

Foliar Application of Zinc Nanoparticles Improves Photosynthetic Performance in Wheat Plants

Prabha Rai Kalal¹, Ayushi Gupta², Anjana Jajoo^{3,4,*}

Abstract

The present study investigated the impact of foliar spray of zinc oxide nanoparticles on physiological and biochemical reactions in wheat (*Triticum aestivum* L.) Foliar spray with zinc oxide nanoparticles at 10 mg/l positively increased pigment content and biomass in comparison to control while a significant reduction in chlorophyll content and biomass accumulation was observed at higher concentration of 1000 mg/l. A study of Chlorophyll a fluorescence parameters at 10 mg/l showed an improved primary photochemistry in plants by improving the performance of water splitting complex at the donor side of Photosystem II. The number of active reaction centers per chlorophyll molecule increased by 31%. However, all these parameters were drastically decreased on subjecting plants to 1000 mg/l. In addition, higher concentration of nanoparticles induced oxidative stress in plants which resulted in significant increase in activities of antioxidants including peroxidase, superoxide dismutase and catalase. A study of lipid peroxidation showed a prominent increase by 20% in malondialdehyde content showing membrane instability in plant cells at 1000 mg/l. However, the activities of these antioxidants were decreased at 10 mg/l owing to low reactive oxygen species levels as compared to control. The results obtained present an important insight to the foliar application of zinc oxide nanoparticles as an alternative to current soil fertilization strategies to improve crop yield of wheat.

Keywords: Zinc oxide nanoparticles, foliar spray, phytotoxic, photosynthesis, Chlorophyll a, fluorescence

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the staple food for over 80% of world population. Looking at its consumption rate and increasing population, higher grain productivity is the need of today. Aiming for increased production requires intense cropping system resulting in a serious depletion of macro and micronutrients from soils [1]. Zinc is an essential micronutrient for plants playing an irreplaceable role

in many physiological processes, including synthesis of many enzymes, metabolic processes, protein synthesis and biosynthesis of chlorophylls in plants [2]. Deficiency of zinc in soil leads to severe reduction in wheat production and nutritional quality of grains [3]. Therefore, soil application of supplementary nutrients to plants in the form of chemical fertilizers is the most common practice. Use of chemical fertilizers is not only expensive but also has many limitations with respect to their availability to the plants. The inorganic nutrients get fixed in soil as insoluble forms and leached out posing serious environmental concerns [4]. An alternative approach for application of these micronutrients is through foliar sprays. Foliar spray of different micronutrients has been reported to be equally or more effective as soil application [5]. In

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addition to that, foliar application has proved to be the fastest way of fulfilling the nutrient demand of plants thereby increasing yield and quality of crop products [3].

In recent years, nanotechnology is showing a great scope in agriculture [6] because of unique physicochemical properties of nanoparticles (NPs) which have high surface area volume ratio and high reactivity due to smaller size [7]. Nowadays, foliar spray of engineered NPs as nanofertilizers, nanopesticides, nanosensors and nanocarriers is often being employed in the agricultural industry as an alternative to bulk fertilizers applied to soil [5]. Foliar spray of nanofertilizers facilitate the slow and steady release of nutrients thereby reducing the loss of nutrients and enhancing the nutrient use efficiency by plants. Foliar sprayed NPs mainly enter the leaves through stomata and are transported to different plant parts via apoplastic and symplastic pathways [8].

Zinc oxide nanoparticles (ZnO NPs) have also proved to be effective in enhancing productivity and absorption of zinc [9]. The required dosage of nano-based zinc fertilizer was found to be 10-fold less than the conventional fertilizers [10]. Studies reported beneficial role of ZnO NPs at lower concentration on seed germination in wheat [11]. Previous research has reported that foliar application of zinc nanofertilizer significantly increased shoot length and root length in millet and cotton [12]. Both positive as well as negative effects of NPs on plants have been shown depending on their size, concentration, exposure time and plant species [13]. At higher concentrations, NPs could decrease the yield and quality of crops by altering mineral nutrition, impairing photosynthesis, generating reactive oxygen species (ROS), damaging cellular membranes, proteins, and nucleic acids and inducing genotoxicity [14]. To our knowledge, there have been very few studies reported on the effects of foliar spray of ZnO NPs on plant photosynthetic parameters.

The present study was undertaken to observe the effects of foliar spray of varying concentrations of ZnO NPs photosynthetic apparatus and anti-oxidant enzymes of wheat plants.

MATERIALS AND METHODS

Nanoparticle Suspension Preparation

ZnO NPs were purchased from Nano Research Lab Jamshedpur, India. NPs were in the size range of 20–30 nm. For preparation of NP suspension, ZnO NPs of 5 mg/l, 10 mg/l, 100 mg/l, 1000 mg/l concentration were suspended in deionized water and dispersed by ultrasonic vibration by running five cycles at (400–500 W) for 30 sec each [15].

Pot Experiment

For soil cultivation, five wheat seeds were sown in each pot. First foliar application of ZnO NPs was made at 15 days after sowing (DAS) followed by second application at 30 DAS with various concentrations to find out the optimum concentration of NPs. Approximately 20 ml of the ZnO NPs suspensions was sprayed to cover the foliage twice (only on the day of treatment) for each experimental pot. The treatments applied were untreated (UT) plants considered as control; 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l treated with ZnO NPs suspension. All the experiments were done three times in three replicates for each treatment.

Pigment Content Analysis

The content of photosynthetic pigments (Chlorophyll *a* and *b*, carotenoids in mg/g fresh leaves) was determined spectrophotometrically (Visiscan167, Systronic). For the extraction of pigments, 100 mg leaf tissues in 5 ml of 80% acetone were used. The calculation was made according to Porra *et al.* [16].

Measurement of Photosynthetic Efficiency

The Chl *a* fluorescence induction kinetics was measured at room temperature using a Plant Efficiency Analyzer (PEA), (Hansatech, England). Light intensity used was 3000 $\mu\text{mol/m}^2/\text{s}$ which is capable to generate maximal fluorescence (F_m). Before measurement, samples were kept in dark for 15 min [17]. Biolyzer HP-3 software was used to prepare energy pipeline leaf model and flux ratios [18].

Biomass Estimation

For biomass analysis, the plants were harvested after 50 days. The shoots were separated, and their fresh weight was measured. For dry weight, the shoots were dried in oven at 70°C until the constant weight was recorded [15].

Extraction and Estimation of Enzymes

All the enzyme analyses were done in leaves of treated plants after 40 DAS. Leaves (100 mg) were homogenized in chilled sodium phosphate buffer (50 mM, pH 7.8) containing 1% polyvinyl pyrrolidone (PVP) and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 15,000 rpm for 15 min. The supernatant was used as the enzyme extract for superoxide dismutase (SOD). SOD (EC 1.15.1.1) activity was assayed as described previously by Beauchamp & Fridovich [19]. The activity was expressed as Units/mg protein. The absorbance was measured at 560 nm.

For peroxidase (POD) (EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6), 100 mg of leaf tissue was crushed in chilled 80% acetone at 4–8°C. POD and CAT were assayed following the method previously described by Zhang et al. [20]. Protein estimation was done following the method of Lowry et al. [21] using Bovine serum albumin (BSA) as standard.

Analysis of Lipid Peroxidation

The lipid peroxidation was measured in leaf tissues by estimating the malondialdehyde (MDA) content. During the process of lipid peroxidation, MDA is formed by the decomposition of polyunsaturated fatty acids. MDA content was measured following method as described by Wang et al. [22].

Statistical Analysis

Graphpad Prism 5.01 software, Ind; La Jolla, CA, USA was used to analyze data. One-way analysis of variance (ANOVA) followed by Dunnett's Comparison test was used for analysis. Significance was determined at $p < 0.001$ (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$) and mean values standard deviation (SD) have been shown. All the experiments were carried out three times in three replicates.

RESULTS AND DISCUSSION

Effect of Foliar Spray with ZnO Nanoparticles on Pigment Content Analysis

Effect of foliar application of ZnO NPs at different concentrations of 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l are shown in Figure 1. The treatments of ZnO NPs positively influenced leaf chlorophyll content in 5 mg/l, 10 mg/l and 100 mg/l treatments. At concentration of 10 mg/l, Chl *a*, Chl *b* and total Chl content increased by 54%, 13% and 41%, respectively as compared to UT control. This increase in Chl content in ZnO treated wheat plants may be because of essential role of zinc in chlorophyll biosynthesis [23]. Also, zinc is involved in the development and repair functions of chloroplast and photosystem II (PSII) [24]. Carotenoid, which plays an important role in plant photo protection mechanism, was enhanced by 25% in 10 mg/l ZnO NPs treated plants as compared to control. However, foliar application of 1000 mg/l ZnO NPs resulted in a 25%, 38% and 29% reduction in Chl *a*, Chl *b* and total Chl content, respectively as compared to control. This decrease in Chl content on application of higher concentrations of zinc may be closely related to the generation of ROS inducing oxidative stress in wheat plants [25]. Additionally, at higher concentration, zinc may be responsible for displacement of some other metals from the active sites of proteins thereby affecting chlorophyll biosynthesis [26]. Decline in chlorophyll content at higher concentration (1000 mg/l) may also be regarded as general response associated with heavy metal toxicity affecting plant growth [27].

Effect of Foliar Spray of ZnO NPs on Chl *a* Fluorescence

Chl *a* fluorescence parameters can provide fair estimate of efficiency of PSII [28]. A typical polyphasic fluorescence transients curve was obtained from UT and ZnO NPs treated wheat plant leaves at different concentrations of 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l after 40 DAS as shown in Figure

2. Parameters such as water splitting complex (F_v/F_o), active reaction center per chlorophyll molecule (RC/ABS), area, minimum fluorescence/maximum fluorescence (F_o/F_m) and driving force (DF) in NPs treated wheat plants showed remarkable changes as compared to control (Table 1). An alteration in these parameters give evidence for the positive effect of ZnO NPs foliar spray on PSII reaction centers (RC) showing a maximum increase at concentration of 10 mg/l. The activity of the water splitting complex of PSII (shown by F_v/F_o ratio) increased by 21% followed by increased number of active reaction center per Chl molecule (RC/ABS) as compared to control. ZnO NPs seem to enhance the efficiency of PSII complex transfer of more light energy to the active RC resulting in more efficient primary photochemistry as compared to control. An increase in area over the fluorescence curve by 29% represents the size of quinone pool size in the electron transport chain. This resulted in an increase in the electron transport from reaction center to the quinone pool [29]. Thus, ZnO NPs treatment led to an overall better photochemical efficiency of PSII RC and reduced energy loss by heat dissipation which is expressed as F_o/F_m (decreased by 15%) in comparison to control wheat plants. Other components that explains the efficiency of dark redox reaction explained as $\psi_o / (1-\psi_o)$ and efficiency of light reactions explained as $\Phi_{po} / (1-\Phi_{po})$ were also enhanced by 24% and 28%, respectively due to ZnO NPs treatment at a concentration of 10 mg/l in wheat plants as compared to control plants (Table 1). A significant increase (by 32%) in DF represents overall forced capability for photosynthesis in plants [30].

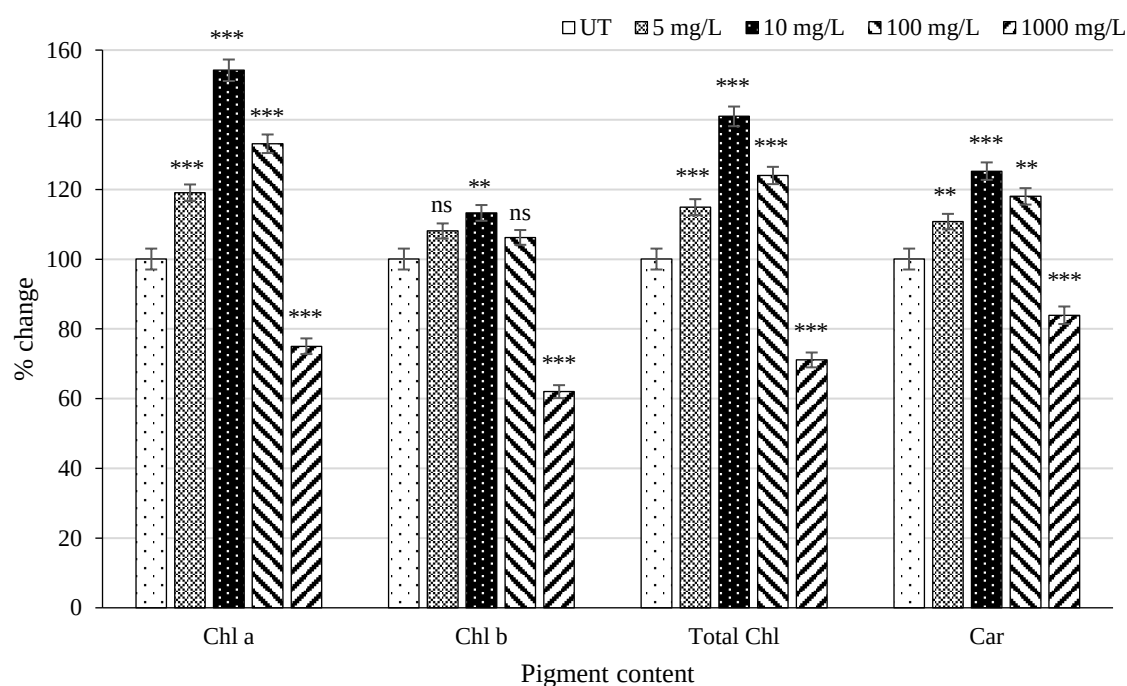


Figure 1. Effect of foliar spray of ZnO NPs on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll content (Total Chl) and carotenoid content (Car) in untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l treated wheat plants. Each experiment was done three times with three replicates. Significance was determined at $p < 0.001$ (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, ns=non-significant) and the results were given as mean values $\pm$ SD.

On the contrary, at higher concentrations (1000 mg/l) parameters such as F_v/F_o , RC/ABS , area and DF showed a dramatic decline by 25%, 11%, 14% and 15%, respectively as compared to control wheat plants. Decrease in all the above parameters depicts a decrease in overall primary photochemistry and increased dissipation in the form of heat (F_o/F_m increased by 18%). In addition to these, performance index PI (abs) which is considered as one of the sensitive indicator of overall primary photochemical reactions increased by 80% in ZnO NPs treated plants at 10 mg/l while it reduced significantly (by 31%) in wheat plants when subjected to higher concentration (1000 mg/l) of ZnO NPs as compared to control plant.

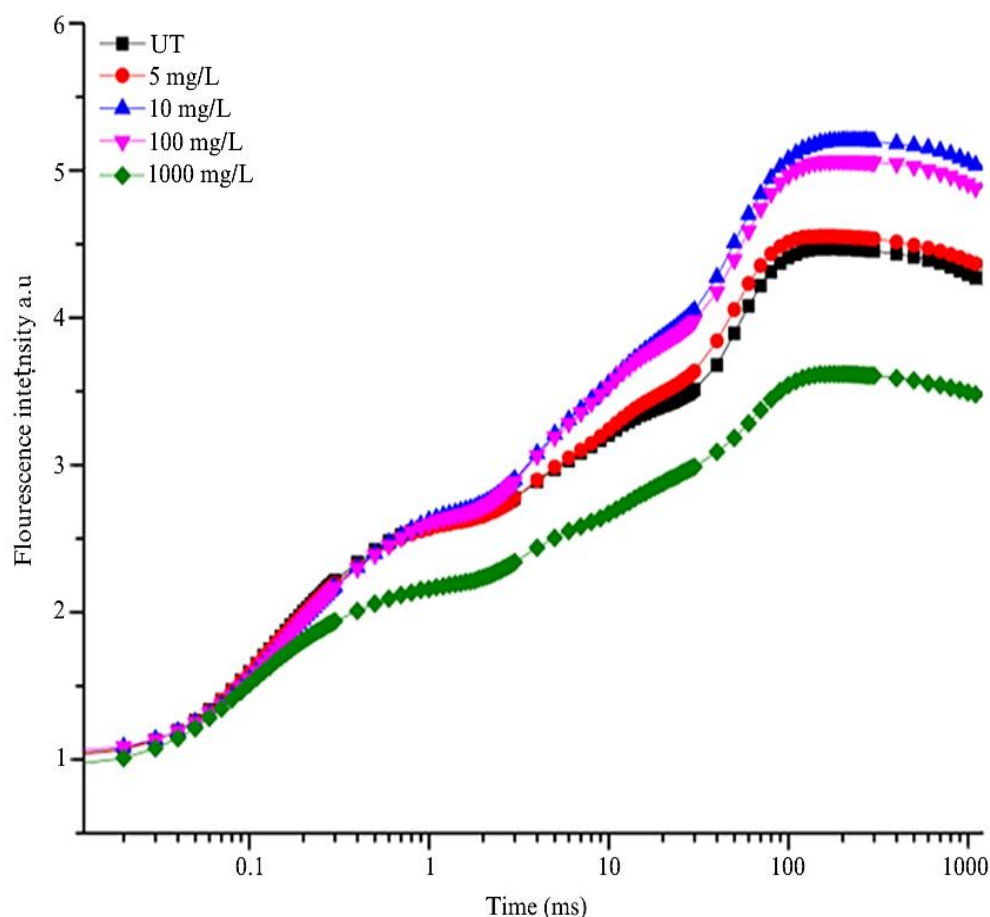


Figure 2. Chlorophyll *a* transient curves (normalized at F_o) on logarithmic scale obtained from untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/L ZnO NPs treated wheat plants.

Table 1. Effect of foliar spray of ZnO NPs on various fluorescence parameters obtained from OJIP curve in untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l wheat plants. Each experiment was done three times in three replicates. Significance was determined at $p < 0.001$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns=non-significant) and the results are given as mean value $\pm$ SD.

Treatments	F_o	Area	RC/ABS	F_v/F_o	F_o/F_m	$\psi_o / (1-\psi_o)$	$\Phi_{P_0} / (1-\Phi_{P_0})$	DF	PI
UT	414 $\pm$ 7	27332 $\pm$ 1823	0.55 $\pm$ 0.02	3.46 $\pm$ 0.3	0.28 $\pm$ 0.02	2.53 $\pm$ 0.1	1.28 $\pm$ 0.02	2.15 $\pm$ 0.3	0.99 $\pm$ 0.2
5 mg/l	418 $\pm$ 5 ⁿ	26133 $\pm$ 1670 ^{ns}	0.57 $\pm$ 0.02 ^{ns}	3.55 $\pm$ 0.3 ^{ns}	0.27 $\pm$ 0.01 ^{ns}	2.60 $\pm$ 0.3 ^{ns}	1.36 $\pm$ 0.02 ^{ns}	2.25 $\pm$ 0.2 ^{ns}	1.10 $\pm$ 0.1 ^{ns}
10 mg/l	408 $\pm$ 4 ⁿ	35290 $\pm$ 1892 ^{**}	0.72 $\pm$ 0.03 ^{**}	4.20 $\pm$ 0.2 ^{**}	0.24 $\pm$ 0.02 ^{**}	3.15 $\pm$ 0.1 ^{**}	1.65 $\pm$ 0.01 ^{**}	2.77 $\pm$ 0.2 ^{**}	1.78 $\pm$ 0.3 ^{**}
100 mg/l	410 $\pm$ 6 ⁿ	32988 $\pm$ 1674 ^{**}	0.69 $\pm$ 0.03 ^{**}	4.06 $\pm$ 0.2 ^{**}	0.24 $\pm$ 0.02 ^{**}	3.04 $\pm$ 0.3 ^{**}	1.59 $\pm$ 0.02 ^{**}	2.68 $\pm$ 0.4 ^{**}	1.63 $\pm$ 0.2 ^{**}
1000 mg/l	493 $\pm$ 4 ⁿ	23640 $\pm$ 1747 ^{**}	0.50 $\pm$ 0.01 ^{**}	2.61 $\pm$ 0.3 ^{**}	0.33 $\pm$ 0.03 ^{**}	1.98 $\pm$ 0.2 ^{ns}	1.22 $\pm$ 0.02 ^{**}	1.83 $\pm$ 0.3 ^{**}	0.69 $\pm$ 0.1 ^{**}

Effect of Foliar Spray of ZnO NPs on Energy Flux Parameters

Energy flow through various components of PSII is studied in the form of energy pipeline leaf model obtained by using Biolyzer software (Figure 3). Table 2 shows parameters such as ABS/CSm, ETo/CSm, TRo/CSm representing the efficiency of light absorption, electron trapping and electron transport per cross section, respectively in ZnO NPs treated and control plants. An increase in ABS/CSm

(by 14%) is correlated to an increase in density of active RC per cross section RC/CSm (indicated by open circles). The increase in absorption further resulted in a significant increase in electron transport (ETo/CSm) and electron trapping (TRo/CSm) by 17% and 16%, respectively at 10 mg/l as compared to control plants. However, at higher concentration (1000 mg/l) all the above parameters (RC/CSm, ABS/CSm, TRo/CSm, ETo/CSm) were observed to be decreased significantly (by 25%, 15%, 14%, and 27%, respectively) as compared to control plants thereby posing phytotoxic effects on wheat plants. Thus, ZnO NPs enhanced photochemistry best at 10 mg/l; but exhibit negative effects on plant photosynthetic efficiency at 1000 mg/l.

Effect of Foliar Spray of ZnO NPs on Plant Growth

Effect of foliar spray of ZnO NPs on dry and fresh weight of aerial biomass at different concentrations is shown in Figure 4. Plants treated with three treatments that is, 5 mg/l, 10 mg/l and 100 mg/l showed noticeable increase in biomass accumulation. At 10 mg/l of ZnO NPs, fresh weight increased by 56% while dry weight increased by 45% as compared to control. However, at 1000 mg/l of ZnO NPs, both fresh and dry biomass accumulation decreased significantly by 15% and 20%, respectively. The increase in biomass at 10 mg/l of ZnO NPs could be due to greater assimilation of Zn^{2+} ions when applied in the form of ZnO NPs allowing greater leaf penetration [31]. Also increase in biomass by ZnO NPs may be because of increased chlorophyll content resulting in improved photosynthesis (discussed earlier).

Effect of Foliar Spray with ZnO NPs on Antioxidant Enzymes Activity

The process of oxidative damage is due to the imbalance process of ROS in plants [32]. Activities of multiple antioxidant enzymes (SOD, CAT and POD) in wheat leaves exposed to ZnO NPs are presented in Figure 5. We observed a concentration-dependent influence of ZnO NPs on the activity of SOD, CAT and POD. At 10 mg/l, activity of POD decreased by 14% as compared to control. However, the activity of POD in plants treated with other ZnO concentrations (5 mg/l and 100 mg/l) were not different from control. Similarly, activity of SOD, involved in conversion of O_2 to H_2O_2 followed by action of CAT decomposing H_2O_2 to H_2O and O_2 molecules, were decreased by 22% and 61%, respectively when subjected to ZnO NPs at 10 mg/l as compared to control. On the contrary, in plants treated with ZnO NPs at 1000 mg/l, the activity of POD, SOD and CAT were increased by 22%, 23% and 11%, respectively as compared to control plants. The rise in antioxidants is a biological marker for increase in oxidative stress (ROS). Increase in oxidative stress at higher concentration of ZnO NPs can be explained due to release of Zn^{2+} ions to a greater extent at 1000 mg/l which are more toxic in nature resulting in increased generation of ROS [33].

Effect of Foliar Spray of ZnO NPs on Lipid Peroxidation

MDA is an important lipid peroxidation byproduct which gives an idea about the extent of damage caused to the cell membrane under stress [34]. Effects of foliar spray on MDA content of plant treated with ZnO NPs at varying concentrations are shown in Figure 6. Under our experimental conditions, MDA levels significantly decreased by 27% in plants treated with ZnO NPs at 10 mg/l followed by subsequent decrease in MDA at 5 mg/l and 100 mg/l by only 11% and 20%, respectively as compared to control plants. This decrease in MDA content in ZnO NP treated plants is in agreement with the earlier observation where zinc was reported to exert stabilizing and protective effect on biomembranes against oxidative and membrane permeability alterations [35]. However, MDA content was observed to be significantly increased (by 20%) in plants treated with increasing concentration (1000 mg/l) of ZnO NPs. Results obtained are supported by subsequent increase in antioxidant enzyme activity in 1000 mg/l treated wheat plants showing them to experience greater extent of oxidative stress (Figure 5). On the contrary, ZnO NPs at lower concentrations (10 mg/l) were more effective in protecting plant biomembrane integrity as a result of decrease in ROS production showing lower levels of oxidative stress in wheat plants [11].

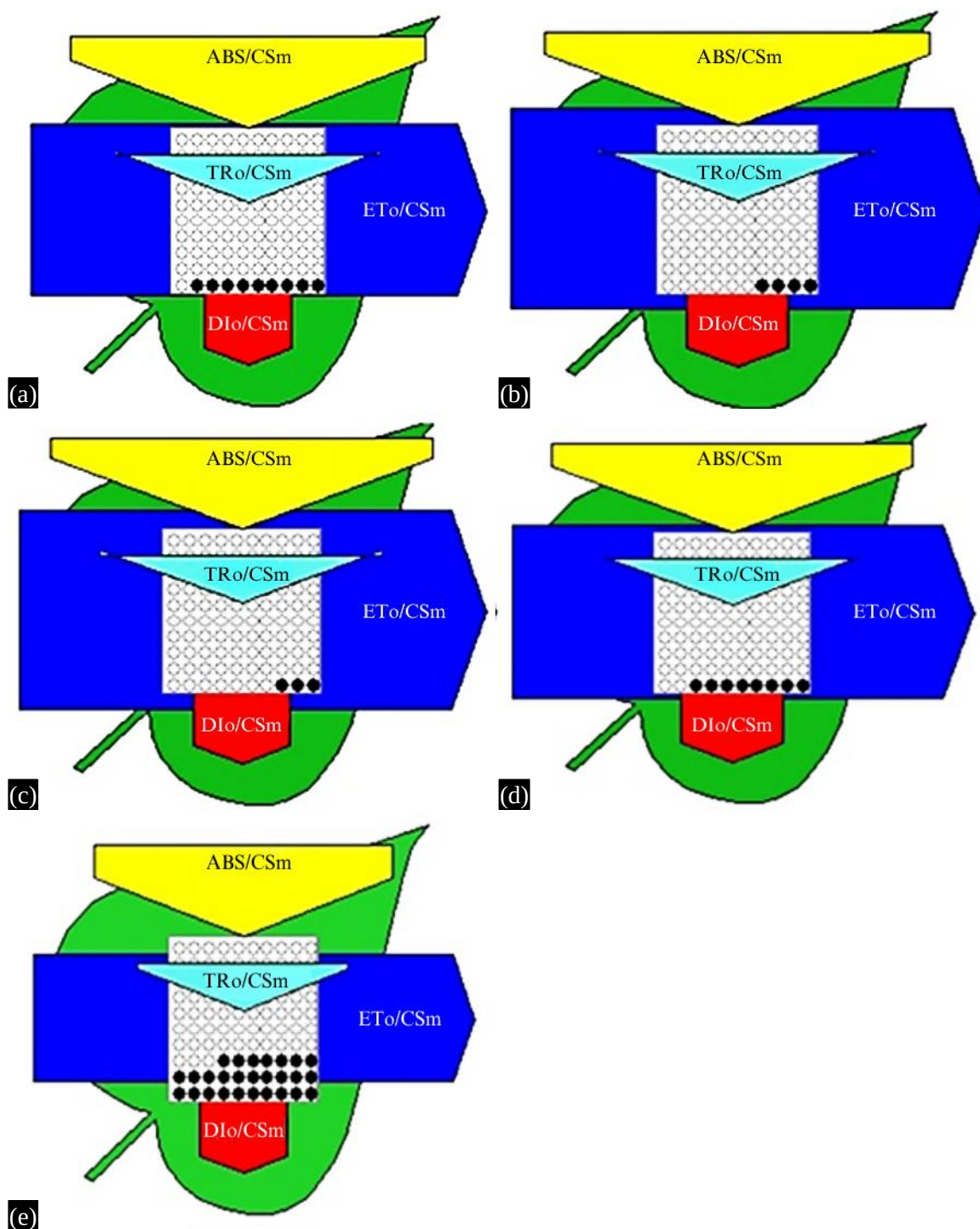


Figure 3. Energy pipeline model showing the proportion of phenomenological energy flux parameters (per cross section CSm) in wheat leaves 40 DAS (a) UT (control), (b) 5 mg/l, (c) 10 mg/l, (d) 100 mg/l and (e) 1000 mg/l treated wheat plants. Width of the corresponding arrow shows relative activity. Active RC (Q_A reducing) are shown by empty circles while black circles indicate the non-active (non- Q_A reducing) RC of PSII. ABS/CSm: Absorption per cross section; TRo/CSm: trapping per cross section; ETo/CSm: electron transport per cross section, DIo/CS: dissipation per cross section.

Table 2. Effects of foliar spray with ZnO NPs on energy flux parameters in untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l treated wheat plants. Each experiment was done three times in three replicates. Significance was determined at $p < 0.001$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns=non-significant) and the results are given as mean value $\pm$ SD.

Treatment	RC/CSm	ABS/CSm	TRo/CSm	ETo/CSm
UT	632.53 $\pm$ 16	2138 $\pm$ 17	1502.03 $\pm$ 6	984.05 $\pm$ 3
5 mg/l	620.50 $\pm$ 10 ^{ns}	2104 $\pm$ 13 ^{ns}	1507.93 $\pm$ 4 ^{ns}	1005.94 $\pm$ 4 ^{ns}
10 mg/l	735.23 $\pm$ 9***	2440 $\pm$ 15***	1741.99 $\pm$ 5***	1150.02 $\pm$ 4***
100 mg/l	685.42 $\pm$ 13 ^{ns}	2178 $\pm$ 13 ^{ns}	1592.98 $\pm$ 5 ^{ns}	1099.00 $\pm$ 7**
1000 mg/l	477.87 $\pm$ 11***	1834 $\pm$ 12***	1297.92 $\pm$ 3***	728.00 $\pm$ 4***

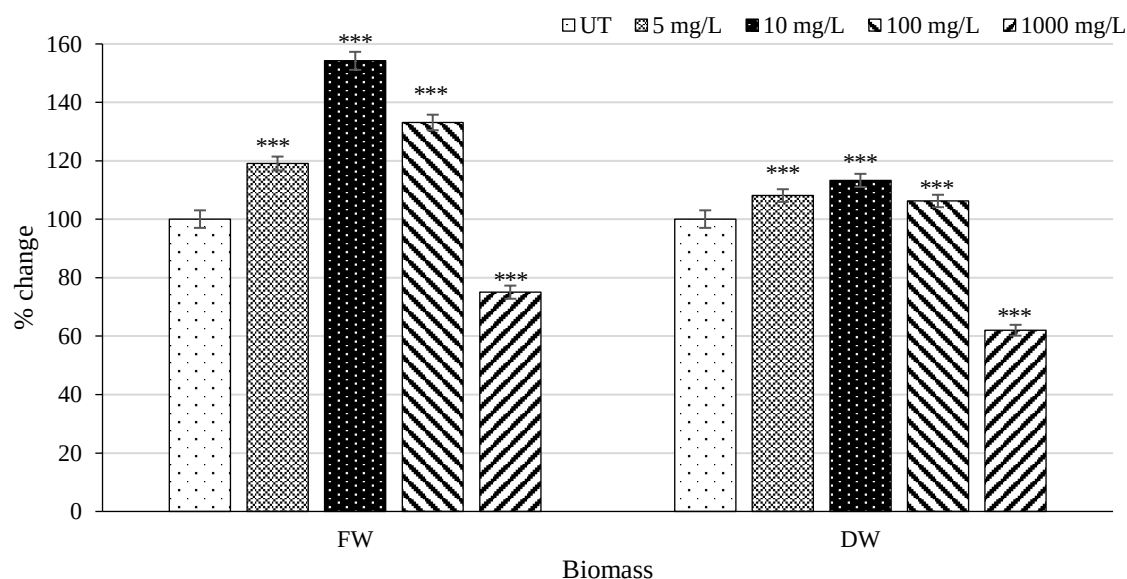


Figure 4. Effect of foliar spray of ZnO NPs on fresh (FW) and dry (DW) biomass of untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l treated wheat plants. Each experiment was done three times in three replicates. Significance was determined at $p < 0.001$ and the results are given as mean $\pm$ standard deviation.

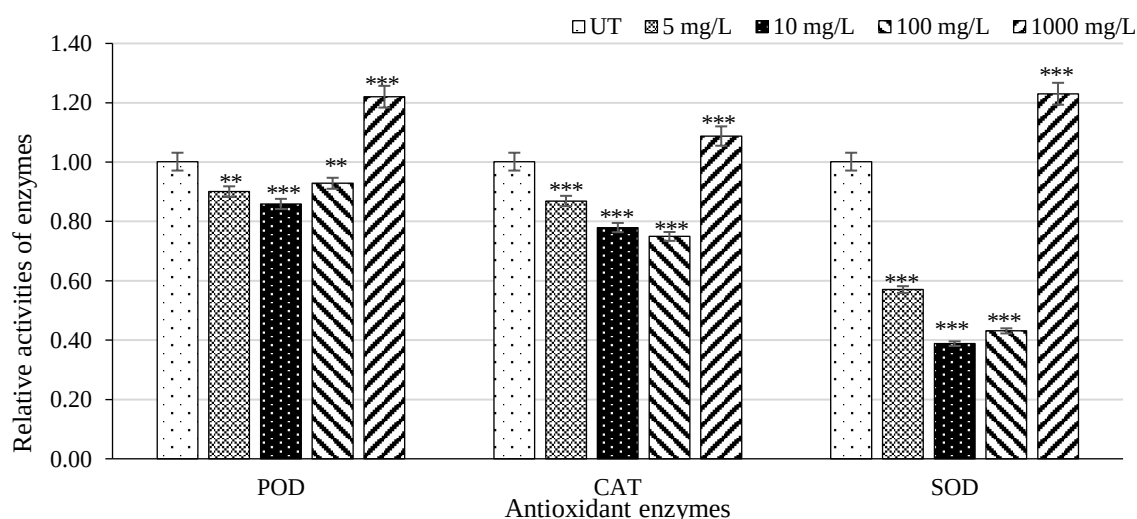


Figure 5. Effect of foliar spray with ZnO NPs on relative activity of peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l treated wheat plants. Each experiment was done three times with three replicates. Significance was determined at $p < 0.001$ (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, ns=non-significant) and the results are given as mean values $\pm$ SD.

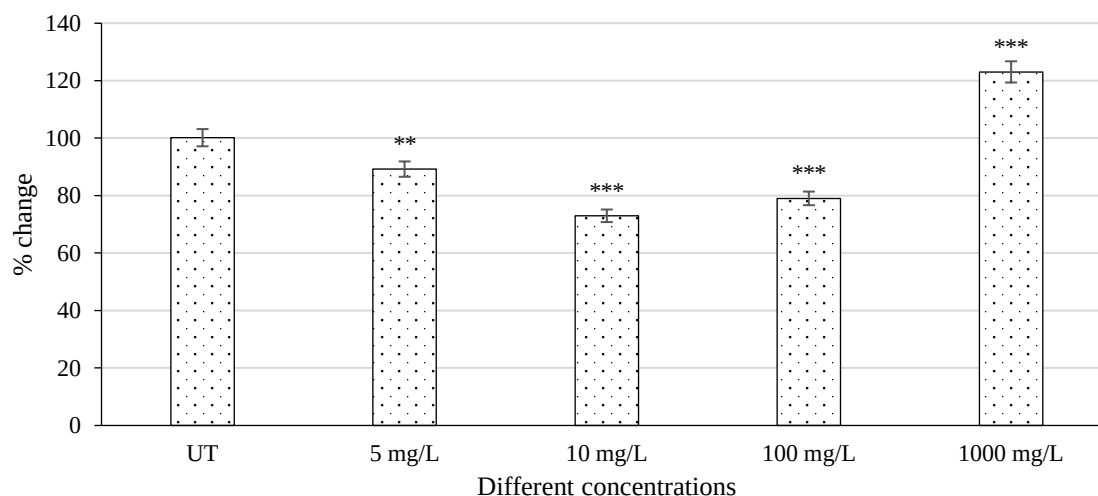


Figure 6. Effect of foliar spray with ZnO NPs on malondialdehyde (MDA) content in leaves of untreated (UT), 5 mg/l, 10 mg/l, 100 mg/l and 1000 mg/l treated wheat plants. Each experiment was done three times with three replicates. Significance was determined at $p < 0.001$ (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, ns=non-significant) and the results are given as mean values $\pm$ SD.

CONCLUSION

The present study highlighted the effects of foliar spray of ZnO NPs on growth and photosynthetic efficiency of wheat plants. ZnO NPs at 10 mg/l, promoted plant growth, increased total chlorophyll leaf content and carotenoid content in treated plants as compared to control. Higher chlorophyll accumulation in ZnO NP treated plants resulted in efficient utilization of light energy thereby indirectly improving light absorption, trapping and electron transport thereby improving overall plant photochemistry. At the same time plants subjected to lower concentrations of ZnO NPs experienced reduced oxidative stress that has again directly resulted in improving the photosynthetic performance. However higher concentration of ZnO NPs (1000 mg/l) exhibited phytotoxic effects affecting plant photosynthesis and growth. Thus from the present data, it can be concluded that foliar application of ZnO NPs at lower concentrations (10 mg/l) can be used as an efficient strategy to improve the quality of crops and also an alternative to soil application.

Abbreviations

ABS: Absorption
 CS: Cross section
 DI_o: Dissipation
 DS, Drought stress
 ET_o: Electron transport
 Fm: Maximum fluorescence
 Fo: Minimum fluorescence
 MDA: Malondialdehyde
 Fv/Fo: Efficiency of water splitting complex
 PEA: Plant efficiency analyser
 PSII: Photosystem II
 PI: Performance index
 RC: Reaction center
 RC/ABS: Active reaction center per chlorophyll molecule
 TR_o: Trapping
 ROS: Reactive oxygen species.

Authors' Contribution

The present study was design by AJ, PR and AG. Experiments were performed and analyzed by PR and AG. Manuscript was written by PR and edited by AJ.

Declaration of Competing Interest

Authors declared no conflict of interest.

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