/ or Cd increased the shoot length, shoot fresh weight and shoot dry weight and decreased the root length, root length /shoot length and root dry weight in comparison with control.

For the transport in plant, shoot and root length are consider the important parameters (Sujing *et al.*, 2012). The results in Table 2 showed that, the length of the

shoots were increased while, the roots length were decreased in all treated plants. The increase of SL is lead to increase in SFW and SDW. In contrast, the

inhibition of RL is lead to reduction in RFW, RDW and RL/SL.

**Table (1):** Zinc and cadmium concentrations in *Vicia faba* ( $\mu\text{g.g}^{-1}$ ) dry weight.

Treatments ( $\text{mg. L}^{-1}$ )	Cd		Zn	
	Shoot	Root	Shoot	Root
Control	Nd	Nd	88.56 $\pm$ 7.30 <sup>h</sup>	146.56 $\pm$ 5.75 <sup>h</sup>
ZnONPs 250	Nd	Nd	377.56 $\pm$ 1.95 <sup>f</sup>	3354.42 $\pm$ 11.76 <sup>f</sup>
ZnONPs 500	Nd	Nd	573.25 $\pm$ 6.53 <sup>c</sup>	4917.60 $\pm$ 571.52 <sup>c</sup>
ZnONPs 1000	Nd	Nd	1224.94 $\pm$ 3.06 <sup>a</sup>	6687.57 $\pm$ 1.73 <sup>a</sup>
Cd 25	10.24 $\pm$ 0.66 <sup>cd</sup>	234.75 $\pm$ 4.40 <sup>c</sup>	87.90 $\pm$ 2.22 <sup>h</sup>	145.89 $\pm$ 1.50 <sup>h</sup>
Cd 50	18.70 $\pm$ 2.40 <sup>a</sup>	671.20 $\pm$ 5.74 <sup>a</sup>	89.34 $\pm$ 3.74 <sup>h</sup>	146.96 $\pm$ 2.11 <sup>h</sup>
ZnONPs 250 + Cd 25	9.18 $\pm$ 0.24 <sup>d</sup>	130.34 $\pm$ 4.27 <sup>f</sup>	416.53 $\pm$ 4.21 <sup>e</sup>	2816.14 $\pm$ 5.52 <sup>g</sup>
ZnONPs 250 + Cd 50	11.88 $\pm$ 0.70 <sup>b</sup>	401.83 $\pm$ 1.57 <sup>b</sup>	247.19 $\pm$ 57.16 <sup>g</sup>	2743.90 $\pm$ 10.17 <sup>g</sup>
ZnONPs 500 + Cd 25	4.22 $\pm$ 0.59 <sup>c</sup>	123.37 $\pm$ 3.19 <sup>f</sup>	353.21 $\pm$ 1.91 <sup>f</sup>	3742.06 $\pm$ 17.94 <sup>de</sup>
ZnONPs 500 + Cd 50	11.59 $\pm$ 1.42 <sup>bc</sup>	329.22 $\pm$ 0.95 <sup>c</sup>	272.69 $\pm$ 0.69 <sup>g</sup>	3503.68 $\pm$ 6.95 <sup>ef</sup>
ZnONPs 1000 + Cd 25	3.32 $\pm$ 0.56 <sup>e</sup>	108.86 $\pm$ 1.70 <sup>g</sup>	737.67 $\pm$ 5.25 <sup>b</sup>	5236.94 $\pm$ 13.88 <sup>b</sup>
ZnONPs 1000 + Cd 50	9.92 $\pm$ 0.88 <sup>cd</sup>	276.58 $\pm$ 11.73 <sup>d</sup>	456.21 $\pm$ 5.05 <sup>d</sup>	3945.56 $\pm$ 20.68 <sup>d</sup>

Data are means  $\pm$  SD of three independent experiments.

<sup>a, b, ...</sup> or <sup>g</sup> indicated a significant difference at  $p \leq 0.05$ .

**Table (2):** Effect of different concentration of ZnONPs and/or Cd on growth parameters.

Treatments ( $\text{mg. L}^{-1}$ )	RL	S L	RL/SL	RFW	RDW	SFW	SDW
Control	6.903 $\pm$ 0.528 <sup>a</sup>	3.571 $\pm$ 0.399 <sup>b</sup>	1.946 $\pm$ 0.222 <sup>a</sup>	0.376 $\pm$ 0.027 <sup>a</sup>	0.030 $\pm$ 0.006 <sup>a</sup>	0.254 $\pm$ 0.036 <sup>b</sup>	0.025 $\pm$ 0.006 <sup>c</sup>
ZnONPs 250	3.440 $\pm$ 0.277 <sup>b</sup>	5.060 $\pm$ 0.752 <sup>a</sup>	0.688 $\pm$ 0.106 <sup>bc</sup>	0.264 $\pm$ 0.024 <sup>b</sup>	0.027 $\pm$ 0.006 <sup>ab</sup>	0.413 $\pm$ 0.050 <sup>a</sup>	0.040 $\pm$ 0.005 <sup>a</sup>
ZnONPs 500	2.916 $\pm$ 0.367 <sup>bcd</sup>	4.471 $\pm$ 0.632 <sup>ab</sup>	0.667 $\pm$ 0.162 <sup>bc</sup>	0.226 $\pm$ 0.012 <sup>bc</sup>	0.020 $\pm$ 0.002 <sup>b</sup>	0.355 $\pm$ 0.059 <sup>ab</sup>	0.033 $\pm$ 0.005 <sup>abc</sup>
ZnONPs 1000	2.796 $\pm$ 0.177 <sup>cd</sup>	4.065 $\pm$ 0.698 <sup>ab</sup>	0.706 $\pm$ 0.163 <sup>bc</sup>	0.178 $\pm$ 0.008 <sup>c</sup>	0.022 $\pm$ 0.007 <sup>ab</sup>	0.329 $\pm$ 0.058 <sup>ab</sup>	0.036 $\pm$ 0.007 <sup>ab</sup>
Cd 25	2.999 $\pm$ 0.138 <sup>bc</sup>	3.675 $\pm$ 0.430 <sup>b</sup>	0.824 $\pm$ 0.102 <sup>b</sup>	0.264 $\pm$ 0.020 <sup>b</sup>	0.023 $\pm$ 0.007 <sup>ab</sup>	0.328 $\pm$ 0.042 <sup>ab</sup>	0.029 $\pm$ 0.004 <sup>bc</sup>
Cd 50	2.515 $\pm$ 0.437 <sup>cd</sup>	4.037 $\pm$ 0.523 <sup>ab</sup>	0.628 $\pm$ 0.131 <sup>bc</sup>	0.268 $\pm$ 0.076 <sup>b</sup>	0.022 $\pm$ 0.005 <sup>ab</sup>	0.332 $\pm$ 0.029 <sup>ab</sup>	0.025 $\pm$ 0.001 <sup>c</sup>
ZnONPs 250 + Cd 25	3.070 $\pm$ 0.302 <sup>bc</sup>	4.360 $\pm$ 0.479 <sup>ab</sup>	0.708 $\pm$ 0.078 <sup>bc</sup>	0.253 $\pm$ 0.025 <sup>b</sup>	0.026 $\pm$ 0.006 <sup>ab</sup>	0.348 $\pm$ 0.054 <sup>ab</sup>	0.034 $\pm$ 0.004 <sup>abc</sup>
ZnONPs 250 + Cd 50	2.620 $\pm$ 0.079 <sup>cd</sup>	3.588 $\pm$ 0.407 <sup>ab</sup>	0.736 $\pm$ 0.072 <sup>bc</sup>	0.220 $\pm$ 0.039 <sup>bc</sup>	0.023 $\pm$ 0.004 <sup>ab</sup>	0.292 $\pm$ 0.074 <sup>b</sup>	0.030 $\pm$ 0.007 <sup>abc</sup>
ZnONPs 500 + Cd 25	2.798 $\pm$ 0.454 <sup>cd</sup>	3.381 $\pm$ 0.666 <sup>b</sup>	0.858 $\pm$ 0.246 <sup>bc</sup>	0.257 $\pm$ 0.018 <sup>b</sup>	0.026 $\pm$ 0.007 <sup>ab</sup>	0.319 $\pm$ 0.034 <sup>ab</sup>	0.034 $\pm$ 0.007 <sup>abc</sup>
ZnONPs 500 + Cd 50	2.746 $\pm$ 0.226 <sup>cd</sup>	4.398 $\pm$ 0.579 <sup>ab</sup>	0.630 $\pm$ 0.083 <sup>bc</sup>	0.239 $\pm$ 0.055 <sup>bc</sup>	0.021 $\pm$ 0.005 <sup>ab</sup>	0.358 $\pm$ 0.062 <sup>ab</sup>	0.037 $\pm$ 0.004 <sup>ab</sup>
ZnONPs 1000 + Cd 25	2.624 $\pm$ 0.421 <sup>cd</sup>	4.332 $\pm$ 1.510 <sup>ab</sup>	0.630 $\pm$ 0.108 <sup>bc</sup>	0.173 $\pm$ 0.067 <sup>c</sup>	0.027 $\pm$ 0.001 <sup>ab</sup>	0.349 $\pm$ 0.159 <sup>ab</sup>	0.037 $\pm$ 0.011 <sup>ab</sup>
ZnONPs 1000 + Cd 50	2.413 $\pm$ 0.318 <sup>d</sup>	4.320 $\pm$ 0.177 <sup>ab</sup>	0.557 $\pm$ 0.052 <sup>c</sup>	0.214 $\pm$ 0.038 <sup>bc</sup>	0.020 $\pm$ 0.005 <sup>ab</sup>	0.305 $\pm$ 0.029 <sup>ab</sup>	0.030 $\pm$ 0.002 <sup>bc</sup>

Data are means  $\pm$  SD of three independent experiments.

<sup>a, b, ...</sup> or <sup>g</sup> indicated a significant difference at  $p \leq 0.05$ .

These results in accordance with the results obtained by Pramod *et al.* (2011) who revealed that the higher dose of ZnONPs suspension reduced root and shoot growth of gram and mung seedlings, which may be duo to toxicity levels of NPs. ZnONPs significantly reduced the biomass of rye-grass, tip of root and different tissues of root. ZnONPs highly adhered on root surface and a few NPs were observed in the apoplast, steel and root endodermis. Zn transporter from root to shoot stay very low through ZnONPs treatments and the author indicated that the ZnONPs toxicity was not correlated directly with their bounded dissolution in the

solution of bulk nutrient or rizosphere (Lin and Xing, 2008). So, this may explain the increasing of SL, SFW and SDW, although the inhibition of RL.

#### c- Germination parameters:

ZnONPs and/or Cd decreased the Mean Daily Germination MDG, Germination Index GI, Coefficient of Velocity of Germination CVG, Promptness index PI, Germination stress tolerance index GSI, Seedling vigor index SVI and Root length stress tolerance index RLSI and increased Mean Germination Time MGT in comparison with the control (Table 3).

**Table (3):** Effect of different concentration of ZnONPs and/or Cd on germination parameters.

Treatments (mg. L <sup>-1</sup> )	FGP	MDG	CVG	MGT	GI	PI	GSI	SVI	RLSI
<b>Control</b>	100	38.89 ± 9.62 <sup>a</sup>	91.67 ± 7.22 <sup>a</sup>	1.10 ± 0.08 <sup>c</sup>	3.39 ± 0.10 <sup>a</sup>	6.83 ± 0.14 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>	1047.34 ± 74.00 <sup>a</sup>	100.00 ± 0.00 <sup>a</sup>
<b>ZnONPs 250</b>	100	33.33 ± 0.00 <sup>ab</sup>	81.02 ± 5.61 <sup>ab</sup>	1.24 ± 0.08 <sup>bc</sup>	3.22 ± 0.10 <sup>ab</sup>	6.58 ± 0.14 <sup>ab</sup>	96.34 ± 2.11 <sup>ab</sup>	849.95 ± 86.79 <sup>b</sup>	49.84 ± 4.02 <sup>b</sup>
<b>ZnONPs 500</b>	100	30.56 ± 4.81 <sup>b</sup>	63.99 ± 5.84 <sup>cd</sup>	1.57 ± 0.14 <sup>a</sup>	2.86 ± 0.13 <sup>c</sup>	6.00 ± 0.25 <sup>c</sup>	87.80 ± 3.66 <sup>c</sup>	738.65 ± 29.90 <sup>bc</sup>	42.24 ± 5.31 <sup>bcd</sup>
<b>ZnONPs 1000</b>	100	33.33 ± 0.00 <sup>ab</sup>	79.55 ± 13.78 <sup>b</sup>	1.29 ± 0.25 <sup>bc</sup>	3.17 ± 0.29 <sup>b</sup>	6.50 ± 0.43 <sup>ab</sup>	95.12 ± 6.34 <sup>ab</sup>	686.11 ± 61.38 <sup>c</sup>	40.51 ± 2.57 <sup>cde</sup>
<b>Cd 25</b>	100	30.56 ± 4.81 <sup>b</sup>	75.19 ± 4.49 <sup>bc</sup>	1.33 ± 0.08 <sup>b</sup>	3.14 ± 0.05 <sup>b</sup>	6.42 ± 0.14 <sup>b</sup>	93.90 ± 2.11 <sup>b</sup>	667.37 ± 45.10 <sup>c</sup>	43.45 ± 2.01 <sup>bcd</sup>
<b>Cd 50</b>	100	30.56 ± 4.81 <sup>b</sup>	75.19 ± 4.49 <sup>bc</sup>	1.33 ± 0.08 <sup>b</sup>	3.14 ± 0.05 <sup>b</sup>	6.42 ± 0.14 <sup>b</sup>	93.90 ± 2.11 <sup>b</sup>	655.24 ± 72.36 <sup>c</sup>	36.44 ± 6.34 <sup>de</sup>
<b>ZnONPs 250 + Cd 25</b>	100	33.33 ± 0.00 <sup>ab</sup>	75.19 ± 4.49 <sup>bc</sup>	1.33 ± 0.08 <sup>b</sup>	3.11 ± 0.10 <sup>b</sup>	6.42 ± 0.14 <sup>b</sup>	93.90 ± 2.11 <sup>b</sup>	742.94 ± 68.44 <sup>bc</sup>	44.47 ± 4.38 <sup>bc</sup>
<b>ZnONPs 250 + Cd 50</b>	100	30.56 ± 4.81 <sup>b</sup>	58.33 ± 0.00 <sup>d</sup>	1.71 ± 0.00 <sup>a</sup>	2.69 ± 0.05 <sup>c</sup>	5.75 ± 0.00 <sup>c</sup>	84.15 ± 0.00 <sup>c</sup>	620.79 ± 46.43 <sup>c</sup>	37.95 ± 1.14 <sup>cde</sup>
<b>ZnONPs 500 + Cd 25</b>	100	33.33 ± 0.00 <sup>ab</sup>	81.02 ± 5.61 <sup>ab</sup>	1.24 ± 0.08 <sup>bc</sup>	3.22 ± 0.10 <sup>ab</sup>	6.58 ± 0.14 <sup>ab</sup>	96.34 ± 2.11 <sup>ab</sup>	617.86 ± 52.68 <sup>c</sup>	40.53 ± 6.57 <sup>cde</sup>
<b>ZnONPs 500 + Cd 50</b>	100	30.56 ± 4.81 <sup>b</sup>	61.87 ± 3.06 <sup>d</sup>	1.62 ± 0.08 <sup>a</sup>	2.81 ± 0.05 <sup>c</sup>	5.92 ± 0.14 <sup>c</sup>	86.59 ± 2.11 <sup>c</sup>	714.44 ± 66.44 <sup>bc</sup>	39.78 ± 3.28 <sup>cde</sup>
<b>ZnONPs 1000 + Cd 25</b>	100	33.33 ± 0.00 <sup>b</sup>	84.26 ± 5.61 <sup>ab</sup>	1.19 ± 0.08 <sup>bc</sup>	3.28 ± 0.10 <sup>ab</sup>	6.67 ± 0.14 <sup>ab</sup>	97.56 ± 2.11 <sup>ab</sup>	695.62 ± 193.16 <sup>c</sup>	38.01 ± 6.10 <sup>cde</sup>
<b>ZnONPs 1000 + Cd 50</b>	100	33.33 ± 0.00 <sup>ab</sup>	81.67 ± 10.10 <sup>ab</sup>	1.24 ± 0.16 <sup>bc</sup>	3.22 ± 0.19 <sup>ab</sup>	6.58 ± 0.29 <sup>ab</sup>	96.34 ± 4.23 <sup>ab</sup>	673.24 ± 48.52 <sup>c</sup>	34.95 ± 4.60 <sup>e</sup>

Data are means ± SD of three independent experiments.

<sup>a, b, c, ...</sup> or <sup>g</sup> indicated a significant difference at  $p \leq 0.05$ .

Seed germination and seedling growth parameters are very important indicators in yield determination (Rauf *et al.*, 2007). Dhanda *et al.* (2004) indicated that seed vigor index and shoot length are among the most sensitive indices to stress, followed by root length and coleoptiles length. In present study, the germination parameters presented in Table (3) indicated that all treatments led to 100 % germination of seeds (FGP) and a significant reduction in all germination indices except MGT. Prapatsorn *et al.* (2011) found that ZnONPs didn't affect rice grains germination but, in a higher concentration reduced the root length. Seed germination needs water imbibitions to start a physiological process (Wierzbicka and Obidzinska, 1998). However, in case of germination seed of Faba bean take place normally but the bad effect is more obvious in the roots, probably due to the coat of faba bean seed, which can act to protect the embryo (Prapatsorn *et al.*, 2011). Also, other nanoparticles like alumina (nano-Al<sub>2</sub>O<sub>3</sub>) at 2000 mgL<sup>-1</sup> could inhibit root elongation of five plant species, *Zea*

*mays* (corn), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot) (Yang and Watts, 2005). Uptake and movement of water in the embryo axis was inhibited by Cd concentration lowered the seedling development in *Suaeda salsa* seeds (Sujing *et al.*, 2012). Many studied reported that Zn<sup>2+</sup> showed an inhibitory effect on root growth and the cell division (Munzuruglu and Geckil, 2002; El-Ghamery *et al.*, 2003; Talgar *et al.*, 2011). This may be associated with several disorders in the event chain of germinative metabolism (Sujing *et al.*, 2012). The inhibition of root elongation caused by heavy metal may be due to metal interference with cell division, in cluding inducement of chromosomal aberrations and abnormal mitosis (Talebi *et al.*, 2014). Talgar *et al.* (2011) reported that, the reduction in the rate of mitotic division in garlic plant was attributed to the mitotic inhibition by ZnONPs, indicated that ZnONPs could interfere with the development of mitosis and cause cytotoxic effects. It might be due to

the inhibition of DNA synthesis at S-phase or a blocking in the G2 phase of the cell cycle (Duan and Wang, 1995; Borboa and De la Torre, 1996; Sudhakar *et al.*, 2001).

This evidence supporting that some engineered nanoparticles could exert physio-chemical toxicity or bad effect on plant depending on their chemical composition, size, surface energy and importantly is the species of plant which resulting in different ways (Prapatsorn *et al.*, 2011).

### CONCLUSION

It could be concluded that increase doses of ZnONPs decrease the content of Cd in the plant, which leads to reduce the Cd toxicity. Doses of ZnONPs (250, 500 and 1000 mg L<sup>-1</sup>) haven't positive effect on germination parameters. Effect of applications of nanotechnology in plant remained need more effort and more study to clear their benefits or harmful.

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