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ASSESSMENT OF EXPOSURE WHEAT *TRITICUM AESTIVUM* L. TO ZINC OXIDE NANOPARTICLES (ZNO): EVALUATION OF OXIDATIVE DAMAGE

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ABSTRACT: Nanoparticles (NPs) are introduced in a growing number of commercial products and their production may lead to their release in the environment. To increase knowledge on Zinc oxide nanoparticles accumulation and impact on plants, we designed a study on wheat *Triticum aestivum* L. Plants were exposed to ZnO-NPs at several concentrations (10; 50; 100; 500; 1000; 1500; 2000 ppm) for 14 days. ZnO nanoparticles were prepared by chemical synthesis method. The crystal structure of the ZnO was investigated by using, x-ray diffraction (XRD). This impact have been studied on roots and leaves by measuring the plant growth, Oxidative stress biomarkers (CAT) and (APX) activities, Oxidative damage indicators such as lipid peroxidation (MDA) and (H_2O_2) content, as well respiratory energy metabolism was examined. Our results highlight a decrease of growth wheat plants and reveals a significantly induction of antioxidant enzymatic activities such as CAT and APX. We noted also a lipid peroxidation supported by a significant dose-related increase in MDA and H_2O_2 level and a significant inhibition of respiratory activity. Triggering of the endogenous antioxidant system and the inhibition of mitochondrial respiratory activity could be explained by the increased enhance production of free radicals causing cellular damage.

Keywords: *Triticum aestivum* L., Zinc nanoparticles, Oxidative stress, Phytotoxicity, Respiratory metabolism.

INTRODUCTION:

Nanotechnology is emerging as a rapidly growing field in the 21st century, with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level (Santhoshkumar *et al.*, 2014). The various commercial uses of nanomaterials for novel applications are increasing exponentially. Nanomaterials are extremely small in size and possess a large surface area per unit of volume**.** These novel physical characteristics of nanomaterials can result in their having drastically different chemical and biological properties compared to the same material in bulk form (Fu *et al*, 2014). Nanomaterials are part of a commercial revolution that has resulted in an explosion of hundreds of new products due to their diverse physico-chemical properties, enabling their usage in a wide range of innovative applications, including some in industry, agriculture, business, medicine, clothing, cosmetics, and food (Gonzalez *et al*, 2008).

Several recent studies have evaluated NPs phytotoxicity as well as their ecotoxicity (Barrena *et al.*, 2009; Guangke *et al.*, 2011). Zinc Oxide nanoparticles are one of the most widely present nanomaterials in human life. Their antimicrobial properties and contribution to mechanical properties of final products made them inevitable in food industry, medical and dental materials (Zeljezic and Mladinic, 2014).

For these reasons, these NPs will surely be released in the environment. The knowledge of their potential effects on human health is rapidly increasing, however little is known on their potential toxicological effects on environment, i.e. destabilization of the ecosystems and trophic transfer, but also on their potential transfer to the food chain via plant ingestion (Gottschalk *et al.*, 2009; Mahmoodzadeh *et al.*, 2013). Many people can get exposed to nanostructures in a variety of manners such as researchers manufacturing the nanostructures. Nanoparticles may interact with macromolecules in unexpected way inducing adverse health effects (Fischer and Chan, 2007).

Hence, nanotoxicology a new subdiscipline of nanotechnology has emerged. There is a keen interest in nanotoxicology research because the processing of nanostructures in biological systems could lead to unpredictable effects (Oberdorster *et al.*, 2007). Concerns over the potential threats of these engineered NPs to the biota and ecosystems have led to numerous toxicological studies to investigate the negative effects of these NPs on various organisms and on water/soil quality (Liu *et al*, 2016). Therefore, understanding the interactions of nanomaterials with biological systems is a particularly important scientific issue. The toxicity of nanomaterials has been studied in different biological systems, both in cell line systems and different organisms (Fischer and Chan, 2007).

Compared to their bulk-size counterparts, engineered nanomaterials possess a small size, high specific surface area, and high surface reactivity, leading to the production of higher levels of ROS

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(Reactive Oxygen Species). Nevertheless, the amount of cellular uptake decreases with the increase in particle size (Wang *et al.,* 2010). Biologically relevant ROS include superoxide anion radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide (H_2O_2). Generation of ROS induced by nanomaterials, directly or indirectly, plays a vital role in genotoxicity (Yin *et al.*, 2012).

Oxidative stress is one of several mechanisms leading to nanotoxicity. Some nano-metal oxides can enhance ROS generation, inducing oxidative stress, DNA damage, unregulated cell signaling, and eventually leading to changes in cell motility, apoptosis, and even carcinogenesis (Valco *et al.,* 2006; Amamra *et al.,* 2015).

Generation of free radicals or reactive oxygen species during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress (Zima *et al.,* 2001).

Indeed, understanding the mechanism of nanomaterials induced toxicity is the first defense for hazard prevention. Antioxidant plays a crucial rule in terminating the oxidative rancidity in food by scavenging the free radical which is generated during oxidation process (Beltran *et al.,* 2004). Enzymatic defenses have evolved to protect against harmful biological oxidants. SODs, peroxidases and catalases are some of the prominent and extensively studied antioxidant enzymes. Also, antioxidants also play an important role in preventing/limiting the damage caused by ROS (Prior *et al.,* 2005). In addition, metal oxide NPs promote reactive oxygen species (ROS) generation, which is a predictive chemical marker of nanotoxicity and an indicator for evaluating ENP phytotoxicity (Adams *et al.,* 2006; Choi and Hu 2008)

Wheat is one of the most important staple cereal crops that cultivates worldwide due to its highly nutritious value and versatility in adaption to a wide range of agro climatic conditions (Aggarwal *et al*, 2015). Generally, wheat is used for bio-indicator or bio-monitor of pollution damage (Weinstein *et al.*, 1990). Because, they are easy to grow and adaptable to environmental stress and can be used for assessment of environmental conditions in different habitats (Lee *et al,* 2013). Among plant species, wheat is known as metal accumulator and to be translocated well to shoots from roots (Tamura *et al.,* 2005). However, the uptake, accumulation, and translocation of engineered nanoparticles (NPs) by wheat are only few investigated.

Thus, the aim of this research was to study effects of ZnO nanoparticles, on a variety of soft wheat plants by study of different Biomarkers of Oxidative stress and Oxidative lipid peroxidation in roots and leaves, also a respiratory activity was evaluated. This study provides new information on nanoparticles effects on plants. This approach enhances our understanding of the effects of the ENPs on this plant.

MATERIALS AND METHODS:

Plant material: The experiments are carried out at the Laboratory of Cellular Toxicology of Annaba, University, Algeria. Wheat seeds *Triticum aestivum* L. HD 1220 obtained from the Algerian Office Inter Cereals (AOIC) El Hadjar Annaba, Algeria, were used as plant model to study toxic effects and biological responses upon exposure to toxic.

Chemical material characterization and suspension: ZnO nanoparticles were synthesized according to Sharma *et al.* (2011) and characterized by the XRD patterns obtained utilizing an X-ray diffractor using X'Pert PRO (PANalytical) system with Cu radiation at wavelength λ=1.5405980 Å at 2θ values between 20° and 80°. The synthesized ZnO-NP resulted in small particle size which is 30 nm. Nanoparticles were first suspended in 18 MΩ deionized water through sonication to prevent aggregation. The stocks were sonicated for 30 min in a temperature controlled sonication bath (150W, 40Hz, 25ºC) (Wang *et al,* 2016). Various concentrations (0; 10; 50; 100; 500; 1000; 1500; 2000 ppm) of ZnO nanoparticles were prepared.

Seedling culture: Seeds were surface sterilized in 7% (v/v) sodium hypochlorite (NaCiO) for 15 min, thoroughly rinsed with de-ionized water and subsequently soaked in same for overnight at 4°C. Seeds of the other plant species were then transferred to filter papers (90-mm diameter, Whatman No.1) placed in Petri dishes (100 mm × 15 mm), that contain 5ml of each concentration of a test solution, with 10 seeds per dish and 1 cm or larger distance between each seed, and allowed to germinate at 25±1ºC in the dark for 48 h.

At 14th day of ZnO exposure, leaves and roots of wheat seedlings were harvested from all the treatments. They were rinsed carefully in deionized water and pressed gently between blotting paper to remove excess water. The seedlings were subsequently used for the measurement of various parameters as described hereafter.

Plant growth parameters: Total length of root and leaves was measured after 14 days of treatment. Roots and leaves were separated and used for recording the parameters (Tiquia *et al.,* 1996)**.**

Biochemical assay:

Estimation of lipid peroxidation: Lipid peroxidation was measured in terms of malondialdehyde (MDA) produced by 2-thiobarbituric acid (TBA) as per method given by De Vos *et al.* (1989). leave/root samples from treated and control seedlings were homogenized in 5 ml of 10% (w/v) trichloroacetic acid (TCA) containing 0.25% (w/v) 2-thiobarbituric acid (TBA). The homogenates were incubated at 95°C for 30 min and immediately cooled in ice bath to stop the reaction. Thereafter, the homogenate was centrifuged at 12000 g for 20 min and absorbance of the supernatant was taken at 532 and 600 nm. The amount of MDA produced was determined by subtracting the absorbance at 600 nm from that at 532 nm and using an absorbance coefficient of 155 Mm cm^{-1} (Kwon *et al.,* 1965).

Estimation of H_2O_2 content: Hydrogen peroxide content from treated and untreated (control) wheat seedlings were determined according to Velikova *et al.* (2000). Leave /root samples were homogenized in 5.0 ml of TCA (0.10% (w/v)) in an ice bath and centrifuged at 12000 g for 15 min. An aliquot (0.75 ml) of supernatant was added to 0.75 ml potassium phosphate buffer (10.0 mM pH 7.0) and 1.5 ml potassium iodide (1.0 M). H_2O_2 content was measured by taking the absorbance of the mixture at 390 nm and using an extinction coefficient of 0.28 $mM^{-1}cm^{-1}$

Estimation of antioxidant enzymes:

Extraction of enzymes was described by Loggini *et al.* (1999). Leaves and roots of wheat (1g of fresh weight) were homogenized in ice cold 50mM phosphate buffer (pH 7.5). The homogenate were centrifuged at 12000g for 20 min, and the supernatants were used for enzyme activity assays.

CAT activity was determinated according to Cakmak and Horst (1991). The assay mixture (3,0ml) consisted of 100µl enzyme extract, 50µl H_2O_2 (300mM) and 2.85ml 50mM phosphate buffer (pH=7.2). CAT activity was assayed by monitoring the decrease in the absorbance at 240 nm as a consequence of H_2O_2 disappearance (ε=39. $4mM^{-1}$ cm^{-1}). **APX** activity was assayed according to the method of Nakano and Asada (1987). The reaction mixture consisted of 100µl enzyme extract, 0,5mM ascorbate, 50mM phosphate buffer (pH=7.2) and 50µl H_2O_2 (300mM). The oxidation of ascorbate was determined by the change in absorbance at 290 nm (ε= 2.8 $mM^{-1}cm^{-1}$).

Measurement of respiratory activity:

Respiratory metabolism was assessed by measuring the respiratory activity of isolated roots of wheat *Triticum aestivum* L. The respiratory activity was monitored using a Clark oxygen electrode (Hansatech Ltd, Kinj's Lym, U, K) coupled to a computer. The reaction medium contains buffer (10 mM phosphate) at pH 7.2 and 0.5 to 1 g of roots. The glass cell, a volume adjustable from 1 to 2 ml is thermostated at 25 ° C ± 0.02°C (Djebar and Djebar, 2000).

Statistical analysis:

The obtained results are represented by the average ± Standard Error. Statistical analysis is performed by the Student "t" test using data analysis software: Minitab (version 16.0) (Dagnelie, *et al*., 1999). The probability were considered significant when *p < 0.05; very significant when **p < 0.01; and very high significant when ***p < 0.001.

RESULTS:

Structural Properties by XRD:

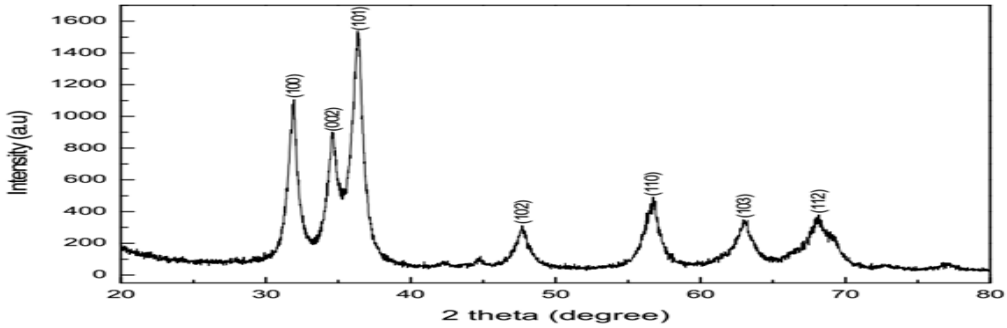

Fig.1. XRD pattern of ZnO nanoparticles.

The peaks pointed out at 2θ values of can be associate with (100),(002), (101), (102), (110),(103) and (112), respectively, correspond to hexagonal zinc item phase of ZnO which are in good agreement with the literature values (JCPDS card no.36-1451).The average ZnO nanoparticles size of 30 nm can be estimated by Scherrer's formula (Ganesh *et al.*,2012). **$D=\lambda/(\beta \cos\theta)$**, where k = 0.9 , λ is the X-ray wavelength (1.5405980 Å) , β is the full width at half maximum of and θ is the Bragg diffraction angle of the diffraction peaks.

Growth responses of wheat to ZnO:

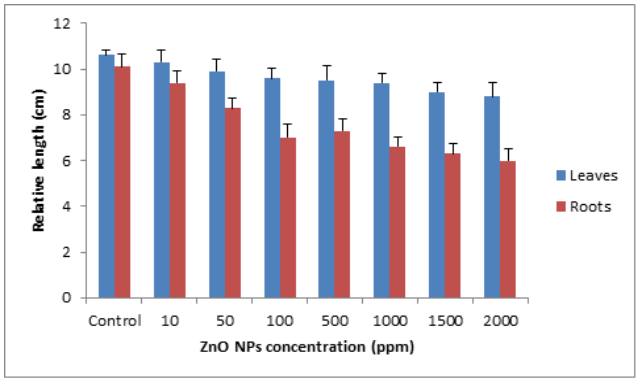

Fig.2. Relative length of roots and leaves *Triticum aestivum* L. after 14 days of treatment with ZnO nanoparticles.

Results are presented as mean±S.E (Standard Error); Significantly different from the control: *p<0.05, **p< 0.01, ***p<0.001.

Total length of root growth of seedling wheat exposed to Zinc NPs after 14 day of treatment **(fig.2)** showed a significant decrease in length root treated with all concentrations compared to the controls. In fact, the root length decreased is in order to (31.68%) and (40.59%) for the higher concentrations (1500 ppm and 2000 ppm), compared to the controls.

However, aerial part length of wheat *Triticum aestivum* L. treated with ZnO NPs at the various concentrations revealed a decrease in the length of leaves plants treated from that of the control after 14 day of treatment. Indeed, for the highest concentrations 2000 ppm length of leaves treated is in order to (8.8 cm) compared to the controls (10.6 cm).

Oxidative stress biomarkers:

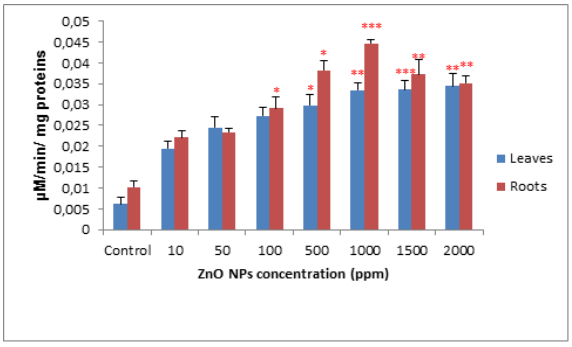

Fig.3. Catalase activity in roots and leaves after treatment with ZnO nanoparticles on *Triticum aestivum* L.

Results are presented as mean±S.E (Standard Error); significantly different from the control: *p<0.05, **p< 0.01, ***p<0.001.

Figure 3 illustrates a dose-dependent increase of CAT activity on wheat plants in the roots and leaves treated with different concentrations of ZnO NPs compared to the control after 14 days of treatment. The level of catalase increased from (0.0101 µM/min/mg of proteins) and (0.0061 µM/min/mg of proteins) in controls to (0.0446 µM/min/mg of proteins and (0.0334 µM/min/mg of proteins) in roots and leaves, respectively for the concentration 1000 ppm.

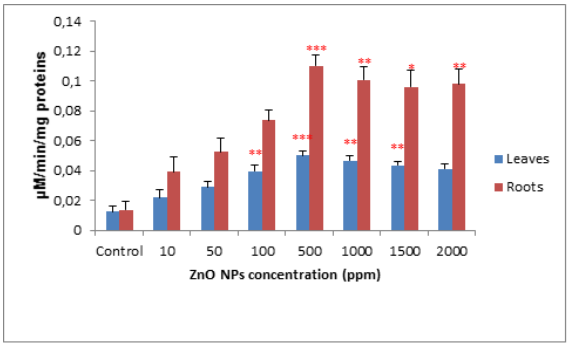

Fig.4. Ascorbate peroxydase activity in roots and leaves after treatment with ZnO nanoparticles on *Triticum aestivum* L.

Results are presented as mean±S.E (Standard Error); Significantly different from the control: *p<0.05, **p< 0.01, ***p<0.001.

Figure 4 showed the impact of ZnO NPs on ascorbate peroxidase activity (APX) in roots and leaves. Thus after 14 days of treatment, ascorbic activity increase and revealed a significant induction particularly for concentration 500 ppm with a level (0. 0.110 µM/min/mg of proteins) for roots and (0.0501 µM/min/mg of proteins) for leaves.

Biochemical assay:

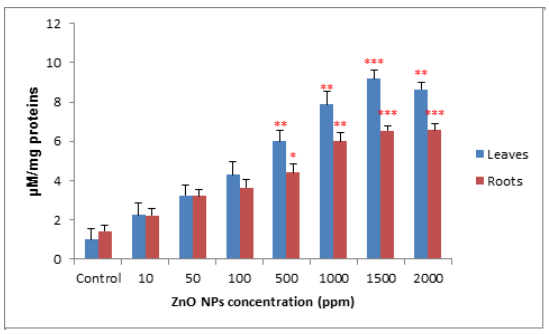

Fig.5. Malondialdehyde in roots and leaves after treatment with ZnO nanoparticles on *Triticum aestivum* L.

Results are presented as mean±S.E (Standard Error); Significantly different from the control: *p<0.05, **p< 0.01, ***p<0.001.

The measurements of MDA level in roots and leaves was dose-dependent increased at different concentrations of ZnO NPs compared with the controls **(fig.5).** At the highest concentration (2000ppm) in the roots, the rate of increased was (6.5727 µM/mg of proteins) and at concentration (1500ppm), it was noted (9.1971µM/mg of proteins) in leaves.

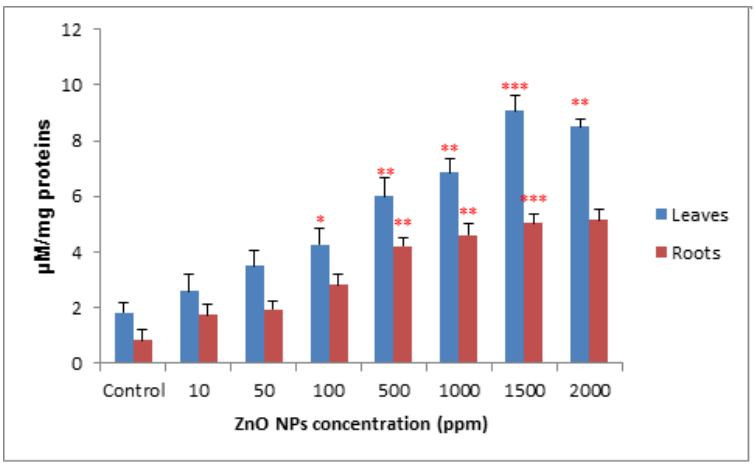

Fig.6. Hydrogen peroxide in roots and leaves after treatment with ZnO nanoparticles on *Triticum aestivum* L.

Results are presented as mean±S.E (Standard Error); Significantly different from the control: *p<0.05, **p< 0.01, ***p<0.001.

The effects of different concentrations of ZnO nanoparticles on the H_2O_2 content in roots and leaves as demonstrated in **figure 6**. The result revealed a significant and dose related increase of H_2O_2 level content in roots and leaves after 14 days of treatment. This increase is in order of (5.1584 µM/mg of proteins) and (8.5025µM/mg of proteins) for the highest concentration 2000 ppm, for roots and leaves respectively compared to the controls.

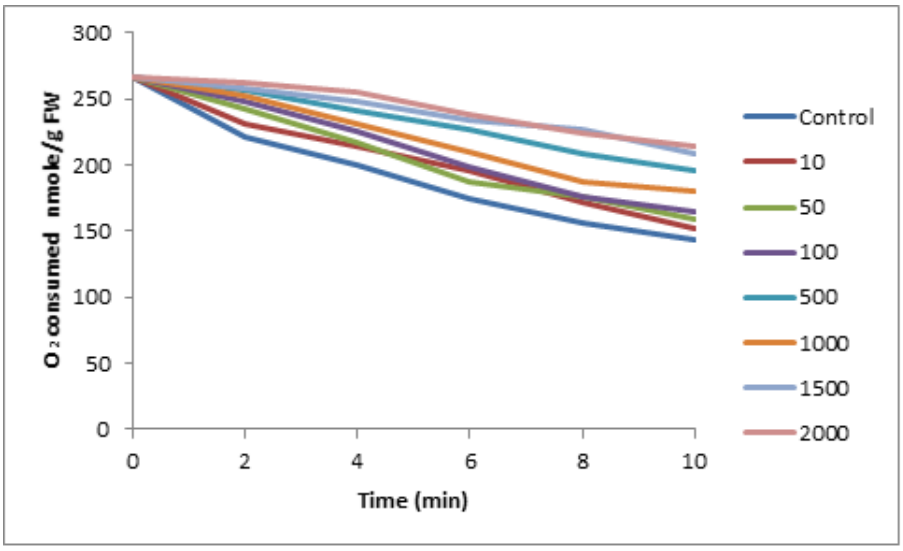

Fig. 7. Respiratory activity in roots after treatment with ZnO nanoparticles on *Triticum aestivum* L.

Results are presented as mean±S.E (Standard Error); Significantly different from the control: *p<0.05, **p< 0.01, ***p<0.001.

Figure 7 represents the evolution of oxygen consumption in roots after 14 days of ZnO NPs treatment. The results indicated a significantly decrease on the roots of wheat plants at all concentration of zinc oxide nanoparticles compared to the controls.

DISCUSSION:

Recent advances in nanotechnology include the incorporation of metallic nanoparticles (NPs) into diverse industrial, medical, and household products (Navarro *et al.*, 2008; Lee *et al.*, 2013). Improper handling and disposal of NP-containing wastes could result in environmental contamination. NPs have the potential for harmful effects on plants (Lin and Xing, 2007; Dimkpa *et al.*, 2012; Nair *et al.*, 2014; Du *et al.*, 2015). As an essential nutrient at low concentrations, Zn is crucial to several biochemical functions in plants such as producing chlorophyll and forming carbohydrates. Thus, Zn is the most yield-limiting micronutrient in crop global production (Fageria, 2009). Plants expose huge interfaces to the air and soil environment. Thus, persistent NPs with crop plants can enter the human food chain (Lee *et al.*, 2013). Hence,

plants are one of the entry points of certain pollutants into the food chain making it an essential study model for hazard and risk assessment (Manier, 2008).

In the present study, the growth of plants shows a decrease in relative length of roots wheat *Triticum aestivum* L. exposed to ZnO nanoparticles. In fact, Mahmoodzadeh *et al.* (2013) reported that nano-ZnO to be one of the most toxic nanoparticles that could terminate root growth of test plants (radish, rape, ryegrass, lettuce, corn, and cucumber). Our results corroborate those of Ren *et al.* (2011); Landa *et al.* (2012) have also observed an increase at the roots of *Mung Bean* and *Arabidopsis thaliana* treated with NPs Fe_2O_3, ZnO and TiO_2 respectively.

Indeed, Lin and Xing (2007) account for ZnO NPs primarily adhere to the root surface and are observed in the apoplast and protoplast spaces in root endodermis.

On the other hand, the nanoparticles of ZnO affect also negatively the growth of leaves of soft wheat plants. These results confirmed by Lee *et al.* (2010) found that a decrease in leaves growth of *A. thaliana* growth under treatment with ZnO, Fe_3O_4 and SiO_2 NPs. Similar results were obtained by Lee *et al.* (2008), *Wu et al.* (2012) and Shi *et al.* (2011). They show that nanoparticles of CuO reduced length leaves in terrestrial and aquatic plants. In addition, multiwall carbon nanotubes have been found to decrease of length leaves in tomato plants (Khodakovskaya *et al.*, 2011).

Plants under the abiotic and biotic stress conditions are reported to increase the production of ROS. For protection against the toxic effects of ROS, plant cells employ antioxidant defence systems, which provide the first line of defence toward any kind of oxidative toxicity at the cellular level (Gill *et al.*, 2010).

In order to assess the extent of oxidative stress in NPs treated plants, the levels of antioxidant biomarkers such as CAT and APX were determined (Faisal *et al.*, 2013).

Catalase is an important enzyme in antioxidant defense systems by converting free radicals H_2O_2 to water and oxygen. Therefore, it provides protection against oxidative damage to the cell (Bai *et al.* 1999). In this study, CAT enzyme activity was significantly increased at all treatment concentrations on roots and leaves of wheat plants. Thus Wang *et al.* (2011) reported an increase in CAT activity in roots of ryegrass under different concentrations of nano-ZnO.

Similar results were recorded by Dimkpa *et al.* (2012); Trujillo-Reyes *et al.* (2014) in roots and leaves to scavenge most of the oxidative stress induced by CuO NPs in *Triticum aestivum* and lettuce treated. Furthermore, CAT activity seemed to be a saturated pattern at the high doses (1000 and 2000 mg/l) NPs. This phenomenon resulted from high dose of NPs that produced too much ROS, which exceeded the scavenging capacity of CAT, inhibited its activity, and produced oxidative damage (Xia *et al.* 2006; Kim *et al.* 2009).

According to Willekens *et al.* (1997) CAT has been suggested to scavenge the bulk H_2O_2, while peroxidases can sequester the remaining H_2O_2. Ascorbate peroxidase is specific to plants and algae that are indispensable to protect chloroplast and other cell organelles from damage by H_2O_2 and $^{\bullet}OH$ (hydroxyl radical) produced from it. These enzymes work independently in different parts of the plants to break up hydrogen peroxide (Rao *et al.*, 2014). Our results demonstrate an increase in ascorbate peroxidase activity in the roots and leaves of plants. Those results are in line with Iannone *et al.* (2016) who reported a high induction of ascorbate activity caused by magnetite NPs in plants of wheat (*Triticum aestivum*). Data regarding, Hernandez-Viezcas *et al.* (2011); Chiahi *et al.* (2016), determined an increase in the peroxidase enzyme level in *Velvet mesquite* and *Triticum turgidum ssp. durum* under treatment of Zinc nanoparticles.

In summary, lack of complete scavenging of the highly reactive ROS through enhanced APX activity, might be one of the crucial factors causing higher membrane damages in leaves of nano-copper stressed barley seedlings *Hordeum vulgare L*. (Shaw *et al*, 2014).

Malondialdehyde (MDA) is an important lipid peroxidation product when plant is in stress conditions of aging or injured. Its content is closely related to plant senescence and stress injury. The extent of the damage of plants membrane system and plant resistance can be known by measuring the MDA level of lipid peroxidation (Arbona *et al.*, 2008; Hossain *et al.*, 2009). Under our experimental conditions, MDA levels significantly increased in nano-stressed on roots and leaves suggesting higher membrane damage. This observation is in agreement with Rico *et al.* (2013) which highlight impact of CeO_2 NPs on rice seeds.

Our results are in concomitant with Rao *et al.* (2014); Salah *et al.* (2015) as observed when *Brassica juncea* and *Oryza sativa L.* have showed greater level of MDA contents in roots and leaves under nano-ZnO stress. In addition, MDA levels of *Zea mays L.* roots treated by Fe_2O_3 NPs were higher than that observed in the control plants **(**Li *et al.*, 2016)

Antioxidant enzymes have long been considered as the first line of defense against ROS generation, however, their actions need to be complemented by that of other ROS scavenging systems during severe stress conditions (Apel and Hirt, 2004). In this work, it was noted a significant level of H_2O_2 content in roots and leaves, although imply triggering of the antioxidant system which was not sufficient to scavenge the excess H_2O_2 generated in response to nano stress. A similar trend was enhanced CAT and APX activity with concomitant increases in H_2O_2 levels have been reported in leaves of rice seedlings subjected to nano-CuO stress (Shaw and Hossain, 2013). This result is in agreement with Zhao *et al.* (2012) observed H_2O_2 accumulation in maize exposed to CeO_2 nanoparticles, despite the increase in catalase and ascorbate peroxidase activities. Moreover, Alayat (2015) also recorded a significant increase in H_2O_2 level on different genotype of wheat plants *Triticum durum, Triticum aestivum and* barley *Hordeum vulgare* under treatment with Cd and Cr bulk.

An important mechanism of nanotoxicity is the generation of reactive oxygen species (ROS), resulting

in the subsequent formation of oxidative stress in tissues (Gonzalez *et al.*, 2008). It has been seen that lipid peroxidation is the essential and primary indicator for the oxidative stress while the induction of ROS may also depends on the mitochondria dysfunction (Yamamoto *et al.*, 2002, 2003). In the present study, roots on wheat plants show a significant decrease in the rate of oxygen consumption correlated with an inhibition of the respiratory metabolism in mitochondrial chain. Bouchlaghem *et al.* (2011), explains the inhibition of respiration, by the disturbance of mitochondrial oxidative phosphorylation due to the effects of metallic dust, tested on mosses and lichens. Our results corroborate the work of Zouainia *et al.* (2016), which showed inhibition of mitochondrial respiratory activity in *Elodea canadensis* planes treated with cadmium and zinc bulk.

NPs may interfered with the components of the cell, which may collapse the plasma-membrane potential and de-activate energy-dependent reactions, leading to depletion of the levels of intracellular ATP-ion transport and even metabolite sequestration (Su *et al.*, 2009). Consequently, this interferes with the electron flow through the respiratory chain, giving rise to the generation of ROS causing damage to the cellular macromolecules (Lapresta-Fernàndez *et al.*, 2012).

CONCLUSION:

In conclusion, as the technological benefits of nanotechnology begin to rapidly move from laboratory to large-scale industrial production, the nanomaterials are used in all various applications. In this work, the results demonstrated that nanoparticles of ZnO disturbed some morphological, enzymatic parameters and in particular the energetic parameters of soft wheat plants *Triticum aestivum* L. We recorded a decrease in the length of the roots and leaves of the plants of soft wheat. Moreover an induction in antioxidant enzymes system CAT, APX which scavenges residual free radicals and increase in product of lipid peroxidation MDA and H_2O_2 content, followed by a decrease of respiratory activity in roots and leaves of wheat plants,

Hence, these results could be explained by the triggering of the antioxidant system caused by a production of ROS. Due to their small size, the NPs were able to cross the root walls and tended to accumulate thereafter, translocated to the leaves and caused morphological damage and enzymatic disturbances enhanced through a peroxidation of the membrane phospholipids following thus the presence of the free radicals.

Our study could help us better understand how NPs impact crop from the aspects of the physiological and biochemical levels and provide the useful information for the hazards NPs into the environment.

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