Nano Zinc-Oxide Enhanced Photosynthetic Apparatus and Photosystem Efficiency of Maize (Zea Mays L.) in Sandy-Acidic Soils

Wiqar Ahmad (wiqar280@yahoo.co.uk)
University of Florida
https://orcid.org/0000-0003-1787-3492

Jaya Nepal
University of Florida

Xiaoping Xin
University of Florida

Zhenli He
University of Florida

Research Article

Keywords: Chlorophyll, Carotenoids, Fluorescence, Nano fertilizer, Nano ZnO, Photosynthesis

Posted Date: January 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1236243/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Nano Zinc-Oxide enhanced photosynthetic apparatus and photosystem efficiency of maize (Zea mays L.) in sandy-acidic soils

Wiqar Ahmad\textsuperscript{a,b}, Jaya Nepal\textsuperscript{a}, Xiaoping Xin\textsuperscript{a}, Zhenli He\textsuperscript{a*},

\textsuperscript{a}Soil and Water Science Department/Indian River Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 2199 South Rock Road, Fort Pierce, FL. 34945, USA

\textsuperscript{b}Department of Soil and Environmental Sciences, the University of Agriculture, Peshawar, AMK Campus, 23200, Mardan, Pakistan.

Authors emails:
Wiqar Ahmad*: wiqar280@yahoo.co.uk / wiqar.ahmad@ufl.edu
Jaya Nepal: j.nepal@ufl.edu
Xiaoping Xin: xinxp1024@ufl.edu
Zhenli He: zhe@ufl.edu

*Corresponding Author: zhe@ufl.edu, wiqar.ahmad@ufl.edu
Postal address: 2199, S ROCK RD. Fort Pierce, FL 34945

Running Title: nano ZnO impact on chlorophyll and photosystems efficiency.

Graphical Abstract:
Nano Zinc-Oxide enhanced photosynthetic apparatus and photosystem efficiency of maize 
(*Zea mays* L.) in sandy-acidic soils

**Abstract**

Conventional Zinc (Zn) fertilization (e.g., zinc sulfate) often leads to poor availability in 
soils. Zinc oxide nanoparticles (nano ZnO) can be a potential solution, but their effect on crop 
photosynthetic activity isn’t well documented. The effects of nano ZnO (50, 100, 150, 200 mg L$^{-1}$) 
and application methods (seed-coating, soil-drench, and foliar-spray) in comparison with 
ZnSO$_4$ recommended dose were evaluated for plant height, biomass, chlorophyll pigments and 
photosystem efficiency in a greenhouse pot experiment. 100 mg L$^{-1}$ of nano ZnO significantly 
increased the chlorophyll (*Chl.*) a, b, a+b, carotenoids (x+c), a+b/x+c, SPAD, leaf *Chl.*, total 
chlorophyll content plant$^{-1}$, plant height and total biological yield (by 18-30%, 33-67%, 22-38%, 
14-21%, 14-27%, 12-19%, 12-23% 58-99%, 6-11% and 16-20%, respectively) and reduced *Chl.* 
a/b (by 6-22%) over the other treatments (p<0.01) irrespective of application methods. Nano 
ZnO applied at 100 mg L$^{-1}$ significantly increased photochemical quenching (qP) and efficiency 
of photosystem II (EPSII) compared to 150 and 200 mg L$^{-1}$ regardless of application methods. 
The positive correlations between *Chl.* a and *Chl.* b ($r^2=0.90$), *Chl.* a+b and x+c ($r^2=0.71$), SPAD 
and *Chl.* a ($r^2=0.90$), SPAD and *Chl.* b ($r^2=0.94$) and SPAD and *Chl.* a+b ($r^2=0.93$) indicates a 
uniform enhancement in chlorophyll pigments; SPAD value, qP, EPSII, and growth and yield 
parameters. This elucidates that the application of nano ZnO at 100 mg L$^{-1}$ promotes corn 
biochemical health and photosynthesis, irrespective of the application method. These findings 
have a great propounding for improving plant growth through nano ZnO bio-fortification in 
acidic Spodosols.
Keywords: Chlorophyll, Carotenoids, Fluorescence, Nano fertilizer, Nano ZnO, Photosynthesis

1. Introduction:

Zinc (Zn) is an essential micro-nutrient required for enhancing the productivity and quality of cereal crops. It is required for several plant physiological functions including enzyme activation, synthesis of chlorophyll pigments and functioning of photosynthesis, and membrane integrity (Nadeem & Farooq, 2019). Therefore, Zn deficiency can potentially impact crop photosynthetic apparatus and efficiency, thereby reducing the quantity and quality of the products. For example, its deficiency has caused severe declines in major cereal crop productivity (Hossain et al., 2019; Bhatt et al., 2020), and threatened the cereal-based cropping systems (Cakmak & Kutman, 2018; Nadeem & Farooq, 2019). The problem is exacerbated in highly alkaline (Recena et al., 2021) or acidic (García-Gómez et al., 2020) soils, where Zn$^{2+}$ is highly fixed, making it unavailable for crop plant uptake. Corn is a major cereal crop and requires the application of Zn for maintaining production. The straw quality and grain Zn bio-fortification rely on supplementation of Zn through chemical fertilization. However, Zn application through conventional methods (e.g., zinc sulfate) often leads to poor availability and low crop uptake due to fixation reactions in soils (Elemike et al., 2019). The Zn uptake efficiency is particularly low when the soil contains low organic matter, low clay content, high carbonate, or low pH (Recena et al., 2021).

Nanoparticles, owing to their minute size and large reactive surface area, offer a potential solution to improve nutrient uptake efficiency in agriculture (Rizwan et al., 2017). As they are readily up-taken by plants, they possess a significant potential to improve crop growth and yield.
(Sabir et al., 2014). Zinc oxide nanoparticles (nano ZnO) can be a viable alternative to enhance Zn uptake in low organic matter, sandy, and highly acidic soils. Although using ZnO nanoparticles for mitigating Zn deficiency and augmenting Zn bio-fortification of different crops has previously been reported (Rizwan et al., 2017; Moghaddasi et al., 2017); their effect on crop photosynthetic activity isn’t well documented. Also, uptake efficiency of nano ZnO through various methods and its possible implications on plant physiological mechanism is not understood well. The availability of Zn also directly affects the efficiency of photosynthesis systems II (PSII) as Zn is vital in the formation and activation of photosynthetic pigments and providing energy through electron transport during light and dark reactions photosynthesis. Corn biomass, stomatal conductance, and quantum yield of PSII were significantly improved through Zn application under well-watered conditions (Wang et al., 2009). Zn deficiency leads to reduced photosynthesis rate (Subba et al., 2014), disruption in chlorophyll membrane, leaf chlorophyll content and reduction in photochemical efficiency of PSII (Chen et al., 2008). The leaf chlorophyll content, chlorophyll a/b ratio, Fv/Fm, Fv/Fo were significantly reduced by zinc deficiency, which indicated the integral efficiency of the PSII was damaged when sufficient Zn was not available (Chen et al., 2008). Nano zinc oxide particles have shown to improve corn growth either at par or better than conventional ZnSO4 (Adhikari et al., 2015; Taheri et al., 2015). However, their effects on plant photosynthetic pigments and efficiency were inconsistent with different crops and fluctuated based on the concentration used (Reddy Pullagurala et al., 2018; Salam et al., 2022). It is, thus, critical to document the effect of nano ZnO application not only in evaluating Zn uptake by crops but also in understanding its implications in crop photosynthetic apparatus and efficiency to identify its optimal level of application.
In this study, the performance of nano ZnO was evaluated for corn plant photosynthetic efficiency using different application modes and rates in sandy-acidic Spodosols. The major objectives were to evaluate the effect of different application rates of nano ZnO on crop growth as evidenced through plant growth, the robustness of photosynthetic apparatus (chlorophyll pigments, carotenoids and antioxidant activity) and photosynthetic efficiency (efficiency of photosystem II), as compared to conventional Zn sulfate application. Besides, Nano ZnO was tested through different application methods (seed coating, soil drench and foliar spray) to understand its effectiveness for photosynthetic performance, growth and Zn nutrition of corn.

2. Material and methods:

2.1. Experimental site and soil sampling:

The pot experiment was conducted at the greenhouse facility of the Indian River Research and Education center (IRREC), University of Florida. The soil representing the order of Spodosol (Ankona series) was sampled from the experimental farm at the research center (properties shown in Table 1). The collected soils were composited and homogenized before transporting them to the laboratory, where it was air-dried and sieved through a 1-mm sieve. The pre-sowing analysis of Spodosol soil to be used for this experiment is shown in Table 1.

| Parameters                      | Unit     | Values  |
|---------------------------------|----------|---------|
| pH (1:2.5 H₂O)                  | -        | 4.81    |
| EC (1:5 H₂O)                    | µS/cm    | 202.15  |
| Total C                         | %        | 0.754   |
| Total N                         | "        | 0.022   |
| Available nutrients*            | mg kg⁻¹  |         |
| P                               |          | 28.94   |
| K                               | "        | 30.3    |
| Ca                              | "        | 211.84  |
2.2. Experimental design:

The pot experiment was a completely randomized design with the following treatments: Control, recommended Zn (11 kg ha\(^{-1}\)) applied through ZnSO\(_4\) fertilizer, nano ZnO – coating (50, 100, 150, 200 mg L\(^{-1}\)), nano ZnO – Soil drench (50, 100, 150, 200 mg L\(^{-1}\)) and nano ZnO – foliar (50, 100, 150, 200 mg L\(^{-1}\)). Zinc Sulfate (ZnSO\(_4\)) was applied at the rate of 11 kg Zn ha\(^{-1}\) (5.5 mg Zn kg\(^{-1}\) soil) for soil application as per local recommendations (Arafat et al., 2016), hereafter referred to as ZnSO\(_4\) applied at the recommended dose. The In total, 14 treatments, including control, were replicated three times, making 42 pots. The pots were rotated randomly biweekly in the greenhouse. Pots were filled with 6 kg of soil each, and basal fertilization of potassium (K) and phosphorus (P) was applied. Nitrogen (N) was applied at the recommended dose in two split doses, 10 and 30 days after germination. All the fertilization was performed as per existing recommendations (Ahmad, 2004). Five corn seeds (Zea mays, variety Dekalb; DKC62-08) per pot were sown (later thinned to two plants per pot) at a depth of about 5 cm on April 13\(^{th}\), 2021 and continued till 75 days after sowing (DAS). Zinc oxide nanomaterial (Alfa Aesar™ ZnO nanopowder, 99% metal basis; MW: 81.37) and Zinc sulfate (ZnSO\(_4\).7H\(_2\)O, MW: 287.54) were obtained from Thermo Fisher Scientific. Irrigation was applied as per plant requirements at regular intervals.

| Element | " | Amount |
|---------|---|--------|
| S       | " | 47.22  |
| Mg      | " | 39.90  |
| Cu      | " | 11.69  |
| B       | " | 1.33   |
| Mo      | " | 0.09   |
| Zn      | " | 14.54  |
| Al      | " | 48.31  |
| Mn      | " | 11.97  |

*Estimated by Mehlich 3 extraction.
2.3. Soil analysis:

The soil samples were analyzed prior to the beginning of the experiment (Table 1). Soil pH (1:2.5 H₂O) and electrical conductivity (EC) were measured using the pH/mV/conductivity meter (AB 200, Fischer Scientific, PA, USA). Soil carbon and nitrogen were analyzed through dry combustion of 50 mg sample at 1200 °C using a dry combustion C/N analyzer (Vario MAX CN Macro Elemental Analyzer, Hanau, Germany). Available macronutrients P, K, Ca, Mg, S and micronutrients (Fe, Zn, Cu, Mn, Al, Mo, B) in soil were extracted through Mehlich 3 extraction method (Mehlich, 1984) and their concentrations were analyzed using an Inductively coupled plasma optical emission spectrometry (ICP-OES, Ultima, J.Y. Horiba Group, Edison, N.J.).

2.4. Growth data and plant analysis:

Data were recorded for plant height, SPAD and fluorescence at 60 DAS. Leaf chlorophyll content was measured from two recently matured leaves per plant using a handheld SPAD chlorophyll meter (Minolta Camera, Osaka, Japan). The fluorescence parameters were recorded under actively photosynthesizing (daytime), and dark-adapted conditions using the pulse modulated chlorophyll fluorometer (OS5p+, Opti-Sciences Inc., Hudson, NH). The key parameters recorded were fluorescence under steady-state conditions i.e., before saturation (Fₛ), maximum fluorescence with actinic illumination at steady-state fluorescence (Fₘₛ), quantum photosynthetic yield of photosystem II (Y), minimum fluorescence (Fₒ), maximal fluorescence (Fₘ) and maximum photochemical efficiency of PSII (Fᵥ/Fₘ) [nighttime]. The following calculations were performed to calculate photochemical quenching (qP) and non-photochemical quenching (qN) based on Oxborough & Baker (1997).
\[ q_P = \frac{F_{ms} - F}{F_{ms} - F_o'} \]
\[ qN = \frac{F_m - F_{ms}}{F_{ms}} \]

Where Fo’ is adjusted minimum fluorescence calculated as,

\[ F_o' = \frac{F_o}{(1 - \frac{F_o}{F_m - F_{ms}})} \]

Chlorophyll and carotenoid contents were estimated by the methods suggested by Lichtenthaler & Buschmann (2001) and Ahmed et al. (2020). In brief, 100 mg of fresh leaf sample was taken during the growth of the crop (60DAS) from each pot, crushed and mixed with 10 mL of 80% acetone. The mixture was protected from the light by covering with parafilm and preserving in the refrigerator in dark conditions overnight (24h) to allow for complete digestion of chlorophyll pigments. The filtrates were then analyzed using the UV-Spectrophotometer (U-3110, Hitachi, Japan) at the wavelength of 663, 645, and 470nm to determine chlorophyll (Chl.) a, Chl. b, and carotenoid (x+c) contents using the following equations.

\[ Chl \text{ a (mg g}^{-1}) = \frac{(12.21 \times A_{663}) - (2.81 \times A_{645}) \times Volume \ (ml) }{weight \ of \ the \ sample \ (FW) \ (g) \times 1000} \]

\[ Chl \text{ b (mg g}^{-1}) = \frac{(20.13 \times A_{645}) - (5.03 \times A_{663}) \times Volume \ (ml) }{weight \ of \ the \ sample \ (FW) \ (g) \times 1000} \]

\[ x + c \ (mg \ g}^{-1}) = \frac{((1000 \times A_{470}) \times volume \ (ml) )}{sample \ weight \ (FW) \ (g) \times 1000} - (3.27 \times Chl \text{ a}) - (104 \times Chl \text{ b}) \right] / 229 \]

Where FW = Fresh weight, A (663, 645, 470) = Absorbance at 663, 645 and 470 nm

Non-enzymatic antioxidant (proline) concentration was calculated following BATES (1973) with slight modification based on ninhydrin (Hayyawi et al., 2020). In short, 100 mg of fresh leaf sample was drenched with 2 mL of 3% sulfosalicylic acid, filtered and added 2mL of
glacial acetic acid followed by incubation in a water bath (90°C) for 1 hour. After cooling, the
samples were added with 4mL of Toluene, followed by shaking (20mins) and incubation at room
temperature to develop the toluene-proline layer. 1mL liquid from the upper toluene-proline
layer was sucked and analyzed at 520nm using the UV-Spectrophotometer. Proline concentration
was calculated in µmol g⁻¹ fresh weight using the given equation.

\[
Proline (\mu mol/g) = \frac{(A_{520} \times 20) \times \text{Volume (ml)}}{\text{weight of the sample (FW) (g) \times 1.47 \times MW}}
\]

Where; A520= Absorbance at 520 nm, MW= molecular weight of proline

2.5. Statistical analysis:

All data were statistically analyzed with Statistix 8.1 software. The data were plotted as
the mean values for each treatment along with the standard errors. All the statistical analysis was
performed at the significance level of 0.05. The treatment effects on different parameters were
assessed through analysis of variance (ANOVA) after testing for the homogeneity of variance,
followed by a post-hoc test [Least Significant Difference (LSD) test] was conducted to identify
significantly different treatments.

3. Results

3.1. Chl. a, Chl. b, Chl. a+b and Chl. a/b ratio

Zinc treatments significantly (p < 0.01) improved maize chlorophyll a (Chl. a), Chl. b,
total Chl. (a+b) concentration, weight ratio of Chl. a and Chl. b, (a/b) over control treatment
(Table 2). The increment at 100 mg L⁻¹ nano ZnO was maximum and higher by 29% and 31%
for Chl. a and 51% and 67% for Chl. b over the recommended ZnSO₄ dose and the control,
respectively. Also, the nano ZnO 100 mg L⁻¹ treatment had the maximum Chl. a+b and a
minimum weight ratio of Chl. a/b indicating a significant (p<0.01) difference of 38% for Chl. a+b and 20% for Chl. a/b ratio with the control. The a/b ratio was significantly (p<0.01) lower for nano ZnO 100 mg L\(^{-1}\) application, and the higher value of a/b ratio of nano ZnO 200 mg L\(^{-1}\) and ZnSO\(_4\) indicate significantly poor performance (p<0.01) among zinc treatments. Chl. a+b for 50 mg L\(^{-1}\) nano ZnO was significantly (p<0.01) lower (by 22% and 18%, respectively) than nano ZnO 100 mg L\(^{-1}\) and 150 mg L\(^{-1}\) but statistically similar with nano ZnO 200 mg L\(^{-1}\). However, Chl. a/b for nano ZnO 50 mg L\(^{-1}\) and 150 mg L\(^{-1}\) were statistically similar to other Zn treatments including 100 mg L\(^{-1}\) ZnO NP, but significantly (p<0.01) lower (by 11% and 13%, respectively) than the control (Table 2). Results (Table 2) further showed that the impact of application methods on Chl. a, Chl. b, Chl. a+b and Chl. a/b values were non-significant. However, with the foliar application, a 4% and 3% improvement in Chl. a, 10% and 1% in Chl. b was observed over the soil drench and seed coating, respectively. Similarly, a 3% and 5% higher Chl. a+b value with foliar application of nano ZnO over the seed coating and soil drench; and a 4% higher Chl. a/b with soil drench each over the seed coating and foliar application was observed, respectively. A significant correlation between Chl. a and b (r\(^2\) = 0.90), a+b and x+c (r\(^2\) = 0.71) was observed.

Interaction between the Zn treatments and application methods for Chl. a and Chl. a+b was non-significant but was significant for Chl. b and Chl. a/b values. It was observed from data (Fig. 1a) that lower to moderate nano ZnO doses were effective for improving Chl. b in maize with the foliar application, higher doses were effective with soil drench and a moderate dose of 100 mg L\(^{-1}\) ZnO NP was significantly higher in Chl. b when applied as seed coating. For Chl. a/b (Fig. 2b), lower to moderate nano ZnO doses resulted in lower Chl. a/b ratio with foliar application and seed coating at 100 mg L\(^{-1}\) ZnO NP treatment was the lowest.
Table 2: Maize biochemical parameters as affected by different doses and modes of application of nano ZnO in comparison with recommended dose of ZnSO₄ applied as soil.

| Zn Treatments | Chl. a | Chl. b | a/b | T. Chl. (a+b) | Carot. (x+c) | a+b/x+c | Proline (μmol g⁻¹ (FW)) |
|---------------|-------|--------|-----|---------------|--------------|---------|------------------------|
| Cont.         | 2.31 b| 0.55 c | 4.19 a | 2.87 c       | 0.51 bc      | 5.61 c  | 10.11 a                |
| ZnSO₄ (RD)    | 2.35 b| 0.61 c | 3.92 ab| 2.96 c       | 0.48 c       | 6.14 bc | 1.72 c                 |
| ZnO NP50      | 2.56 b| 0.69 bc| 3.78 bc| 3.24 bc      | 0.56 ab      | 5.77 c  | 9.65 a                 |
| ZnO NP100     | 3.02 a| 0.92 a | 3.5 c  | 3.95 a       | 0.58 a       | 6.84 a  | 9.09 ab                |
| ZnO NP150     | 3.00 a| 0.82 ab| 3.70 bc| 3.82 a       | 0.59 a       | 6.44 ab | 3.68 bc                |
| ZnO NP200     | 2.94 a| 0.76 b | 3.94 ab| 3.70 ab      | 0.60 a       | 6.18 bc | 5.99 abc               |
| LSD (p<0.05)  | 0.34**| 0.16**| 0.38* | 0.48**       | 0.06**       | 0.58**  | 5.66*                  |

Methods of Application

| (MoA)          | Chl. a | Chl. b | a/b | T. Chl. (a+b) | Carot. (x+c) | a+b/x+c | Proline (μmol g⁻¹ (FW)) |
|----------------|-------|--------|-----|---------------|--------------|---------|------------------------|
| Coating        | 2.68  | 0.74   | 3.80| 3.42          | 0.56         | 6.13 ab | 5.55                   |
| Soil           | 2.65  | 0.68   | 3.94| 3.34          | 0.56         | 5.92 b  | 8.73                   |
| Foliar         | 2.76  | 0.75   | 3.77| 3.51          | 0.54         | 6.44 a  | 5.84                   |
| LSD (p<0.05)   | ns    | ns     | ns  | ns            | ns           | 0.41*   | ns                     |

Interaction (Zn * MoA)

| (Zn * MoA)     | Chl. a | Chl. b | a/b | T. Chl. (a+b) | Carot. (x+c) | a+b/x+c | Proline (μmol g⁻¹ (FW)) |
|----------------|-------|--------|-----|---------------|--------------|---------|------------------------|
| ns             | *(Fig. 2) | *(Fig. 3) | ns  | ns            | ns           | **(Fig. 4) | ns                     |

FW= Fresh weight, Chl. a: Chl. a, Chl. b: Chl. b, T.Chl.: total Chl., Carot.: carotenoids, ZnO NP50: ZnO NP 50 mg L⁻¹, ZnO NP100: ZnO NP 100 mg L⁻¹, ZnO NP150: ZnO NP 150 mg L⁻¹, ZnO NP200: ZnO NP 200 mg L⁻¹. In a column, means followed by different letters vary significantly at the p<0.05.
Figure 1 Interaction effect of nano ZnO treatments and their application methods on (a) Chl. b concentration (b) Chl. a/b ratio (c) Chl. a+b/x+c ratio and of maize crop
3.2. Carotenoids (x+c), Chl.-carotenoid ratio (a+b / x+c) and proline

Results (Table 2) showed significant (p<0.01) difference amongst Zn treatments in carotenoids (x+c), the weight ratio of total Chl. (a+b) to carotenoids (a+b / x+c) and proline in maize crop. The nano ZnO treatments were statistically similar in x+c but significantly (p<0.01) higher than the ZnSO₄. However, the extent of x+c increased with increasing dose of nano ZnO and was maximum for 200 mg L⁻¹ followed by 150, 100 and 50 mg L⁻¹ nano ZnO treatments. The weight ratio of Chl. a+b / x+c for nano ZnO 100 mg L⁻¹ was maximum followed by 150 and 200 mg L⁻¹ while all the three were significantly (p<0.01) higher (by 27%, 22%, and 13%, respectively) than the lowest Chl. a+b / x+c ratio in the control. Furthermore, the 100 and 150 mg L⁻¹ nano ZnO doses were significantly (p<0.01) higher in Chl. a+b / x+c ratio (by 22% and 14%, respectively) than the nano ZnO 50 mg L⁻¹ (Table 2). The Zn control had the highest and ZnSO₄ the lowest proline concentration while both differed significantly (p<0.01) with a 5-fold difference approximately. Amongst the nano ZnO solution concentrations, 50 mg L⁻¹ was highest in proline, statistically similar to the control, significantly (p<0.01) higher (by 162%) than 150 mg L⁻¹ and 6% and 60% higher than the nano ZnO 100 mg L⁻¹ and 200 mg L⁻¹ ZnO NP treatments, however, they were statistically similar (Table 2).

Application methods were non-significant for x+c and proline but their impact was significant on Chl. a+b / x+c ratio. Soil drench had an edge (p>0.05) of 1.7% and 5% in x+c and 57% and 50% in proline over seed-coating and foliar application, respectively. Foliar application was significantly (p<0.05) higher in Chl. a+b / x+c ratio (by 10%) than soil drench but had an edge of 6% (p>0.05) over the seed coating of the nano ZnO while the latter two methods were statistically similar (Table 2). The interactions between Zn treatments and their application methods affecting the x+c and proline concentration were non-significant but highly significant.
(p<0.01) for Chl. \( a+b / x+c \) ratio. Data (Fig. 1) revealed that the performance of the nano ZnO 100 mg L\(^{-1}\) was significantly (p<0.01) higher compared to other treatments applied at either method except for the nano ZnO 150 mg L\(^{-1}\) applied as foliar. In soil drench, the higher nano ZnO doses (150 and 200 mg L\(^{-1}\)) performed superior over the nano ZnO 100 and 50 mg L\(^{-1}\), ZnSO\(_4\) and the control treatments to a non-significant extent. However, with foliar application, the nano ZnO 100 and 150 mg L\(^{-1}\) were significantly higher than the ZnSO\(_4\) and the control treatments.

### 3.3. SPAD value, Leaf Chl. (nmol cm\(^{-2}\)) and total Chl. (\(\mu\)mol plant\(^{-1}\))

Based on SPAD analysis, application of Zn significantly (p<0.01) increased leaf Chl. and total Chl. content plant\(^{-1}\) over the non-Zn treatment whilst nano ZnO recorded a further significant (p<0.01) increase over the ZnSO\(_4\) (Table 3). Amongst the nano ZnO treatments, 100 mg L\(^{-1}\) was higher in SPAD and leaf Chl. by 8% (p<0.05) and 12% (p<0.05) than 50 mg L\(^{-1}\), by 3% (p>0.05) and 4% (p>0.05) than 150 mg L\(^{-1}\) and by 4% (p>0.05) and 6% (p>0.05) than 200 mg L\(^{-1}\), respectively. The maximum Chl. content plant\(^{-1}\) recorded with 100 mg L\(^{-1}\) nano ZnO was statistically similar with 150 mg L\(^{-1}\) but significantly (p<0.01) higher than the 50 mg L\(^{-1}\) (by 35%), 200 mg L\(^{-1}\) nano ZnO (by 17%), ZnSO\(_4\) (by 58%) and the Zn control (by 99%).

**Table 3: Maize SPAD and fluorescence parameters as affected by different levels and modes of nano ZnO in comparison with ZnSO\(_4\) applied as soil**

| Zn Treatments | SPAD  | Chl. Conc.   | Total Chl. | EPSII | Y (II) | qP  | qNP  |
|---------------|-------|--------------|------------|-------|-------|-----|------|
| Cont.         | 37.9 d| 31.2 d       | 37.3 e     | 0.8120 b| 0.694 b| 0.865 b| 0.135 a|
| ZnSO\(_4\) (RD)| 40.3 c| 34.0 c       | 47.0 de    | 0.8143 a| 0.706 ab| 0.903 a| 0.097 b|
| ZnO NP50      | 41.8 bc| 35.9 bc      | 54.8 cd    | 0.8139 ab| 0.716 a| 0.915 a| 0.085 b|
Carot.: carotenoids, ZnO NP50: ZnO NP 50 mg L\(^{-1}\), ZnO NP100: ZnO NP 100 mg L\(^{-1}\), ZnO NP150: ZnO NP 150 mg L\(^{-1}\), ZnO NP200: ZnO NP 200 mg L\(^{-1}\), EPSII: efficiency of photosystem II, Y (II)=quantum photosynthetic yield of PSII, qP=photochemical quenching, qNP= non-photochemical quenching. In a column, means followed by different letters vary significantly at the p<0.05.

Results further indicated the impact of the application methods and their interaction with Zn treatments on the SPAD, leaf Chl. and total Chl. plant\(^{-1}\) of the corn crop was non-significant (Table 3). However, seed coating of nano ZnO showed up to 8\% (p>0.05) and 5\% (p>0.05) improvement in total Chl. plant\(^{-1}\) over the soil drench and foliar application methods. SPAD value had a significantly higher correlation with extractable Chl. a \((r^2=0.90)\), Chl. b \((r^2=0.94)\) and Chl. a+b \((r^2=0.93)\) and support the data recorded for Chl. a, Chl. b and Chl. a+b with spectrophotometer (Fig 2).

3.4. Efficiency of (EPSII) and quantum photosynthetic yield (Y) of photosystem II

Zinc treatments significantly (p<0.05) improved the efficiency of photosystem II (EPS II) as well as the quantum photosynthetic yield of PSII (Y) over the control. As for the EPS II, the 100 mg L\(^{-1}\), 150 mg L\(^{-1}\) nano ZnO and the ZnSO\(_4\) were statistically similar but significantly (p<0.05) higher than the control, however, nano ZnO 100 and 150 mg L\(^{-1}\) higher by 0.14\% and 0.16\%,
respectively, over the ZnSO$_4$. It was evident from the data (Table 3) that EPS II was lowered at a concentration above 150 mg L$^{-1}$ and below 100 mg L$^{-1}$ nano ZnO. The Y (II) with all nano ZnO doses was significantly (p<0.05) higher than the control, however, it had a non-significant improvement (1.8%) over the ZnSO$_4$ (Table 3). Amongst the nano ZnO treatments, the maximum Y (II) was recorded with 100 mg L$^{-1}$ nano ZnO, which was significantly (p<0.01) higher (by 3.6%) over the control followed by 50, 200 and 150 mg L$^{-1}$ nano ZnO with 3.2%, 2.4% and 2.3% increase, respectively. The effect of application methods and their interaction with Zn treatments on the Y(II) and EPS II was non-significant.

3.5. Photochemical quenching (qP) and non-photochemical quenching (qN)

Results (Table 3) indicated a highly significant (p<0.01) increase in photochemical quenching (qP) because of Zn treatments and vice versa for non-photochemical quenching (qN). The highest qP and lowest qN were recorded for 100 mg L$^{-1}$ nano ZnO which was significantly (p<0.01) higher (by 6%) over the control and non-significantly higher over the other Zn treatments and vice versa in qN. Also, qP in the ZnSO$_4$ treatment was higher significantly (p<0.01; by 4%) and qN lower by the same extent over the control treatment. Amongst the nano ZnO treatments, 100 mg L$^{-1}$ was the highest qP and lowest qN. Neither significant difference in qP and qN was observed for methods of application nor its interaction with Zn treatments.

3.6. Correlation amongst different photosynthetic and fluorescence parameters

Amongst different photosynthetic and fluorescence parameters, a significant correlation was evident from our study (Fig. 2). Results showed a highly significant correlation between Chl. a and Chl. b ($r^2 = 0.90$); and Chl. a+b and carotenoids x+c ($r^2=0.82$), indicating a uniform effect of Zn treatments on these photosynthetic pigments. Significant correlation between SPAD value and extracted Chl. a ($r^2 = 0.90$), SPAD value and extracted Chl. b ($r^2 = 0.94$) and SPAD
value and extracted Chl. a+b \( (r^2 = 0.93) \) indicates validation of our results for the photosynthetic pigments. Significant correlation between Chl. a and photochemical quenching (qP) \( (r^2 = 0.46) \) and Chl. b and qP \( (r^2 = 0.57) \) indicate a significant effect of increased photosynthetic pigments (a and b) on photosynthetic apparatus. There was a significant correlation between the qP and quantum yield of photosystem II \( (Y) \) \( (r^2 = 0.92) \) and qP and EPS II \( (r^2 = 72) \) and a significant correlation between the \( Y \) and EPS II \( (r^2 = 0.60) \) indicate improved efficiency of the photosynthetic apparatus through improved photochemical quenching.

**Figure 2** Correlation between Chl. a and Chl. b, Chl. a+b and \( x+c \), Chl. a and SPAD, Chl. b and SPAD, Chl. a+b and SPAD, Chl. a and photochemical quenching (qP), Chl. b and qP, qP and \( Y \), qP and EPS II and EPSII and \( Y \).

### 3.7. Growth parameters: plant height and biological yield

All Zn treatments except nano ZnO 50 mg L\(^{-1}\) significantly (\( p<0.01 \)) improved the plant height over Zn control. Biological yield with nano ZnO treatments was significantly (\( p<0.01 \)) higher over the ZnSO\(_4\) applied as soil and Zn control. Although all nano ZnO doses were similar for plant height and biological yield (Fig. 3), the nano ZnO 100 mg L\(^{-1}\) recorded the maximum 11\% and 6\% increase in plant height and biological yield, respectively. The impact of application methods for Zn treatments on plant height and biological yield and its interactions with Zn
treatments was non-significant. However, a 2% improvement (p>0.05) in plant height was noted with seed coating and foliar application over the soil drench and a 4% increase (p>0.05) in biological yield with foliar application over the seed coating and soil drench were recorded.

![Figure 3: Plant height and biological yield among Zn treatments applied through different methods](image)

**Figure 3:** Plant height and biological yield among Zn treatments applied through different methods

4. Discussion

4.1. Crop biochemical response to different levels of Nano ZnO

Zinc is associated with enzymes’ activation, structural and catalytic components of proteins. As a co-factor for normal development of pigment biosynthesis (Broadley et al., 2007), its deficiency disrupts the chlorophyll synthesis (Kösesakal & Ünal, 2009), which can only be restored by optimum Zn intake (Sadeghzadeh, 2013). Leaf chlorophyll estimation is a less time-
consuming and cost-effective practice used to predict the crop physiological condition under different environments (Yu et al., 2021). Improved chlorophyll with nano ZnO 100 mg L\(^{-1}\) compared to other nano ZnO doses and ZnSO\(_4\) suggested improved Zn availability to crop at this concentration. It could be presumed as an optimum concentration under the current soil conditions. Crop response to applied fertilizer varies according to soil conditions, type, source and amount of fertilizer (Kihara et al., 2016; Islam et al., 2018) and mode of application (Santos et al., 2020). However, methods of application in our study were non-significant. According to other researchers, nano ZnO at low doses may act as Zn fertilizer and provide Zn\(^{2+}\) for plant uptake (Liu et al., 2016). Although ZnSO\(_4\) improved the chlorophyll and photosynthetic efficiency compared to Zn control, low pH soil and failure to significantly alleviate soil Zn deficiency limited its absorption for crops Chl. a, b and carotenoids improvement (Subba et al., 2014). The decrease in the effectiveness of nano ZnO higher and lower than 100 mg L\(^{-1}\) to improve biochemical parameters might be due to Zn toxicity and deficiency stress, respectively (Subba et al., 2014). Higher than 100 mg L\(^{-1}\) nano ZnO could be biochemically suppressing the crop that could have resulted in reduced chlorophyll synthesis (Szopiński et al., 2019). Nanoparticle application at higher concentrations negatively affects the terrestrial and aquatic plants and animals (Rajput et al., 2018) and in acid soils, they are more toxic than alkaline soil (Shen et al., 2015).

4.2. Correlation among SPAD, chlorophyll and carotenoid pigments and its implications

The Chl. a/b ratio is a marker of pigments functionality and adoption of a photosynthetic system to light (Lichtenthaler et al., 1981). Chloroplast, which is responsible for photosynthesis, develops from Proplastids (Charuvi et al., 2012), however, in the absence of light, it may also
develop from other types of plastid, e.g., etioplast. When leaves are exposed to light, pale etioplasts are converted into green chloroplasts by plant cells to acquire photosynthetic competence (Armarego-Marriott et al., 2019). \(Chl.\ b\) is less rapidly accumulated than \(Chl.\ a\) during greening and the \(Chl.\ a/b\) ratio turns high, which reverses immediately after greening (Armarego-Marriott et al., 2019), and continues to reduce gradually until it reaches a final ratio of 4.3 reported for fully expanded leaves (Schöttler et al., 2017). Low \(Chl.\ a/b\) ratio means fully developed green leaves, while a high \(a/b\) ratio (4.0 - 10) implies greening of etiolated leaves (Hartmut et al., 2005). Amongst Zn treatments, significantly \(p<0.01\) lower \(Chl.\ a/b\) ratio for 100 mg L\(^{-1}\) nano ZnO (15% lower than the control) (Table 2) indicated less etiolating fully developed green leaves marking its improved performance for chlorophyll formation and functioning of the photosynthetic apparatus.

Higher SPAD values and total chlorophyll content for nano ZnO over the ZnSO\(_4\) (Table 3) indicate significant \(p<0.05\) improvement in the crop’s physiological conditions. In contrast, the results for nano ZnO 100 mg L\(^{-1}\) (Table 3) advocate this dose for further test under different environmental conditions. Increased Zn availability up to optimum level can improve chlorophyll content, crude proteins and Zn content (Samreen et al., 2017). Thus, a higher SPAD value with 100 mg L\(^{-1}\) nano ZnO indicates this concentration is more synchronous for higher nutrient accumulation than the other ZnO doses. Higher SPAD value is tantamount to healthier plants in certain plant species (Minolta, 2009). Reduction in Chlorophyll a, b and total Chlorophyll with higher nano ZnO doses might be because of stress developed (Noor et al., 2018). Besides higher \(Chl.\ a+b\) and \(x+c\), balanced and favorable performance of input is indicated by a relative increase in \(a+b/x+c\) ratio. A higher \(a+b/x+c\) ratio indicates more greenness of the plant with reduced chances of senescence and vice versa, while it usually ranges from 4.2
(less green) to 5 (more green) under sun-leaves and 5.5 (less green) to 7 (more green) in shade-leaves (Hartmut et al., 2005). In our results, a significantly (p<0.01) higher a+b/x+c ratio for nano ZnO 100 mg L$^{-1}$ (Table 2) confirms its biochemical advantage over the other doses. Plant growth, chlorophyll contents, crude proteins, and Zn contents were higher when Zn's availability was increased (Samreen et al., 2017). Decreased Chl. a+b/x+c ratio marks the senescence, stress and damage to the photosynthetic system in plants, faster break down of chlorophyll than carotenoids where values lower up to 3.5 exhibits more yellow than green leaves and values below 3 indicate leaves senescence (Hartmut et al., 2005). Also, higher Chl. a+b/x+c ratio with 150 mg L$^{-1}$ and 200 mg L$^{-1}$ nano ZnO applied through soil drench indicate soil matrix effect on Zn availability and uptake. Zinc deficiency in plants affects photosynthesis due to altered chloroplast pigments (Kösesakal & Ünal, 2009).

### 4.3. Antioxidant enzymatic activity: Proline

Proline accumulation in plants indicates disturbed physiological conditions because of biotic and abiotic stresses where its presence increases the stress tolerance of plants (Senthilkumar et al. 2021). Proline typical range in plant tissues (0.5-50 μmol g$^{-1}$) indicates increasing stress from lower towards higher value (Carillo & Gibon, 2011). Proline production is one of the mechanisms for acclimation to stress, higher proline content indicates stress exposure and vice versa (Nazar et al., 2015). Under heavy metals stress, proline acts as a metal chelator and protects enzymes from Zn and Cd toxicity by forming complexes with these metals (Sharma et al., 1998). The highest proline concentration in Zn control (5 times higher than the ZnSO$_4$) indicates other stress factors present in soil, such as heavy metals like Al (Table 1), Pb, Cu and Cd. Boosted antioxidant activities were reported in plants exposed to Pb (Hussain et al., 2021), Hg and Cd (Cruz et al., 2021). Amongst the nano ZnO doses, the highest proline concentration
was noted for 50 mg L\(^{-1}\) (higher by 162\% than the 150 mg L\(^{-1}\)) and beyond 150 mg L\(^{-1}\), it increased again. Although the overall content was very low for all treatments than the critical limit (5 μmol g\(^{-1}\)), further reduction with Zn application indicates nano ZnO or ZnSO\(_4\) induced relief for crops from already present stress factors in soil. These findings agreed with Hussain et al. (2021), showing reduced proline content with a higher nano ZnO dose (20 mg L\(^{-1}\)). Alia & Saradhi (1991) reported Zn as the weakest proline accumulator, as evidenced from our results from ZnSO\(_4\) treatments; however, Sun et al. (2020) reported enhanced proline content with enhanced nano ZnO. Proline concentration did not vary with application methods.

**4.4. The efficiency of photosystem II**

Reduction (p<0.05) in the efficiency of photosystem II (EPS II) above 150 mg L\(^{-1}\) and below 100 mg L\(^{-1}\) nano ZnO (Table 3) confirm the concentration-dependent impact on crop’s physiological parameters and tallies well with SPAD (Table 3) and extracted chlorophyll data (Table 2). Significant correlation between Chl. a and photochemical quenching (r\(^2\)=0.45) and Chl. b and photochemical quenching (r\(^2\)=0.57) (Fig. 2) confirmed that increased Chl. a and Chl. b concentration with 100 mg L\(^{-1}\) nano ZnO resulted in increased efficiency for PS II. The maximum efficiency of photosystems II noted with 100 mg L\(^{-1}\) nano ZnO is clear proof of better Zn availability within these limits which may have increased the number of reaction centers in photosystems.

Reduced efficiency of PSII suggests toxic effects of supplemental Zn from higher nano ZnO concentrations (200 mg L\(^{-1}\)) over and above the required limits, which is deemed to have reduced the flow of electrons from PSII to PSI (Santos et al., 2021). More importantly, the lower (50 mg L\(^{-1}\)) and moderate (100 and 150 mg L\(^{-1}\)) concentration treatments either improved or maintained the physiological functions of the crop suggesting their absorption and utilization by
plants better than ZnSO₄ (Prasad et al., 2012). The findings emphasized for an optimum ZnO
dose application to plants that could supplement Zn requirements for growth and development
and its structural and enzymatic activities (Subba et al., 2014; Singh et al., 2018).

4.5. Growth parameters: Plant height and biological yield

Significantly (p<0.01) improved plant height, and biological yield over the Zn control
through Zn treatments of the crop is supported by increased chlorophyll content (Table 2) and
photosynthetic efficiency (Table 3) which might have higher photosynthetic assimilates than Zn
deficient plants. The same holds good for further significant improvement in plant height and
biological yield with nano ZnO over the recommended ZnSO₄ dose. This could be due to much
higher Zn uptake from nano ZnO than the Zn²⁺ treated plants (Zhang et al., 2015). However, the
nano ZnO 100 mg L⁻¹ concentration seems to have maximum Zn availability with abiotic stress
mitigation on the plant, as is evident from significantly lower proline content (Table 3) and
therefore, secured the maximum growth. Sun et al. (2020) revealed that 100 mg L⁻¹ nano ZnO
improved plant resistance to stress conditions and supported higher plant growth compared to the
control. Increased photochemical quenching at 100 mg L⁻¹ nano ZnO level could have enhanced
the rate of photophosphorylation to meet ATP requirements for other physiological activities of
the plants which could have increased the ultimate crop growth up to an optimum concentration
(Singh et al., 2018; Del Buono et al., 2021). Furthermore, this study confirms the fundamental
role of a certain amount of Zn as a nutrient for optimum growth and produce (Sadeghzadeh,
2013), cell elongation, membrane structure, stability and environmental stress tolerance and
protection (Marreiro et al., 2017; Tufail et al., 2017; Bafaro et al., 2017; Tufail et al., 2017).
5. Conclusion

The enhancement in chlorophyll pigments, SPAD value, photochemical quenching and efficiency of photosystems, growth and yield elucidate that nanoforms of zinc oxide play a positive role in the biochemical health and functioning of maize up to a particular concentration. Crop’s positive response towards nano-ZnO was more pronounced at 100 mg L\(^{-1}\) than its lower and higher doses and the conventional ZnSO\(_4\) fertilizer recommended dose. Either seed coating, soil drench, or foliar spray can be used for nano ZnO application. Notwithstanding, foliar application had a non-significant edge over the other methods. Thus, nano ZnO up to 100 mg L\(^{-1}\) can be recommended to improve crop’s biochemical health and functioning, irrespective of the application modes, in Zn deficient acidic Spodosol soil for improving maize crop growth through Zn bio-fortification.

6. Acknowledgments

The authors acknowledge the Higher Education Commission (HEC) of Pakistan for supporting Dr. Ahmad’s visit to the University of Florida, United States as Post Doc Research Scholar and the University of Florida for conducting this research. The authors also thank Mr. Brian Cain for assistance with sample analysis.

7. Declaration of competing interest:

No financial or personal interests appear to exist amongst the authors that might influence the work reported in this paper.

8. Funding statement:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
9. References

Adhikari, T., Kundu, S., Biswas, A. K., Tarafdar, J. C., & Subba Rao, A. (2015). Characterization of Zinc Oxide Nano Particles and Their Effect on Growth of Maize (Zea mays L.) Plant. *Journal of Plant Nutrition*, 38(10), 1505–1515. https://doi.org/10.1080/01904167.2014.992536

Ahmed, N., Habib, U., Younis, U., Irshad, I., Danish, S., Rahi, A. A., & Munir, T. M. (2020). Growth, chlorophyll content and productivity responses of maize to magnesium sulphate application in calcareous soil. *Open Agriculture*, 5(1), 792–800. https://doi.org/10.1515/opag-2020-0023

Ahmad, N. (2004). Fertilizer use by crop in Pakistan. Retrieved from: https://www.fao.org/3/y5460e/y5460e00.htm#contents

Alia, & Saradhi, P. P. (1991). Proline Accumulation Under Heavy Metal Stress. *Journal of Plant Physiology*, 138(5), 554–558. https://doi.org/10.1016/S0176-1617(11)80240-3

Arafat, Y., Shafi, M., Khan, M. A., Adnan, M., Basir, A., Arshad, M., ... & Shah, J. A. (2016). Yield response of wheat cultivars to zinc application rates and methods. *Pure and Applied Biology*, 5(4), 1.

Armarego-Marriott, T., Kowalewska, Ł., Burgos, A., Fischer, A., Thiele, W., Erban, A., Strand, D., Kahlau, S., Hertle, A., Kopka, J., Walther, D., Reich, Z., Schöttler, M. A., & Bock, R. (2019). Highly resolved systems biology to dissect the etioplast-to-chloroplast transition in tobacco leaves. *Plant Physiology*, 180(1), 654–681. https://doi.org/10.1104/pp.18.01432

Bafaro, E., Liu, Y., Xu, Y., & Dempski, R. E. (2017). The emerging role of zinc transporters in cellular homeostasis and cancer. *Signal Transduction and Targeted Therapy*, 2(February), 1–12. https://doi.org/10.1038/sigtrans.2017.29

BATES, L. S. (1973). *SHORT COMMUNICATION Rapid determination of free proline for water stress studies*. 207, 205–207.

Bhatt, R., Hossain, A., & Sharma, P. (2020). Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: A review. *Open Agriculture*, 5(1), 176–186. https://doi.org/10.1515/opag-2020-0018

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., Lux, A. (2007). Zinc in plants. *New Phytol.*, 173, 677–702.

Cakmak, I., & Kutman, U. B. (2018). Agronomic biofortification of cereals with zinc: a review. *European Journal of Soil Science*, 69(1), 172–180. https://doi.org/10.1111/ejss.12437

Carillo, P., & Gibon, Y. (2011). *PROTOCOL: Extraction and determination of Proline*. -. https://www.researchgate.net/publication/211353600

Charuvi, D., Kiss, V., Nevo, R., Shimoni, E., Adam, Z., & Reich, Z. (2012). Gain and loss of photosynthetic membranes during plastid differentiation in the shoot apex of arabidopsis. *Plant Cell*, 24(3), 1143–1157. https://doi.org/10.1105/tpc.111.094458

Chen, W., Yang, X., He, Z., Feng, Y., & Hu, F. (2008). Differential changes in photosynthetic
capacity, 77 K chlorophyll fluorescence and chloroplast ultrastructure between Zn-efficient and Zn-inefficient rice genotypes (Oryza sativa) under low zinc stress. *Physiologia Plantarum*, 132(1), 89–101. https://doi.org/10.1111/j.1399-3054.2007.00992.x

Cruz, Y., Villar, S., Gutiérrez, K., Montoya-Ruiz, C., Gallego, J. L., Delgado, M. del P., & Saldarriaga, J. F. (2021). Gene expression and morphological responses of Lolium perenne L. exposed to cadmium (Cd2+) and mercury (Hg2+). *Scientific Reports*, 11(1), 1–11. https://doi.org/10.1038/s41598-021-90826-y

Del Buono, D., Di Michele, A., Costantino, F., Trevisan, M., & Lucini, L. (2021). Biogenic zno nanoparticles synthesized using a novel plant extract: Application to enhance physiological and biochemical traits in maize. *Nanomaterials*, 11(5). https://doi.org/10.3390/nano11051270

Elemike, E. E., Uzoh, I. M., Onwudiwe, D. C., & Babalola, O. O. (2019). The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. *Applied Sciences (Switzerland)*, 9(3), 1–32. https://doi.org/10.3390/app9030499

García-Gómez, C., García-Gutiérrez, S., Obrador, A., & Fernández, M. D. (2020). Study of Zn availability, uptake, and effects on earthworms of zinc oxide nanoparticle versus bulk applied to two agricultural soils: Acidic and calcareous. *Chemosphere*, 239. https://doi.org/10.1016/j.chemosphere.2019.124814

Hayyawi, N. J. H., Al-Issawi, M. H., Alrajhi, A. A., Al-Shmgani, H., & Rihan, H. (2020). Molybdenum Induces Growth, Yield, and Defence System Mechanisms of the Mung Bean (Vigna radiata L.) under Water Stress Conditions. *International Journal of Agronomy*, 2020. https://doi.org/10.1155/2020/8887329

Hossain, A., Mottaleb, K. A., Farhad, M., & Deb Barma, N. C. (2019). Mitigating the twin problems of malnutrition and wheat blast by one wheat variety, “BARI Gom 33”, in Bangladesh. *Acta Agrobotanica*, 72(2). https://doi.org/10.5586/aa.1775

Hussain, F., Hadi, F., & Rongliang, Q. (2021). Effects of zinc oxide nanoparticles on antioxidants, chlorophyll contents, and proline in Persicaria hydropiper L. and its potential for Pb phytoremediation. *Environmental Science and Pollution Research*, 28(26), 34697–34713. https://doi.org/10.1007/s11356-021-13132-0

Islam, S., Hamid, F. S., Shah, B. H., Zaman, Q., Khan, N., Ahmad, F., & Aftab, S. (2018). Response of Organic & Inorganic Fertilizers to the Growth, Yield and Soil Nutrient Status in Tomato (Lycopersicon esculentum). *Open Academic Journal of Advanced Science and Technology*, 2(1), 1–4. https://doi.org/10.33094/5.2017.2018.21.1.4

Janet C King, Kenneth H Brown, Rosalind S Gibson, Nancy F Krebs, N. M. L., & Jonathan H Siekmann, and D. J. R. (2016). Biomarkers of Nutrition for Development (BOND)-Zinc review. *Journal of Nutrition*, 146(4), 858S-885S. https://doi.org/10.3945/jn.115.220079.

Kihara, J., Nziguheba, G., Zingore, S., Coulibaly, A., Esilaba, A., Kabambe, V., Njoroje, S., Palm, C., & Huising, J. (2016). Understanding variability in crop response to fertilizer and amendments in sub-Saharan Africa. *Agriculture, Ecosystems and Environment*, 229, 1–12. https://doi.org/10.1016/j.agee.2016.05.012
D. A., Campos, L. F. C., Ribon, A. A., Lopes, T. A., & Boas, R. L. V. (2020). Chemical characteristics of soil after application of tannery sludge as fertilizer in the sugarcane crop. *Australian Journal of Crop Science, 14*(4), 641–648. https://doi.org/10.21475/ajcs.20.14.04.p2234

Santos, E. F., Pongrac, P., Reis, A. R., Rabêlo, F. H. S., Azevedo, R. A., White, P. J., & Lavres, J. (2021). Unravelling homeostasis effects of phosphorus and zinc nutrition by leaf photochemistry and metabolic adjustment in cotton plants. *Scientific Reports, 11*(1), 1–14. https://doi.org/10.1038/s41598-021-93396-1

Schöttler, M. A., Thiele, W., Belkius, K., Bergner, S. V., Flügel, C., Wittenberg, G., Agrawal, S., Stegemann, S., Ruf, S., & Bock, R. (2017). The plastid-encoded Psal subunit stabilizes photosystem i during leaf senescence in tobacco. *Journal of Experimental Botany, 68*(5), 1137–1155. https://doi.org/10.1093/jxb/erx009

Senthilkumar, M., Amaresan N., S. A. (2021). *Plant-Microbe Interactions: Laboratory techniques*.

Sharma, S. S., Schat, H., & Vooijs, R. (1998). In vitro alleviation of heavy metal-induced enzyme inhibition by proline. *Phytochemistry, 49*(6), 1531–1535. https://doi.org/10.1016/S0031-9422(98)00282-9

Shen, Z., Chen, Z., Hou, Z., Li, T., & Lu, X. (2015). Ecotoxicological effect of zinc oxide nanoparticles on soil microorganisms. *Frontiers of Environmental Science and Engineering, 9*(5), 912–918. https://doi.org/10.1007/s11783-015-0789-7

Singh, A., Prasad, S. M., & Singh, S. (2018). Impact of nano ZnO on metabolic attributes and fluorescence kinetics of rice seedlings. *Environmental Nanotechnology, Monitoring and Management, 9*(July 2017), 42–49. https://doi.org/10.1007/s11783-015-0754-2

Subba, P., Mukhopadhyay, M., Mahato, S. K., Bhatia, K. D., Mondal, T. K., & Ghosh, S. K. (2014). Zinc stress induces physiological, ultra-structural and biochemical changes in mandarin orange (Citrus reticulata Blanco) seedlings. *Physiology and Molecular Biology of Plants, 20*(4), 461–473. https://doi.org/10.1007/s12298-014-0254-2

Sun, L., Song, F., Guo, J., Zhu, X., Liu, S., Liu, F., & Li, X. (2020). Nano-ZnO-induced drought tolerance is associated with melanin synthesis and metabolism in maize. *International Journal of Molecular Sciences, 21*(3), 1–18. https://doi.org/10.3390/ijms21030782

Szopiński, M., Sitko, K., Gieroń, Ż., Rusinowski, S., Corso, M., Hermans, C., Verbruggen, N., & Małkowski, E. (2019). Toxic effects of Cd and Zn on the photosynthetic apparatus of the Arabidopsis halleri and Arabidopsis arenosa pseudo-metallophytes. *Frontiers in Plant Science, 10*(June), 1–13. https://doi.org/10.3389/fpls.2019.00748

Taheri, M., Qarache, H. A., Qarache, A. A., & Yoosefi, M. (2015). The Effects of Zinc-Oxide Nanoparticles on Growth Parameters of Corn (SC704). *STEM Fellowship Journal, 1*(2), 17–20. https://doi.org/10.17975/sfj-2015-011

Tufail, A., Li, H., Naeem, A., & Li, T. X. (2017). Leaf cell membrane stability-based mechanisms of zinc nutrition in mitigating salinity stress in rice. In *Plant Biology* (Vol. 20, Issue 2). https://doi.org/10.1111/plb.12665
Wang, H., Liu, R. L., & Jin, J. Y. (2009). Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. *Biologia Plantarum, 53*(1), 191–194. https://doi.org/10.1007/s10535-009-0033-z

Yu, S., Zhang, N., Kaiser, E., Li, G., An, D., Sun, Q., Chen, W., Liu, W., & Luo, W. (2021). Integrating chlorophyll fluorescence parameters into a crop model improves growth prediction under severe drought. *Agricultural and Forest Meteorology, 303*(October 2020), 108367. https://doi.org/10.1016/j.agrformet.2021.108367

Zhang, R., Zhang, H., Tu, C., Hu, X., Li, L., Luo, Y., & Christie, P. (2015). Phytotoxicity of ZnO nanoparticles and the released Zn(II) ion to corn (Zea mays L.) and cucumber (Cucumis sativus L.) during germination. *Environmental Science and Pollution Research, 22*(14), 11109–11117. https://doi.org/10.1007/s11356-015-4325-x