Exogenous application of zinc oxide nanoparticle (ZnONPs) alleviates the cadmium toxicity in *Triticum aestivum*

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Abstract

The increased industrialization and urbanization have greatly deteriorated the environment by releasing the toxic substances and pollutants in the environment. Among the pollutants the industrial runoff primarily contain toxic heavy metals such as cadmium (Cd), arsenic (As), lead (Pb) and chromium (Cr) of which Cd toxicity and its accumulation in the environment has led to the environmental pollution thus leading to its toxicity and deterioration to the environment. Due to its elevated level the Cd contamination is leading to the damaging effect on plant system and the whole thing is dependent on the plant weather animals and human. Thus the mitigating approach is needed to release the stress situation thus the use of nanoparticle as a mitigating agent is practiced this days. Therefore the current study deals with the toxicity impact of Cd on *Triticum aestivum* evident from growth, pigment and oxidative stress and mitigating effect of ZnONPs against Cd toxicity.

Key words: Cadmium; growth; photosynthetic pigment and oxidative stress.

1.0.Introduction

Environmental pollution due to heavy metals (HMs) has become extensive owing to increase in mining and industrial activities enhanced in the 20th century. This HMs pollution is regarded as one of the major concerns for the ecosystem and triggers various toxic impacts on plants and ecological chain. It has become one of the significant factors responsible for decreased crop productivity. Due to ever increasing population, industrialization and food demands, this condition has further got worse. Among these HMs cadmium (Cd) is one of the major contaminants, which ranked seventh position in between the top contaminants (Yang et al. 2004). It generally presents in the soil with an average value more than 1 mg K/g (Peterson and Alloway, 1979). Cadmium is not an essential element for plant development, but it easily penetrates the plant root cell and translocate from root to shoot (Seregin and Ivanov 2001). It affects several molecular, bio-chemical and physiological mechanism as well as causes growth retardation of tissue, leaf chlorosis and necrosis (Clemens et al., 2002). It also affects the stomatal opening, transpiration, and photosynthesis (Gabrielli and Sanita` di Toppi, 1999) and also decreases the nitrate absorption and transportation of mineral from root cell to stem shoot (Hernandez et al., 1996). Cadmium also alters the permeability of plasma membrane and reduces the water conduction (Barcelo et al., 1986) as well as stimulates the oxidative burst in plants (Seregin and Ivanov,2001). Nanotechnology is considered as a revolutionary technology which is used in several sectors including agriculture. Nanotechnology enhances the agriculture productivity with the significance of nano fertilizer, nano pesticides or nano herbicides and nano-coating. The ZnONPs has considered as a vital supplement in the form of essential nutrient as well as a co-factor for nutrient mobilizing enzymes (Raliya et al., 2015). It has been reported in various studies that ZnONPs (1 ~ 20 ppm) enhance the plant growth and reduces the oxidative stress (Mahajan et al., 2011; Hussain et al., 2017). Due to increased surface area and complexing capability, NPs may adsorb pollutants, subsequently changing then transport, bioavailability and toxicity of HMs in plants. Therefore, in the current study we have hypothesized that ZnONPs may mitigate the Cd toxicity in wheat by enhancing growth and Zn concentrations whereas reducing Cd concentrations in plants.

2.0 Materials and Methods

2.1. Plant growth and conditions

Seeds of *Triticum aestivum* (wheat) purchased from certified seed agency of Agra, Uttar Pradesh. After sterilization with sodium hypochlorite the seeds were washed thrice with distilled water and kept in dark. The germinated seeds were then shifted to plant growth chamber under controlled humidity and temperature under 300 μmol photons $m^{-2}s^{-1}$.

2.2. Selection of doses

Following 15 days of acclimatization, uniform sized seedlings were exposed to Cd stress i.e. (20 ppm) pointing to LC 30 and ZnONPs (30 µM) alone and in combination with Cd. The, over all set- up comprises of (i) control (ii) 20 ppm Cd (iii) ZnONPs (iv) Cd +ZnONPs. All the experiments were done after 8 days of seedlings growth. The ZnONPs at 30 µM promoted (20%) growth that was selected for the study.

2.3.Growth behaviour

Growth was determined by analyzing fresh mass of shoot and root respectively.

2.4. Total chlorophyll content

The total chlorophyll content **(**Chl *a* + Chl b) and carotenoids (Car) were estimated by following the method of Lichtenthaler (1987).

2.5.Protein content

The estimation of protein content was done by following the method of Lowery et al., 1981.

2.6.Oxidative stress biomarkers (superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and MDA contents

2.7. The oxidative stress biomarkers i.e. (SOR; $O_2^{\bullet -}$) H_2O_2 and MDA contents were analysed by following the method of Elstner and Heupel (1976) Velikova et al. (2000) and Hodges et al., (1999) respectively.

2.8.Statistical analysis

Duncan multiple range test (DMRT) was performed for the significant differences among treatments at $P<0.05$ levels.

3.0. Experimental findings

3.1. Shoot and root fresh mass

The results related to growth have been presented in Figure 1. The results clearly state that the growth of tested seedling was significantly affected under Cd stress (20 ppm) i.e. it declined SFM by 28% and RFM 26 % respectively. However exogenous supplementation of ZnONPs alleviated the negative impact caused by Cd stress i.e. ZnONPs alone promoted the growth by 20 % and further on combining with Cd dose the toxicity was lessened i.e. it only showed 12 % and 14% in SFM and RFM respectively. Pointing towards beneficial role of ZnONPs, in reducing Cd stress in plants.

3.2. Total chlorophyll content

The result related to pigment content i.e. total chlorophyll and carotenoids content has been presented in Figure 2. The results reveals that the total Chl content was considerably declined by 30 % under selected dose of Cd while Car content was declined by 21 % with respect to control. However, exogenous supplementation of ZnONPs alone raised total Chl and Car content by 22% and 26 % respectively, further along with Cd dose the toxicity was reduced i.e. ZuONPs caused enhancement in the pigment content under similar condition along-with Cd it showed only 15 and 19% reduction in total Chl and Car.

3.3. Protein content

The result related to protein content has been presented in Figure 3 result clearly shows that under Cd toxicity the protein content considerably declined by 33 %. But conversely under exogenous supplementation of ZnONPs alone raised the protein content by 18% and under combined treatment with Cd the reduction in protein content was found to be only 9 % . Thus concentrating on the advantageous aspect of ZnONPs, in extenuating Cd induced toxicity.

3.4. Reactive oxygen species

The enhanced Cd stress causes overproduction of reactive oxygen species (ROS) i.e. SOR and H_2O_2 and the result has been depicted in Figure 4. The outcome evidently suggests that under tested dose of Cd the SOR and H_2O_2 content raised by 40 and 64% respectively. However exogenous supplementation of ZnONPs diminishes the generation of ROS i.e. by 30 and 35% alone conclusively along with Cd the SOR and H_2O_2 generation was reduced i.e. increase of only 22 and 30% in SOR and H_2O_2 content was observed respectively.

4.0. Discussion

The current study focuses the alleviating role of ZnONPs in alleviating Cd toxicity in crop plant particularly *Triticum aestivum*. The Cd toxicity caused a substantial reduction in the growth (Figure 1) that is related through augmented Cd accumulation consequently prompting damaging effect on photosynthetic pigment content (Figure 2) and protein contents (Figure 3) and thus alterating the rate of photosynthesis and disturbing the electron leakage that eventually clues to oxidative stress (Figure 4) equivalent to our conclusions studies have also been stated by Gill et al., (2012). Clemens et al., 2002 also reported parallel decrease in growth under Cd stress. The photosynthesis rely upon the eminence of the photosynthetic pigment,(Chl *a* and Chl b) which are essential whereas Car is a accessory pigment and involved in photo protection. Under Cd stress the photosynthetic pigment content pointedly declined due to replacement of co-factors required for the Chl biosynthesis or degradation of enzyme involved in chlorophyll synthesis (Barcelo et al., 1986), thus modification in pigment content and photosynthetic activity might be the possible motive for the reduction in growth. The reduced photosynthetic efficacy points towards the leakage of electron and eventually generation of reactive oxygen species (ROS). Elevated ROS levels leads to membrane damage and finally cell death (Clemens et al., 2002). Following our findings the Cd stress caused the oxidative burst in *T. aestivum*, due to apparent enrichment in SOR and H_2O_2, levels which possibly be due to diminishing of photosynthetic process and leakage of electron or amplified Cd level that accelerated free radical formation (Hussain et al., 2018). The enhanced values of oxidative stress (Figure 4) feasibly linked to greater membrane damage, which eventually plugs that Cd hastens the damage of membrane lipids, similar results were also reported by (Hussain et al., 2018).

However, exogenous supplementation of ZnONPs considerably enhanced the growth and productivity in plant and decreases the generation of reactive oxygen species i.e. $O_2^{\bullet-}$ and H_2O_2, as these findings also suggests that ZnONPs has ability of shielding plants growth under Cd stress by plummeting oxidative stress (Dimpka et al., 2014). Hence, the supplementation of ZnONPs alongside the Cd stress balances the nutrient content in plant that might be motive of increased chlorophyll content, carotenoids as well as repair in photosynthesis. Consequently leading to improved plant growth. Exogenously applied ZnONPs also reduces the generation of

ROS as its triggers the antioxidant defense system in plant cell, that upholds redox homeostasis of cell and sustains and balance between oxidants and antioxidants (Gill and Tuteja 2010).

5.0. Conclusion

The present study concludes that Cd toxicity remarkably reduced the growth of *T. aestivum* owing to augmented Cd accumulation as well as diminuted pigment content and enhanced oxidative stress that finally leads to cell death and reduces crop yield. Whereas adding of ZnONPs remarkably improved the growth of tested plant due to reduced Cd accumulation as well as declining the impairment in the pigment content, protein and reduced oxidative stress. Henceforth, the study endorses that the Cd toxicity in plants can be alleviated *via* application of ZnONPs to increase the plant growth and productivity under heavy metal stress.

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Conflicts of interest

No conflict of interest

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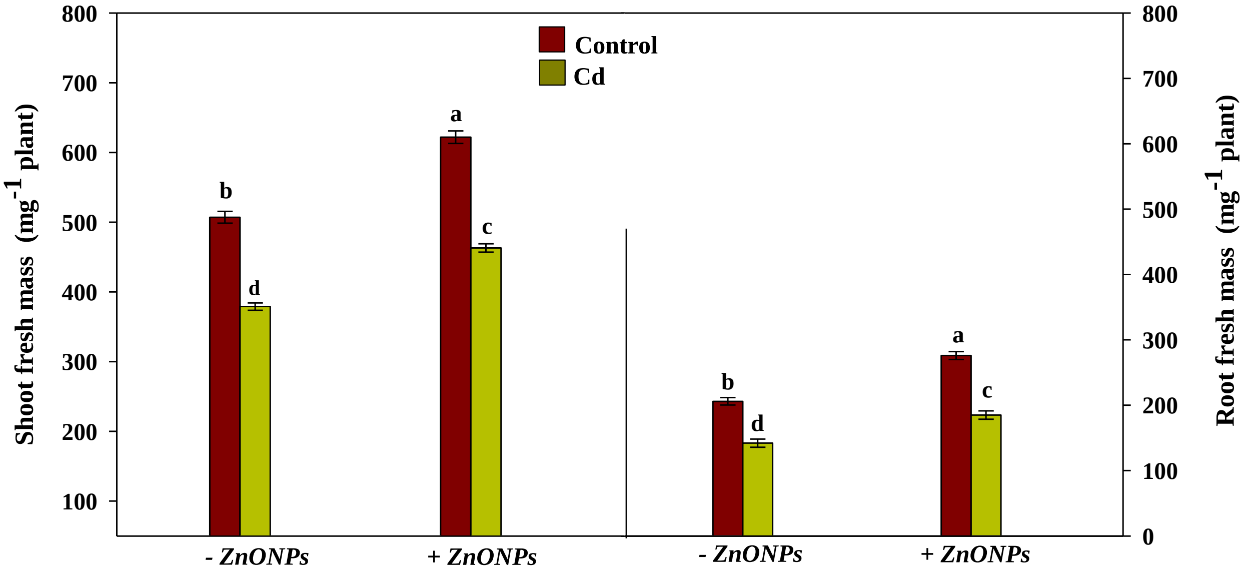

Figure 1. Growth (Fresh mass of shoot and root) of *T. aestivum* seedlings under Cd stress supplemented with or without ZnONPs. Data are means ± standard error of three replicates. Bars with different letters show significant differences at p<0.05 between treatments according to the Duncan's multiple range test.

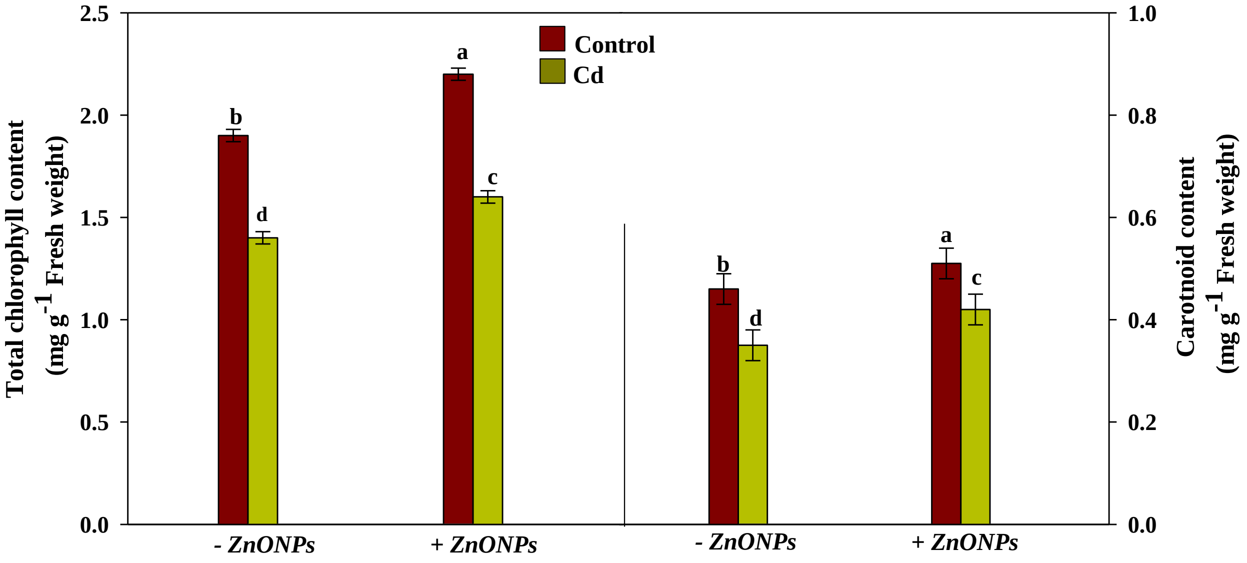

Figure 2. Photosynthetic pigment (total chlorophyll and carotenoid) of *T. aestivum* seedlings under Cd stress supplemented with or without ZnONPs. Data are means ± standard error of three replicates. Bars with different letters show significant differences at p<0.05 between treatments according to the Duncan's multiple range test.

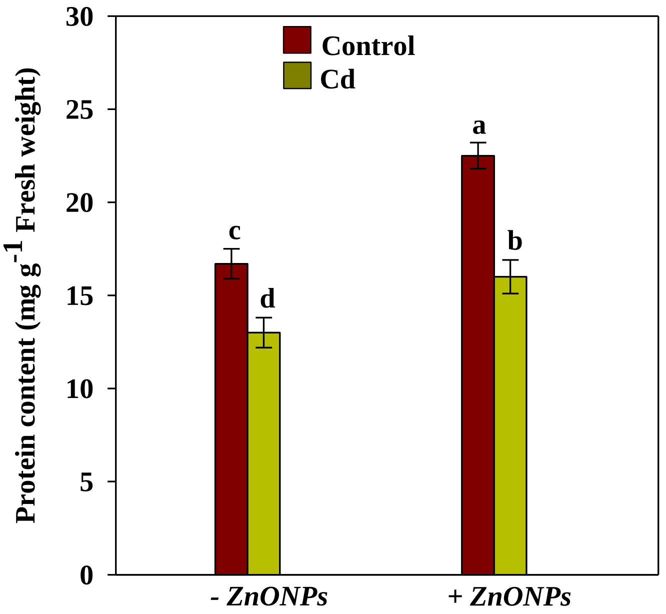

Figure 3. Protein content of *T. aestivum* seedlings under Cd stress supplemented with or without ZnONPs. Data are means ± standard error of three replicates. Bars with different letters show significant differences at $p<0.05$ between treatments according to the Duncan's multiple range test.

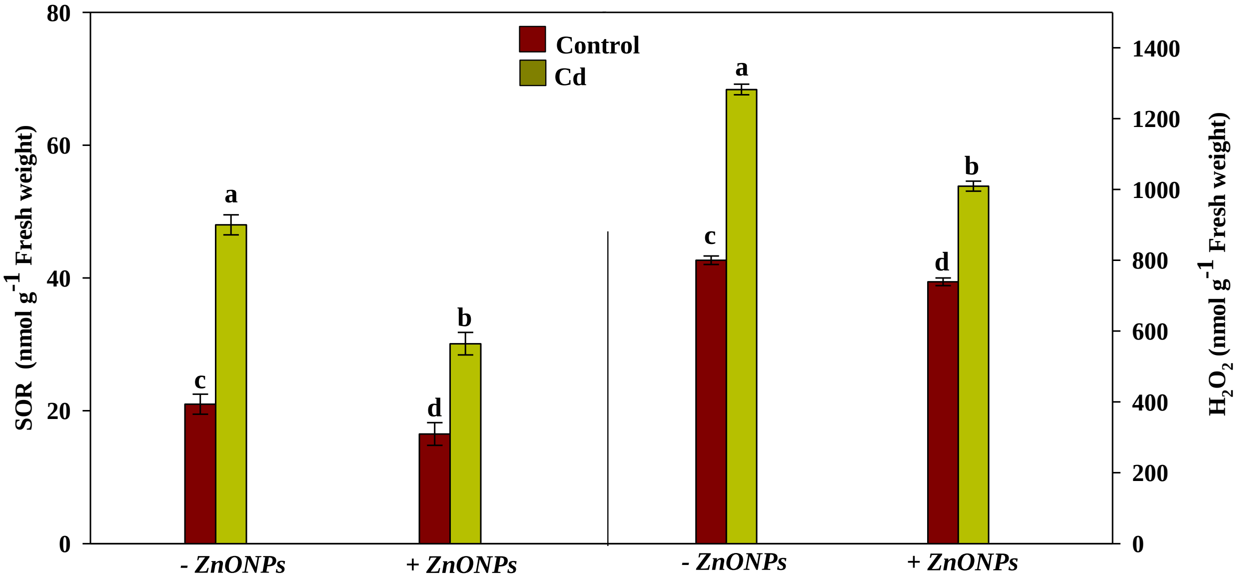

Figure 4. Superoxide radicle (SOR) and hydrogen peroxide (H_2O_2) content of *T. aestivum* seedlings under Cd stress supplemented with or without ZnONPs. Data are means ± standard error of three replicates. Bars with different letters show significant differences at $p<0.05$ between treatments according to the Duncan's multiple range test.