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RESEARCH PAPER

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Impact of Zinc oxide nanoparticles on pigments and oxidative stress markers in *Triticum aestivum*

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ABSTRACT

Plants bear many challenges due to enforcing condition related environmental pollutants. The attentively use of metallic nanoparticles (MNPs) in commercial product cause serious risk to environment causing a major concern for sustainable agriculture. Plants are the primary producer in ecosystem and being a sessile they are the essential component of biotic system and are greatly exposed to many factors like metal and non-metal toxicity. This may lead to adversely affect the plant growth and development through MNPs induced Phytotoxicity of cereal crops.The present study investigates the role of zinc oxide nanoparticles (ZnO NPs) toxicity in wheat *(Triticum aestivum L.).* Hydroponically grown wheat seedlings were given different treatment concentrations (0.0, 1.0, 4.0, 10.0 mgL^{-1}) treatment represented stress condition which was displayed by total chlorophyll, carotenoids, anthocyanin, malondialdehyde (MDA), H_2O_2, content of wheat shoot and root. Moreover, ZnO NP application increase uptake and accumulation of ZnO NPs in *Triticum aestivum* (root and shoot). Supplementation with ZnO NPs significantly alter the plant photosynthetic pigment (total chlorophyll and carotenoids), anthocyanin content while oxidative stress marker (MDA and H_2O_2) has also been analyzed causes increase MDA and H_2O_2 levels in a dose-dependent manner.
Keywords: Zinc oxide nanoparticles (ZnO NPs), wheat (*Triticum aestivum*), pigments, oxidative stress markers.

INTRODUCTION

Nanotechnology is the branch that based on the use of nanoparticles which range in size from 1 to 100 nm and have special characteristics because of their high surface area, shape, and electric charge (Ekar et al., 2024). As reported by Kumar, (2023) the global market for nanotechnology was valued at 1.76 billion dollars in 2020 and is expected to grow to 33.63 billion dollars by 2030.

The widespread usage of NPs in a variety of fields, including electronics, cosmetics, and both therapeutic and diagnostic medical applications, can be attributed to their small size and vast surface area (Najahi et al.,2021). Nanomaterials are being used in various techniques to enhanced sensitivity of methods based on atomic resolution capabilities, viz., tandem electron microscopy, scanning transmission electron microscopy, and scanning tunneling microscopy. This has contributed to the exponential rise and growing interest in nanotechnology (Missaoui et al.,2018).

Zinc oxide nanoparticles (ZnO NPs) are extensively utilized in various fields due to their unique properties such as they are used in cosmetics as part of sunscreens, lotions and creams because of its antimicrobial and UV-blocking qualities. Additionally, the antibacterial, anticancer, and wound-healing potential of ZnO NPs are being investigated. These are also widely utilized in the textile and electronics industries as components of optoelectronic devices, solar cells, and sensors (Dey et al., 2025). With potential advantages for crop development, yield, and stress tolerance, zinc oxide nanoparticles (ZnO NPs) have become an attractive agricultural tool (Faizan et al.,2020). It can lessen the need for fertilizers by increasing nutrient uptake and usage. Some studies indicate that excessive concentrations of ZnO NPs might be harmful to biodiversity and ecosystems, particularly to plants, soil microbes, and aquatic and terrestrial life. Zinc oxide nanoparticles can build up in water and soil, endangering the ecosystem over time (Bordin et al., 2024; Thangavelu et al., 2024: Rajput et al., 2021)

According to some studies high concentrations of zinc oxide nanoparticles (ZnO NPs) can decrease plant growth, biomass and yield (Khan et al., 2021; Singh et al., 2013). Nanoparticles can induce oxidative stress, leading to cell damage and altered antioxidant enzyme activity and also disrupt nutrient uptake and balance and can reduce chlorophyll content and inhibit photosynthesis (Siddiqui et al., 2025; Rai and Quezada (2024); Parada et al., 2019; Neogi et al.,2025; Kumar et al.,2025).

Extensive used of nanoparticles in agriculture has led to gain concerns about its impact on productivity of major cereals crops (Mridha and sarkar 2025). Some studies suggest that high concentrations of nanoparticles can be toxic to plants, causing reduced growth, biomass, and yield. As reported by that silver nanoparticles can reduce shoot and root length, decrease biomass, and alter metabolic and defense protein expression in wheat. Some previous studied showed negative effect of NPs such as AgNPs, Al_2O_3 NPs, SiO_2 NPs and ZnO NPs on several different parameters of plants effecting growth and development and also total productivity of some economically and valuable crops (Riahi-Madvar et al., 2012; Thwala et al.,2013; Ma et al., 2014; Zhang etal.,201 5).

Wheat is a versatile crop with various economical uses. It is a staple food in many countries. Wheat is used as nutritious feed for livestock, poultry and aquaculture. Wheat starch and gluten are used in textiles, paper and adhesives. The present study aimed to investigate the impact of ZnO NPs on pigments and oxidative stress markers in seedlings of *T. aestivum*.

MATERIALS AND METHODS

Procurement of ZnO NPs

ZnO NPs were procured from Sigma Aldrich (USA). The ZnO NPs, size <50nm.

Raising nursery

Wheat seeds var. HD-3086 (Pusa Gautami), were procured from Indian Council of Agricultural Research, ICAR-IARI, New Delhi. Seeds were double sterilized using alcohol and sodium hypochlorite. After every sterilization seeds were washed thrice with deionised water, then soaked in sterile water for 24h. For germination seeds were placed for 5 days at 24±2̊ C in plastic petridish containing moist filter paper. Germinated *T. aestivum* seedlings with same heights and healthy shoots were selected for further experimentation. ZnO NPs(100μgml^{-1}) were suspended in 5% Hoagland solution by probe sonication (Sonics Libra Cells, Sonics and material INC., New Town USA) and served as stock solution. 12 d old *T. aestivum* seedlings were exposed in triplicate with different concentrations of ZnO NPs(0.0-10.0μgml^{-1}) in 5% Hoagland solution for 21d in growth chamber light intensity 115μmolm^{-2} s^{-1}, light and dark : 14 and 10 h temperature 24±2̊ C. *T.aestivum* seedlings in 5% Hoagland served as control. Each replicate consists of 250ml conical flasks containing 200ml 5% HS solution containing different concentrations of ZnO NPs. After 21d of initiation of experiment seedlings were harvested washed several times with 10mM EDTA followed by 4 washings of deioninsed water, blotted to removed water and used for the analyses of various parameters.

Uptake of Zn by roots and shoots of T. aestivum seedlings

ZnO NPs uptake was monitored by zinc uptake and was estimated by measuring Zinc concentrations in roots and shoots of seedlings exposed to ZnO NPs using protocol described in Kumar et al.,2023. Zn concentrations in roots and shoots were measured (ICP-MS 7700 series, Agilent, U.S) and expressed as μg g^{-1} DW.

Photosynthetic pigments and Anthocyanin content

The impact of ZnO NPs on total chlorophyll and carotenoids were recorded by using protocols described by Arnon (1949) and Duxbury and Yenstch (1956), respectively. Anthocyanin levels were estimated as per protocol described by Nakata and Ohme-Takagi. (2014). Anthocyanin content in shoots has been reported as Absorbance 530 nm/g FW.

Oxidative markers

Malondialdehyde (MDA) and H_2O_2 levels in roots and shoots of seedlings of *T. aestivum* exposed to ZnO NPs (0.0-10.0μg ml^{-1}) were estimated according to Heath and Packer (1968) and Velikova et al., (2000), respectively.

Statstical analysis

One way ANOVA followed by Duncan's multiple range test (DMRT) was used to test the statistical significance ($p<0.05$) and variability of the mean (n=3± SD) data (Gomez and Gomes 1984).

RESULTS AND DISCUSSION

Zn accumulation

T. aestivum seedlings exposed to ZnO NPs (0.0-10.0μg ml^{-1}) accumulate Zn in concentrations dependent manner in shoots and roots. The high concentration of Zn confirmed the uptake of ZnO NPs in test seedlings.

Minimum concentration of Zn in roots (530.65µgg^{-1}DW) and shoots (296.4265µgg^{-1}DW) was observed in seeds exposed to 1.0 µgml^{-1} compared to untreated control plants. However maximum Zn levels in roots and shoots were 1612.52 µgg^{-1}DW and 1402.86µgg^{-1}DW, respectively (Fig.1).

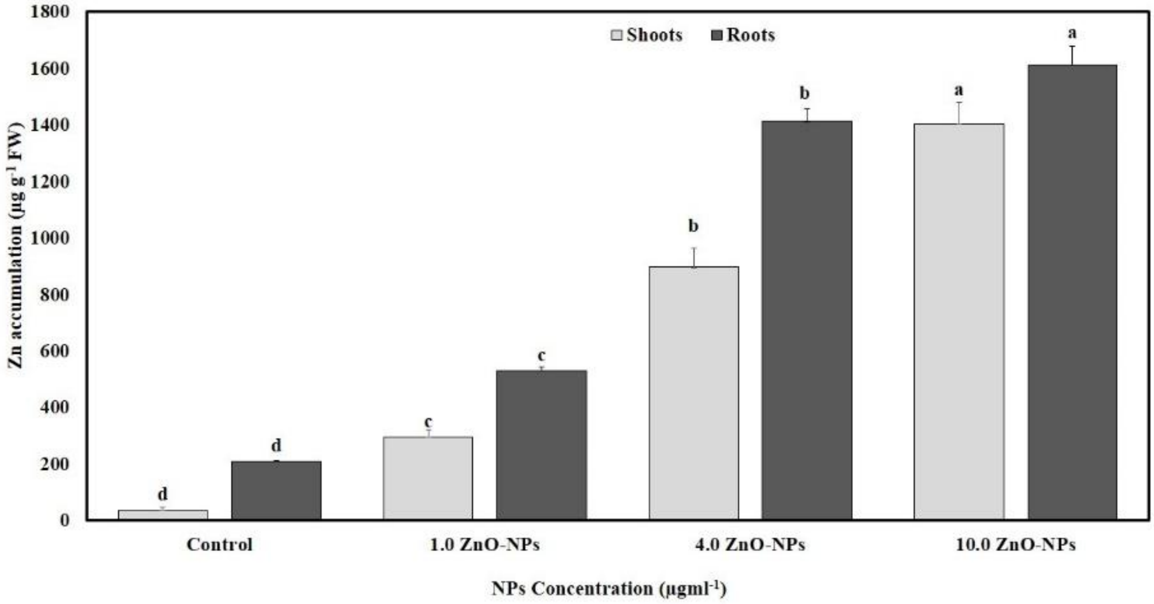

Fig 1. The above figure depicts significant ($p < 0.05$) changes in accumulation of Zinc in the shoots and roots of *T. aestivum* when exposed to ZnO NPs (0.0-10.00µg ml^{-1}) for 21d. Values are mean ± SD (n=3). One way ANOVA in complete randomized block design (separately for shoots and roots) significant at $p<0.05$. Different superscripts on bars for each plant tissue depict significant ($p<0.05$) difference between means for that tissue according to Duncan's multiple range test.

Photosynthetic pigments and Anthocyanin content

ZnO NPs (0.0-10.0µgml^{-1}) significantly DMRT ($p<0.05$) reduced the total chlorophyll content in concentration dependent manner (Fig.2). However, carotenoids contents in ZnO NPs treated seedlings of *T. aestivum* enhanced significantly when concentrations of ZnO NPs were increased in nutrient solution (Fig.3). Our results are in line of previously reported results with ZnO NPs who find that ZnO NPs reduced the chlorophyll content of *Pisum sativum* (Mukharjee et al.,2014). Wang et al.,2016 also reported the reduced photosynthetic pigments in *Arabdiopsis* when exposed to ZnO NPs, ZnO NPs have been reported to act as antioxidants for mitigating oxidative stress. The increased carotenoids level may be attributed to the up regulation of ZDS gene (Zeta-carotene-desaturase gene) which encodes a key enzyme in the carotenoid's biosynthesis pathway of plants and algae. The up regulation of ZDS gene by ZnO NPs has been in *Arabiodopsis* had been documented by Wang et al., 2016.

Thwala et al.,2013; Bandyopadhyay et al., 2015; Henandez –Viezcas et al.,2011 simultaneously reported that due to toxicity caused by ZnO NPs the oxidative stress increased in plants which could be reason for the inhibition effect of ZnO NPs. Moreover, Wang et al., 2016 had reported the decreased levels of genes which are responsible for chlorophyll synthesis and also for carotenoids synthesis.

Geranyl Geranyl Pyrophosphate Synthase 6 (GGPS6), Phytoene Synthase (PSY) and Phytoene desaturase (PDS) are the genes required for biosynthesis of carotenoids and these genes as reported by Shang et al.,2018, he also concluded that for the conversion of GGPP to phytoene, Psy plays role as catalyst for the conversion of GGPP to phytoene during biosynthesis of carotenoids.

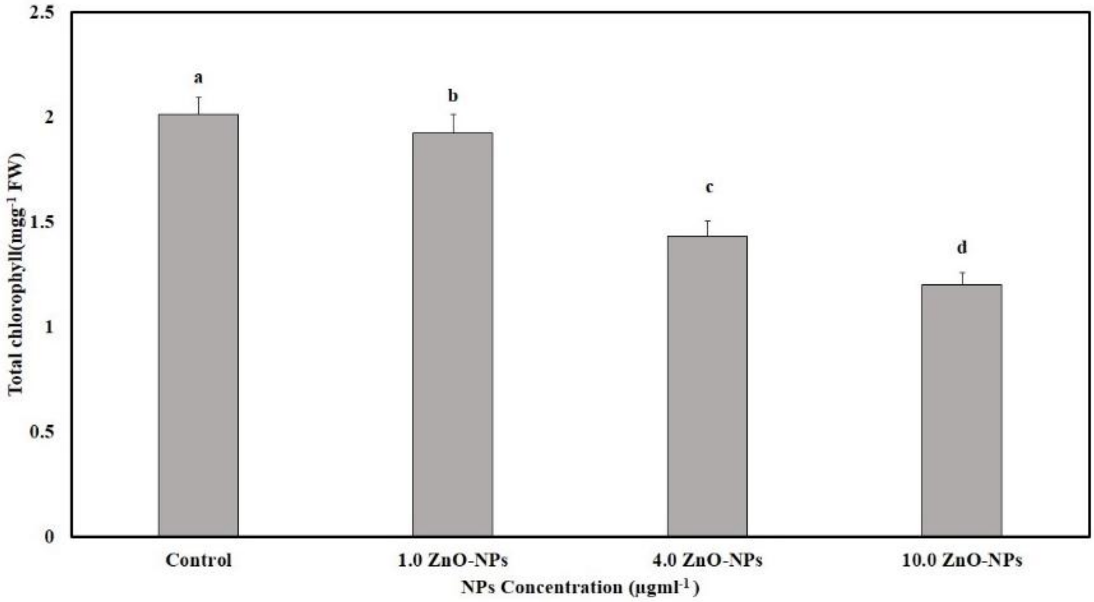

Fig 2. The above figure depicts significant ($p < 0.05$) changes in total chlorophyll in the shoots of *T. aestivum* when exposed to ZnO NPs (0.0-10.00µg ml^{-1}) for 21d. Values are mean ± SD (n=3). One way ANOVA in complete randomized block design significant at $p<0.05$. Different superscripts on bars for each plant tissue depict significant ($p<0.05$) difference between means for that tissue according to Duncan's multiple range test.

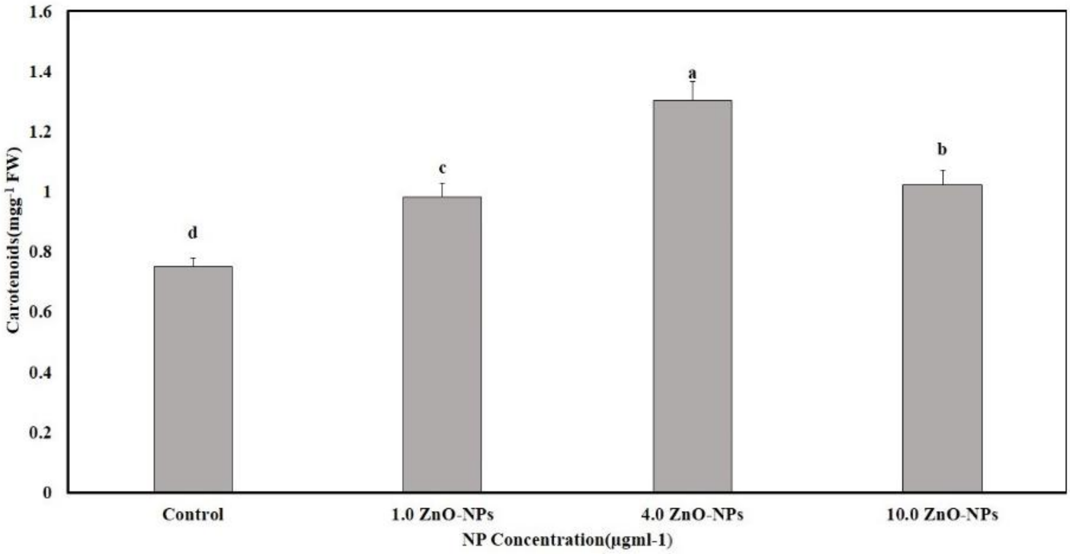

Fig 3. The above figure depicts significant ($p < 0.05$) changes in carotenoids content in the shoots of *T. aestivum* when exposed to ZnO NPs (0.0-10.00µg ml^{-1}) for 21d. Values are mean ± SD (n=3). One way ANOVA in complete randomized block design significant at $p<0.05$. Different superscripts on bars for each plant tissue depict significant ($p<0.05$) difference between means for that tissue according to Duncan's multiple range test.

In our research Anthocyanin content was maximum (4.0A_{530} nm/g FW when seedlings of *T. aestivum* was exposed to ZnO NPs (1.0µgml^{-1}) compared to untreated control seedlings while Anthocyanin content was found minimum (1.80A_{530} nm/g FW) at concentration 10.0µgml^{-1} ZnO NPs as shown in Fig 4. Earlier studies reported ZnO NPs leads to increase anthocyanin content in various plants and it may often induced responses during stress like formation of Reactive Oxygen species. (Syu et al.,2014). Anthocyanins are pigment acting as efficient scavengers of ROS in plants.

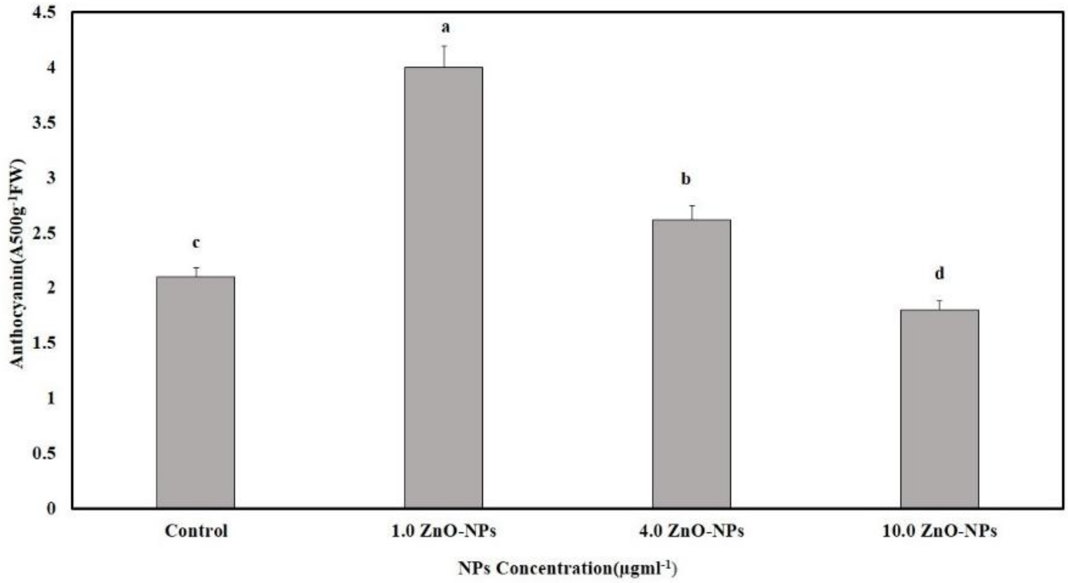

Fig 4. The above figure depicts significant ($p < 0.05$) changes in anthocyanin content in the shoots of *T. aestivum* when exposed to ZnO NPs (0.0-10.00µg ml^{-1}) for 21d. Values are mean ± SD (n=3). One way ANOVA in complete randomized block design significant at $p<0.05$. Different superscripts on bars for each plant tissue depict significant ($p<0.05$) difference between means for that tissue according to Duncan's multiple range test.

Accumulation of MDA and H_2O_2

The important indicators for measuring stress on plants are the MDA and H_2O_2 contents. The results of the H_2O_2 and MDA contents in the treatment of nanoparticles are shown in Figs.5 and 6 respectively. The higher concentrations of both H_2O_2 and MDA in roots and shoots of seedlings of *T. aestivum* (1.0-10.0µg ml−1 ZnO NPs) was due to oxidative burst caused by ZnO NPs. Previous studied on ZnO NPs exposure in duckweed had been reported to induced Reactive oxygen and nitrogen species (ROS/RNS) (Thwala et al.,2013). In this study, we have demonstrated the accumulation of Zn nanoparticles in both roots and shoots of seedlings of *T.aestivum* after exposure of ZnO NPs (0.0-10.00µg ml^{-1}). Hence, we suggest that ZnO NPs (≥ 1.0 µg ml^{-1}) induced photocatalytic activity results in severe oxidative burst in leaves due to direct exposure of light. Roots of *T.aestivum* were not directly in contact with light. Therefore, lower H_2O_2 accumulation and MDA contents in roots of *T. aestivum* in comparison to shoots was observed in concentration dependent manner. Our results are in line with Kumar et al.,2023 who has documented that roots of wheat accumulated more Ti-/TiO2 NPs than leaves but production of ROS was lower in roots than leaves due to lesser photocatalytic activity in dark grown roots. According to Hosseini et al .,2013 zinc induced the production of free radicals in *Zea mays*.

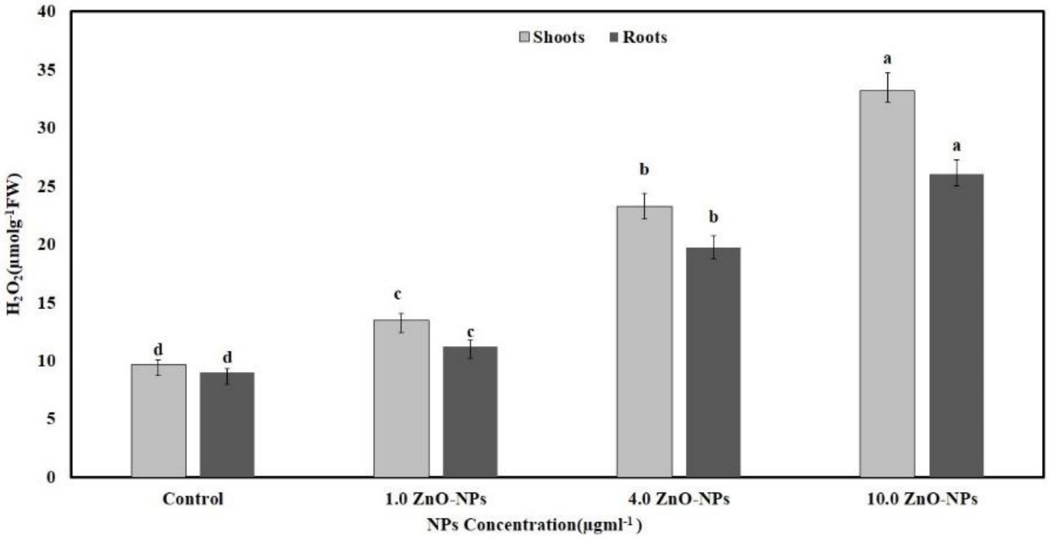

Fig 5. The above figure depicts significant ($p < 0.05$) changes in H_2O_2 content in the shoots and roots of seedlings of *T. aestivum* when exposed to ZnO NPs (0.0-10.00µg ml^{-1}) for 21d. Values are mean ± SD (n=3). One way ANOVA in complete randomized block design (separately for shoots and roots) significant at $p<0.05$. Different superscripts on bars for each plant tissue depict significant ($p<0.05$) difference between means for that tissue according to Duncan's multiple range test.

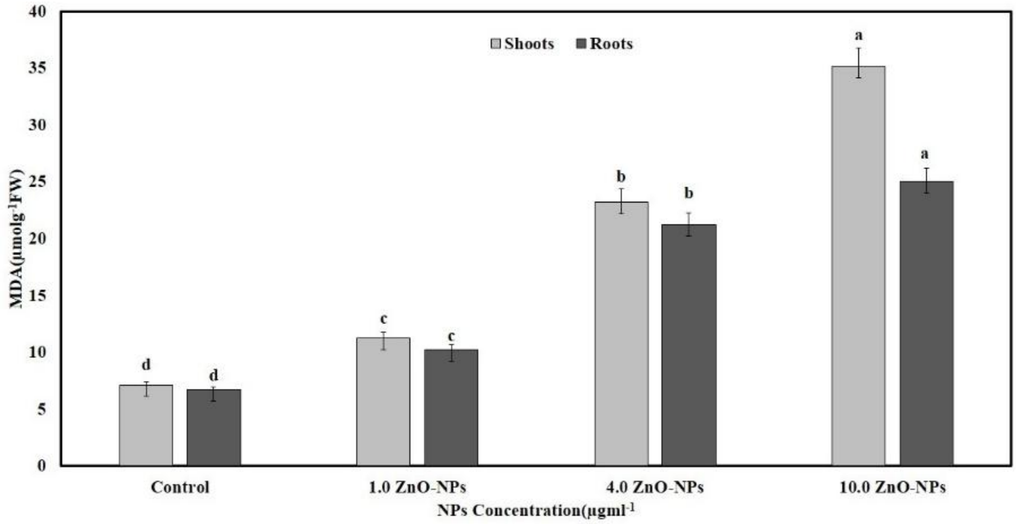

Fig 6. The above figure depicts significant ($p < 0.05$) changes in MDA content in the shoots and roots of seedlings of *T. aestivum* when exposed to ZnO NPs (0.0-10.00µg ml^{-1}) for 21d. Values are mean ± SD (n=3). One way ANOVA in complete randomized block design (separately for shoots and roots) significant at $p<0.05$. Different superscripts on bars for each plant tissue depict significant ($p<0.05$) difference between means for that tissue according to Duncan's multiple range test.

MDA formation in plants exposed to adverse environmental conditions is an indicator of lipid peroxidation in biological systems. Also, MDA formation may be partially due to ROS production in plants (Liu et al., 2015). Under various stress conditions plants show responses such as generation of reactive oxygen species (ROS).

At low levels H_2O_2 act as a signaling molecule and at high levels it may cause oxidative burst to components of cells. MDA generally produced when ROS like hydrogen peroxide damage plasma membrane and other cellular components. Moreover, high level of MDA is indicator of stress in plants which leads to damage of plants cells. In our result, as H_2O_2 levels increases when shoots and roots of *T. aestivum* are exposed to ZnO NPs (1.0-10.0μg ml^{-1}) in concentration dependent manner. Similar trend was observed in MDA level also,when seedlings of *T.aestivum* exposed to ZnO NPs (1.0-10.0μg ml^{-1}) value of MDA increases in both shoots and roots in concentration dependent manner.

In control H_2O_2 level was 9.7μmolg^{-1}FW and 9.0μmolg^{-1}FW in shoots and roots of seedlings of *T. aestivum.* Further when seedlings of T. aestivum exposed to ZnO NPs(1.0μg ml^{-1},4.00μg ml^{-1} and 10.0μg ml^{-1}), the level of H_2O_2 increases (13.45μmolg^{-1}FW,23.25μmolg^{-1}FW and 33.21μmolg^{-1}FW) respectively in shoots of seedlings of test plant. However, in roots level of H_2O_2 was found less in comparision to shoots when seedlings of *T.aestivum* exposed to ZnO NPs(1.0μg ml^{-1},4.00μg ml^{-1} and 10.0μg ml^{-1}) as shown in Fig 5.Increased level of H_2O_2 and MDA indicates the production of ROS (Kumar et al.,2025).

CONCLUSION

ZnO NPs have many uses in various fields including agriculture but up to certain concentration. Its use must be carefully evaluated and regulated to ensure sustainable and safe application. Further investigations are needed to determine the ZnO NPs threshold in current agricultural environments to avoid environmental pollution and human health risks associated with short-term high doses of ZnO NPs entering agricultural soils.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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