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INFLUENCE OF PHYTO-MEDIATED ZINC OXIDE NANOPARTICLES ON GROWTH OF (*ZEA MAYS* L.)

IQRA NASEER[1], SUMERA JAVAD[1*], ANIS ALI SHAH[2], AMINA TARIQ[1] AND ASMA AHMAD[1]

[1]Dept. of Botany, Lahore College for Women University, Lahore, Pakistan
[2]Department of Botany, University of Education, Lahore, Pakistan
**Corresponding author's email: Zif_4@yahoo.com*

Abstract

The agricultural sector is being affected by environmental extremes nowadays and causing issues to food security globally. To resolve these issues, researchers are taking much interest in nanofertilizers and related products nowadays. Maize is known to be an important cereal crop after wheat and rice worldwide. Lack of micronutrients especially zinc in cereal crops is a problem in today's agriculture. The use of plant-based zinc nanoparticles in the form of nano-fertilizers can reduce this problem. The current study mainly focuses on the green synthesis of zinc nanoparticles and their effects on *Zea mays* growth. The formation of Zinc oxide nanoparticles was confirmed by UV-Vis spectroscopy, Fourier Transform Infrared spectroscopy, Scanning Electron Microscopy, and particle size analysis. The effect of concentration of synthesized zinc oxide nanoparticles (0.2-1000 mgL^{-1}) was studied on germination, seedling parameters (root and shoot length, fresh, and dry seedling weight), and biochemical parameters (Chlorophyll, sugar, proline, phenolic, and protein content) of *Z. mays* seeds under lab conditions. The pot experiment was then conducted with the selected concentration of zinc oxide nanoparticles from the petriplate experiment and zinc sulfate salt as a control. Growth and yield parameters were studied for grown maize plants. Results indicated that spherical-shaped zinc oxide nanoparticles with an average size of 68-89nm were obtained, where phenolics were the main chemicals present at the surface of NPs. Zinc oxide nanoparticles improved seed germination significantly (92%) as compared to the control treatment (68%). Root length (26.6cm±0.9) and shoot length (12.1cm ±1) were also enhanced in pots of nanoparticle treatment as compared to control i.e., 23.4±0.9 and 7±1 respectively. Zinc oxide nanoparticles also enhanced sugar content (455ug/g±9.08), protein content (17.1µg/g±0.2), proline content (343.71µg/g±1), and chlorophyll content (682.96ug/g±44) of maize seedlings as compared to control 155, 10.3, 206, 252ug/g of plant material respectively. Results of the pot experiment showed that Zinc oxide nanoparticles treatment significantly increased the germination percentage (25%), shoot length (50%), root length (22.2%), leaf area (34.3%), cobs per plant (33%), chlorophyll content (18.4%), Sugar content (2.5%), Phenolic content (3.4%), proline content (36.3), MDA content (20%), starch content (33%), and zinc content (110%) significantly as compared to control. Hence our results indicated that zinc oxide nanoparticles can be utilized as nanofertilizers to induce early germination in plants and to enhance seedling growth and biochemical processes for *Zea mays*.

Key words: Corn, Zinc, Germination, Seedlings, Zinc oxide nanoparticles.

Introduction

Today world is facing a traumatic condition in agriculture due to a drastic gap in demand and production. To fulfill the enhanced food demand of the increasing population, more production is needed. But seems difficult because of the growing population, decreasing fertile land, and increasing pollution (Usman *et al*., 2020). Furthermore, multiple crop production causes a reduction or partial loss of soil fertility. In this situation of reduced fertility of the soil, fertilizers play their role because plants require macro and micronutrients to grow well and yield more (Yousaf *et al*., 2017; Kalaji *et al*., 2014). Therefore, crop yield is increased by adding nutrients to soils in the form of fertilizers which increases the cost of crop production. Furthermore, the use of excessive chemical fertilizer also adds to environmental pollution and poor land management, causing soil degradation in all aspects (Sher *et al*., 2022; Brevik & Burgess, 2014). Conventional fertilizers are not easily accessible to plants because of certain environmental factors which include leaching, waterlogging, degradation, and many more leading to excessive use of fertilizers. To address this problem, more efficient and effective methods of supplying nutrients to plants, are required. A sustainable agriculture system demands precise and smart delivery of nutrients to plants with the least or minimized side effects on the environment and upgrading the soil status (Abbasifar *et al*., 2020; Gao *et al*., 2020). Nanotechnology offers the solution to this problem in a variety of ways, although sometimes controversial, as toxic effects of nanofertilizers are reported by several researchers (Ranjan *et al*., 2021; Kamran *et al*., 2020; Paramo *et al*., 2020) where many researchers also report positive effects of nano fertilizer on plant growth (Kashyap *et al*., 2023; Neto *et al*., 2020; Song & Kim, 2020; Sun *et al*., 2020). Therefore, it requires more research data on various plants in different climates and soil types with different types, sizes, and concentrations of nanoparticles (Rastogi *et al*., 2017). Micronutrients in nano forms and nanofertilizers can be applied in agricultural fields and their doses can be optimized for a particular type of crop in specific soil and climate. The controlled and smart delivery of nutrients improves plant growth and its metabolic processes, and these nutrients are site-specific and of low cost as well (DeRosa *et al*., 2010; Nair *et al*., 2010). Nanoparticles' physiochemical properties enhance the metabolic rates of plants (Giraldo *et al*., 2014). Most of the cereal farms in the world are deprived of important micro and macro nutrients due to multiple crop production and many other reasons.

Cultivation on such soils leads to the loss of resources in all aspects. In this situation, poor mineral soils can be supplied with nanoparticles, as nanofertilizers, to stimulate plant growth (Mahapatra *et al*., 2021). The use of nanomaterials in agriculture is increasing nowadays. The nanotechnology-based approach is hoped to regulate the nutrients in the soil, finally going towards getting a series of benefits from agriculture (Acharya *et al*., 2018). Nanoparticles' size range is between 1 to 100 nm and they may be 1D, 2D, or 3D nanoparticles. Matter shows different and innovative characteristics at the nanoscale level rather than the macroscopic level. The change in properties is due to the reduced size and increased surface area causing a change in interactions between molecules (Qureshi *et al*., 2018). There are many earlier research reports available that described enhancement in seed germination and plant growth with the application of silver, zinc, titanium, metal sulfide, iron oxide, and silicon nanoparticles on wheat and corn (Irshad *et al*., 2021; Kalal & Jajoo, 2021; Afsheen *et al*., 2020; Tovar *et al*., 2020; Khan *et al*., 2019; Taheri *et al*., 2016; Yasmeen *et al*., 2015). As zinc nanoparticles are eco-friendly, non-toxic, and have compatibility with living cells, therefore, these can be preferred and utilized in the agricultural industry safely (Wadhwani & Jain, 2015). Zinc is an essential micronutrient for plants and animals. Plants face insufficient chlorophyll and underdeveloped growth under zinc scarcity because it is part of carbohydrate, protein, and chlorophyll synthesis. Zinc enhances the structural and physiological capability of the photosynthetic system in plants (Abbasifar *et al*., 2020). Many enzymatic reactions occur in plants in the presence of zinc (Alloway, 2008) because it is part of various enzymatic parts in plants including lyases, hydrolases, oxidoreductases, ligases, and hydrolases (Auld, 2001). This micronutrient also plays an important physiological role in maintaining the functional and structural integrity of membranes, growth regulation, and detoxification of reactive oxygen species (Khanam *et al*., 2018). In microform, zinc sulphate, and zinc chloride are available as a zinc supplement to the soil but these are not performing up to the mark due to their lesser solubility in soil solution (Latef *et al*., 2016). Nanoparticles can be prepared by biological, chemical, or physical methods whereas biological methods include the synthesis of nanoparticles from plants or microbes. Extensive work is going on green synthesis of nanoparticles including Zinc oxide nanoparticles as it is considered the cost-effective and environmentally safe method for the synthesis of nanoparticles (Jadoun *et al*., 2021).

Maize is globally the third most important cereal crop after wheat and rice with an estimated production of 780 metric tons/year. It is a source of food as well as used for diesel production. It is proposed to be part of environment-friendly lithium ion, redox flow, and solid-state batteries (Jin *et al*., 2021). However, it is a highly sensitive crop and prone to zinc deficiency. Currently, due to many factors, maize yield potential and nutrient content are reducing. It is also hugely exported worldwide due to its great economic value including corn oil, popcorn, starch, corn flour, poultry feed, dairy feed, and many other products. With the population outburst, there is the threat of food shortage in coming years so it is necessary of hour to use nano agronomy on an industrial scale. Germination is one of the most important steps for the establishment of plants in agriculture and is fundamental for crop quality (Khalaki *et al*., 2020; Acharya *et al*., 2018; Savassa *et al*., 2018). The rapid development of seedlings ensures fast expansion of the leaves and elongation of the roots, which favor the uptake of nutrients, their translocation through the transpiration flow, and biomass production (Chandrasekaran *et al*., 2020; Acharya *et al*., 2018; Mahakham *et al*., 2016). Young seedlings are one of the most vulnerable stages of the plant life cycle and these are exposed to many environmental stress conditions or pathogens as a result of slow germination, resulting in decreases in vigor and crop productivity. This causes great economic losses for farmers (Acharya *et al*., 2019). Seed germination is an important factor for determining the density of the final plant if planted seeds germinate completely and vigorously (Baal *et al*., 1990). ZnO NPs can be used in agriculture to improve crop yield. Intensive research work on the use of nanofertilizers in various soils for different plants is a requirement of the present day. Crop improvement for food security is the main problem addressed in the present study. Therefore, the main aim of the present study was to examine the effect of green synthesized ZnO NPs to increase overall plant growth and overcome nutrient deficiency. It was hypothesized that Zinc oxide nanoparticles as biofertilizers can help the plant overcome growth and nutrient deficiencies such as zinc in maize plants.

It's a first-time report of the effect of zinc oxide nanoparticles on the "OP" variety of Pakistan grown locally. This work included the study of zinc content in grains when treated with nanoparticles. This can enhance the food fortification of minerals.

Material and Methods

The plant material required was pea and corn. Pea (*Pisum sativum* Linn.) was acquired from the indigenous market while the "OP white" variety of *Zea mays* seeds was very generously provided by the NARC (National Agricultural Research Centre), Islamabad, Pakistan.

Green synthesis of ZnO nanoparticles: Plant material i.e., pea (*Pisum sativum* Linn.) was weighed (10 g) and the extract was prepared in distilled water (100 mL). The mixture was heated in MDS-6G Microwave extractor for 3 minutes at 600 W and filtrate was used for the synthesis of nanoparticles with a 0.4 M solution of Zinc sulfate ($ZnSO_4$). The solution was mixed in the ratio of 3:1 (30 mL of plant extract + 10 mL of $ZnSO_4$) dropwise. After 6 hours, the mixture was centrifuged for 10 minutes at 13000 rpm. The supernatant was discarded and pallets were stored after thorough washing. This process was repeated to get the required amount of nanoparticles.

Characterization of ZnO NPs: UV-visible spectroscopy analysis was done to confirm the formation of nanoparticles. It was practiced at regular intervals of mixing of plant extract and salt solution by defining the wavelength range from 200-700nm. The analysis was done by using a dual-beam UV-visible spectrophotometer (BMS, UV-2600). Maximum absorption peaks were checked and compared with reported literature (Sangeetha *et al*., 2012).

FTIR technique was used to observe various functional groups of plant extract involved in the synthesis of ZnO NPs within the wavelength range of 500cm^{-1} to 4000cm^{-1}. s. FTIR spectrophotometer (IRT racer-100) was used for this study and samples were prepared with pallets observed in section 2.1 and potassium bromide powder (Xiong *et al.*, 2006). The size of nanoparticles was observed by a particle size analyzer (BT 90 Nano laser). For this purpose suspension of the pallet was made and this was then diluted in the ratio of 1:50 µL and sonicated for 10 minutes before analysis (Akbari *et al.*, 2011).

This technique was used to identify the surface morphology/shape of ZnONPs. For this purpose, a smear of NPs was made on slides. Slides were dried for a while and then coated with platinum to make them conductive. Prepared slides were subjected to SEM (JSM- 6480 at 20 kV) for characterization. SEM graphs were studied for the estimated size and shape of synthesized ZnO NPs.

Petriplate experiment and treatments: For one hour, petri plates (diameter 5.5 inches) were sterilized in a drying oven at 120ºC. Corn seeds were sterilized with 10% sodium hypochlorite solution for 10 minutes and were washed thoroughly with sterilized water multiple times to get rid of extra sodium hypochlorite.

Later, the stock solution of characterized ZnONPs (1000mg/L) was made in distilled water as 1g/L, and a range of concentrations were prepared i.e., 0.2, 0.5, 0.8, 1, 20, 60, 100, 200, 400, 600, 700, 800, 900, 1000mg/1000mL of zinc oxide nanoparticles whereas control treatment contained only distilled water.

For each treatment, seeds were dipped in the relative strength of nanoparticles e.g., for the control treatment seeds were dipped in double distilled water and for the experimental, seeds were dipped in relative nanoparticle suspensions for 2 hours. Then 5 mL of each test solution was poured onto the filter paper of the respective petri plate (size 14 mm) with proper labeling and 25 seeds/petri plate were transferred with a 1cm distance between them. Nanoparticle solution was added to each petri dish at a regular interval of 3days. All treatments were applied in three replicates. Plates with seeds were placed in proper light under lab conditions.

Effect of ZnO NPs on the morphological and biochemical parameters of Z. mays seedlings: Petri plates were observed on the daily basis to record their germination data. The germination percentage for all treatments was recorded on the 4th day of the experiment. After 2 weeks seedlings were analyzed for other morphological parameters i.e., shoot and root length, fresh and dry seedling weight.

Furthermore, seedlings were also biochemically analyzed for chlorophyll (Arnon, 1949), protein (Lowry *et al.*, 1951), proline (Bates *et al.*, 1973), sugar (DuBois *et al.*, 1956), and MDA content (Heath & Packer, 1968). Based on these morphological and biochemical parameters of the Petri plate experiment, a dose of ZnONps was selected for the next pot experiment.

Pot experiment: A pot experiment was also carried out, in which two treatments were used i.e., one experimental (ZnONPs 1000 mg/L selected from petriplate experiment) and the second one is a control macro zinc in the form of zinc sulfate salt solution (1000 mg/L). Clay pots (14 inches in diameter) with 6Kg soil per pot were used. Seeds were sown in February and the total experiment was continued for 90 days. Treatments were given as soil drench after every 15 days. Morphological and biochemical parameters were studied for both treatments. Plants were studied for germination percentage, days to germinate, Shoot length, Leaf area, cobs per plant, chlorophyll content (Arnon, 1949), sugar content (DuBois *et al.*, 1956), phenolic content (Rebey *et al*., 2012), proline content (Bates *et al*., 1973), MDA content (Heath and Packer, 1968), starch content (Smith and Zeeman, 2006) and zinc content of grains (Ozturk *et al*., 2006).

Statistics: Data thus generated was in three replicates and analyzed with the help of SPSS statistical software (IBM Software Company, USA) through one-way ANOVA. Later on, to check the significance at the 5% level Duncan’s multiple range test was applied.

Results

Synthesis and characterization of ZnONPs from pea plant extract: In our investigation, ZnONPs were synthesized using plant extract of the pea plant. FTIR, UV-Vis spectroscopy, particle size analysis, and SEM analysis were used as characterization techniques to confirm the synthesis of NPs. Salt reagent when mixed with plant extract, gave rise to zinc oxide nanoparticles, which was indicated by the color change of the salt solution and confirmed by the absorption spectrum of UV spectrophotometry within a range of 200nm to 700nm. Maximum absorption wavelength $\lambda_{(max)}$ was observed as 350nm ±1.1 (Fig. 1a). While particle size analysis showed the formation of nanoparticles of an average size of 79.8 nm (Fig. 1b) with almost uniform distribution. A small peak is also visible at an average size of 240nm. Fourier transform infrared spectroscopy was utilized to study the functional groups of phytochemicals mainly involved in the reduction and capping of the nanoparticles produced from plant extract.

FTIR absorption spectrum exhibited significant absorption bands at 3752, 1700, 1653, 1558, 1457, 1233, and 1075 cm^{-1} which parallels the hydroxyl (OH), carbonyl (C=O), alkene (C=C), nitro-oxide (NO), C-H, S=O, and C-O respectively (Fig 1c). A major peak was observed in the range of 3629 to 3752cm^{-1} which is mainly related to the hydroxyl (OH) group of alcohols and phenols. Results of particle size analysis showed that ZnONPs formed by water extract of pea seeds were having mono disperse distribution with an average size of 68nm (Fig. 1c) while small peaks were present at the 200-300 nm range as well. SEM analysis showed small particles of almost equal size and round shape (Fig. 1d).

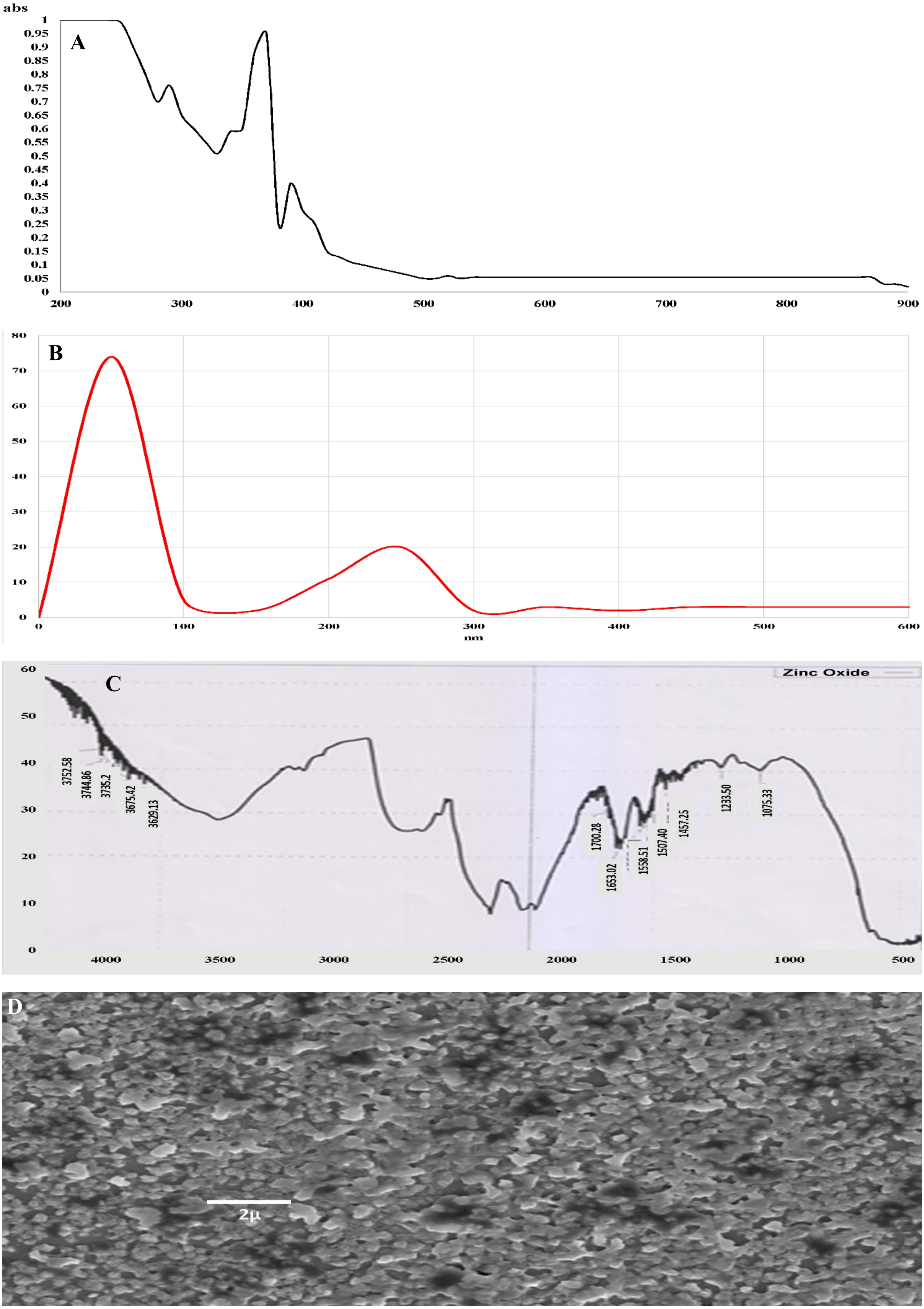

Fig. 1. **a)** UV Vis spectrum of ZnO NPs **b)** Particle size analysis of ZnO NPs **c)** FTIR spectrum of ZnO NPs **d)** SEM analysis of ZnO NPs.

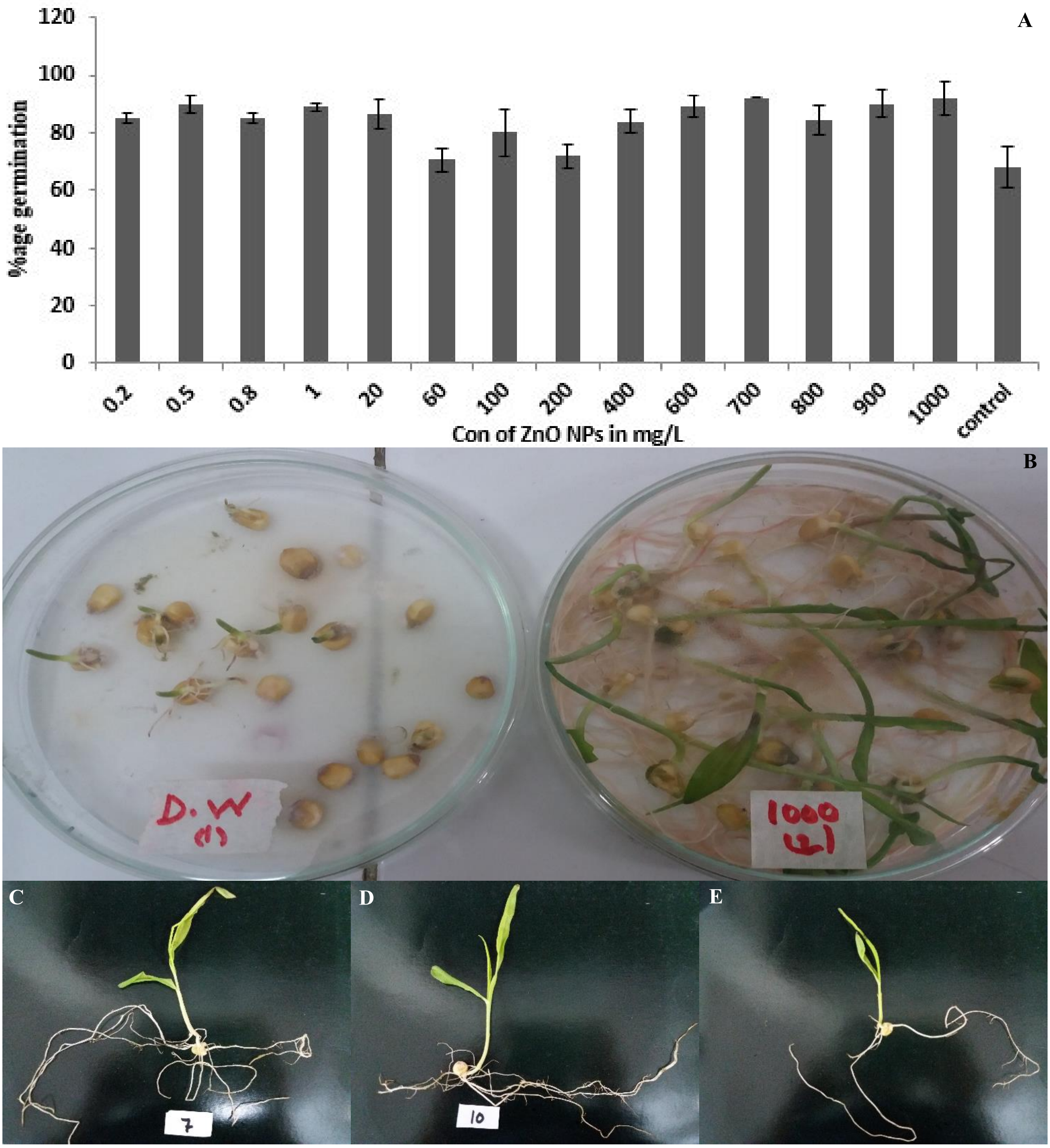

Fig 2. **a)** Effect of ZnO NPs on seed germination of *Z.mays*. **b)** 3 days old Seedlings treated with distilled water and ZnO NPs colloidal solution (1000mg/L) **c)** 15 days old seedlings treated with 700mg/L of ZnONPs. **d)** 15 days old seedling treated with 1000mg/L of ZnO NPs. **e)** 15 days old seedling treated with distilled water.

Lab experiment to optimize ZnONPs dose: Keeping in view the significance of fertilizers for nutrient-deficient plants preliminary research work was done in the lab and effects of ZnO NPs on germination and biochemical processes were observed in *Zea mays* seedlings. Once confirmed the synthesis of ZnO NPs in the present investigation, various concentrations were made out of them and the effect of these green zinc nanoparticles was studied on the germination of corn seedlings. Results showed that ZnO NPs improved the germination percentage and development of maize seedlings. Germination parameters of maize seedlings were regularly recorded. All the treatments showed a significant germination percentage (Fig. 2a). It has been observed that nanoparticles-treated seeds germinated earlier (2 to 3 days of seeds sowing) in comparison to control (5 to 6 days on average to germinate). Maximum germination (92%) was recorded in seeds treated with 700 mg/L to 1000 mg/L concentration of Zn ONPs. On the other hand, the control group had a significantly lesser germination percentage (68%) on the 4th day. Overall results showed that ZnO NPs enhanced the germination rate of the *Z. mays* seeds (Fig. 2a). ZnO NPs also positively affected the number of roots in maize seedlings as compared to control as depicted in (Figs. 2 b-e). After 15 days of seed germination root and shoot length of *Z. mays* seedlings were measured. Enhanced root length (27.57cm ± 2.3) and shoot length (26.68 cm ± 2.7) were observed in seedlings under 700 mg/L and 1000 mg/L doses of nanoparticles (Fig. 3a). Fresh and dry seedling weights were also improved by treating with nanoparticles. Maximum fresh seedling weight (1 g ± 0.07) was found in seedlings treated with 60 mg/L of ZnO NPs as compared to control (0.7 g ± 0.05). Seedlings were allowed to dry under lab conditions after 15 days. After 1-week, the dry weight of the seedlings was measured. Maximum seedling dry weight (0.23 g ± 0) was observed in seedlings treated with 1000 mg/L of ZnO NPs as compared to control (0.16 g ± 0.07) (Fig. 3 b).

Nanoparticles play a very significant role in improving the physiological and biochemical parameters of the plant. In the present study, the chlorophyll content of maize seedlings was enhanced with the increase in nanoparticle concentration (1000 mg/L). Chlorophyll a was found to be highest (661.297 µg/g ± 239) in 800mg/L of NPs-treated leaves. In the same way, Chlorophyll b (926.645 µg/g ± 211) was recorded highest in 800 mg/L of ZnO NPs treated leaves while carotenoids were found highest (810.125 µg/g ± 14.7) in 1000 mg/L of ZnO NPs treated leaves (Fig. 3 c).

However maximum sugar content (455 µg/g of plant material only) of *Z. mays* leaves was observed at the nanoparticle's dose of 400 mg/L and then it started to decrease. At higher doses of ZnO NPs (700 mg/L) sugar content was recorded lowest (80 µg/g) in leaves of *Z. mays* seedlings. It was examined that at lower doses of nanoparticles (up to 400 mg/L) sugar content was significantly improved meanwhile higher doses of ZnO NPs reduced the amount of sugar content in leaves (Fig. 4a).

Nanoparticles also significantly increased protein content in *Z. mays* seedlings. Maximum protein content (17.1 µg/g) was estimated in 900mg/L of ZnO NPs treated leaves as compared to control (10.3 ug/g) (Fig. 4b). Proline content was also improved. Maximum proline content (343 µg/g) was observed in seedlings treated with a higher concentration of ZnO NPs (900 mg/L) as compared to the control which was (206 µg/g) (Fig. 4c).

MDA content was also analyzed for maize seedlings in the present study and it varied from 2 to 3.4 %. The highest MDA content in ZnONPs treated plants was 3.5% which was a 3% increase in quantity from the control (3.4%), while it was lower in some ZNONPs treatments (Fig. 4d).

Pot experiment to compare ZnONPs and ionic zinc for *Zea mays* L. growth: Furthermore, conc. of ZnONPs with maximum and optimized responses (1000mg/L) was selected for pot experiment with control treatment as comparison i.e., 1000mg/L of $ZnSO_4$. Results showed that treatment of maize plants with ZnONPs increased germination percentage by 19% (Fig. 5a), shoot length by 55% (Fig. 5b), root length by 37.5% (Fig. 5b), leaf area by 33% (Fig. 5c) and cobs per plant by 33% (Fig. 5d) from control treatment of zinc salt of macro-particles. There was a significant increase in all the studied growth parameters of maize plants when treated with 1000 mg/L of ZnONPs.

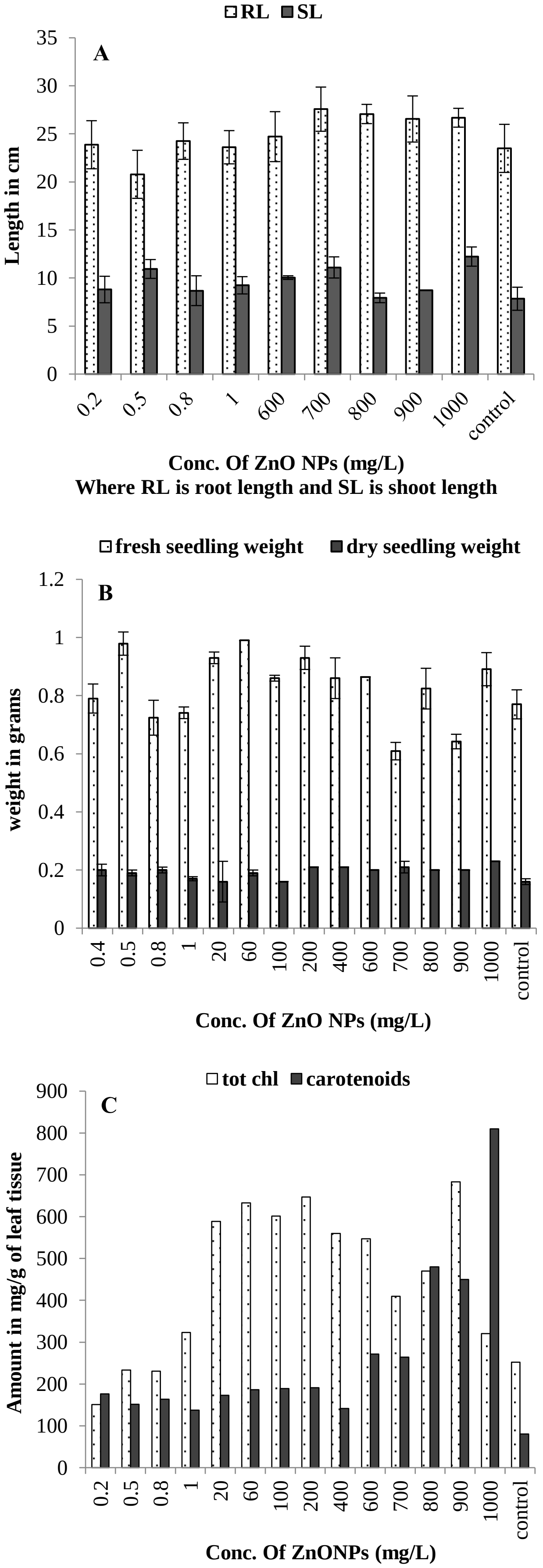

Fig. 3. **a)** Effect of ZnO NPs on root and shoot length of *Z. mays*. **b)** Effect of ZnO NPs on fresh and dry seedling weight of *Z. mays*. **c)** Effect of ZnO NPs on chlorophyll content of *Z. mays* seedling leaves.

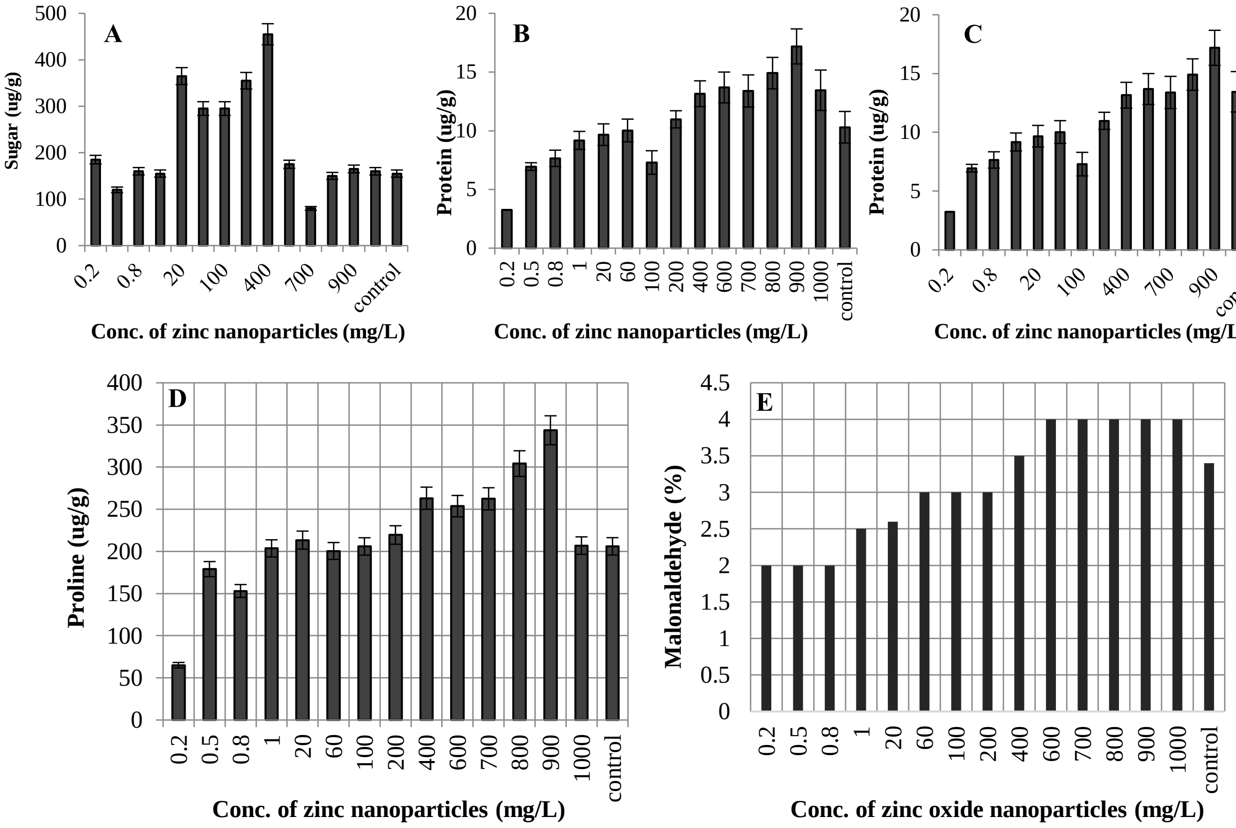

Fig. 4. Effect of ZnO NPs on a) sugar content b) protein content c) proline content d) MDA content of *Z. mays* leaves.

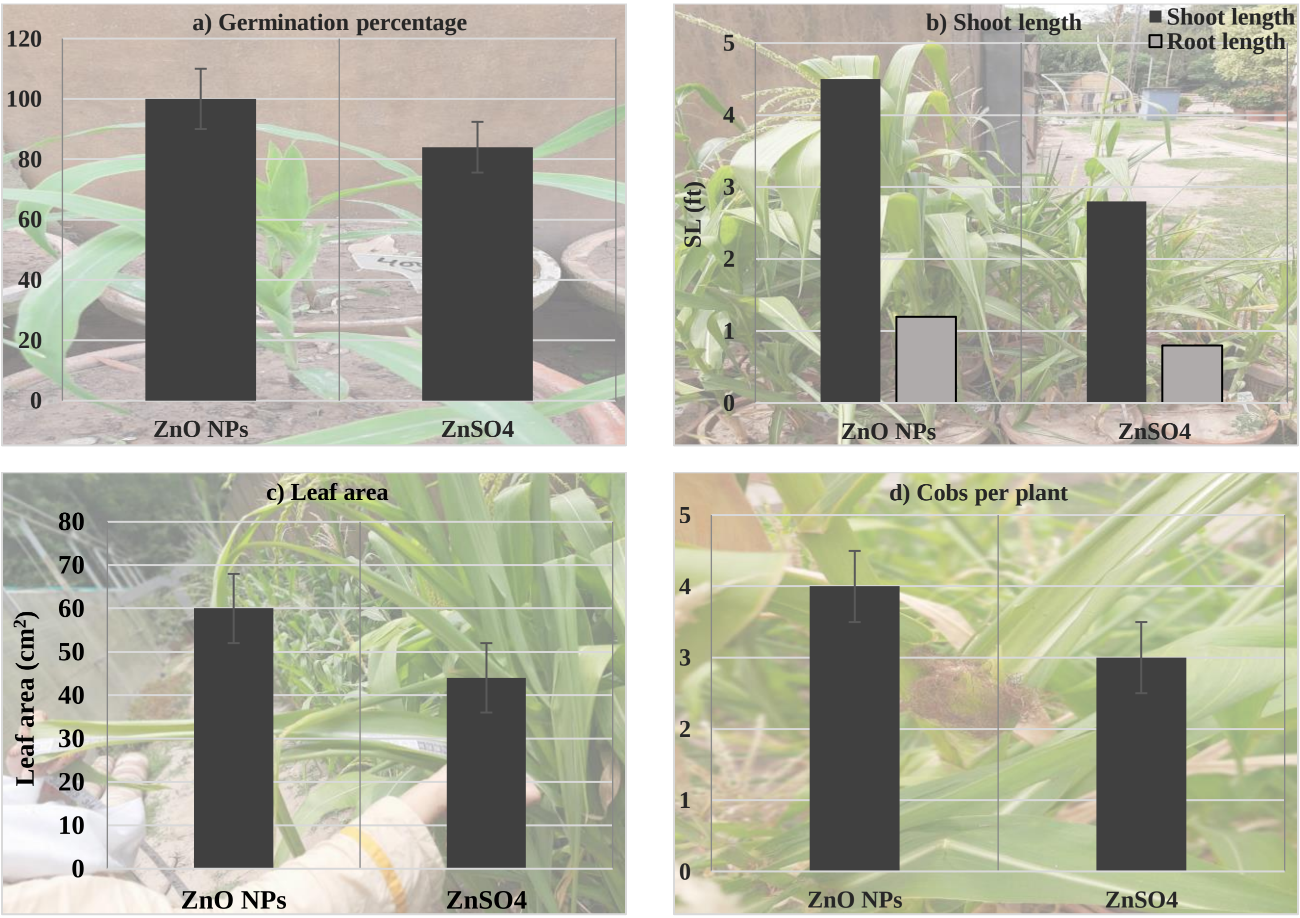

Fig 5. Pot experiment growth parameters a) germination percentage b) Shoot length c) Leaf Area d) Cobs per plant e) seed weight per plant f) number of seeds per plant.

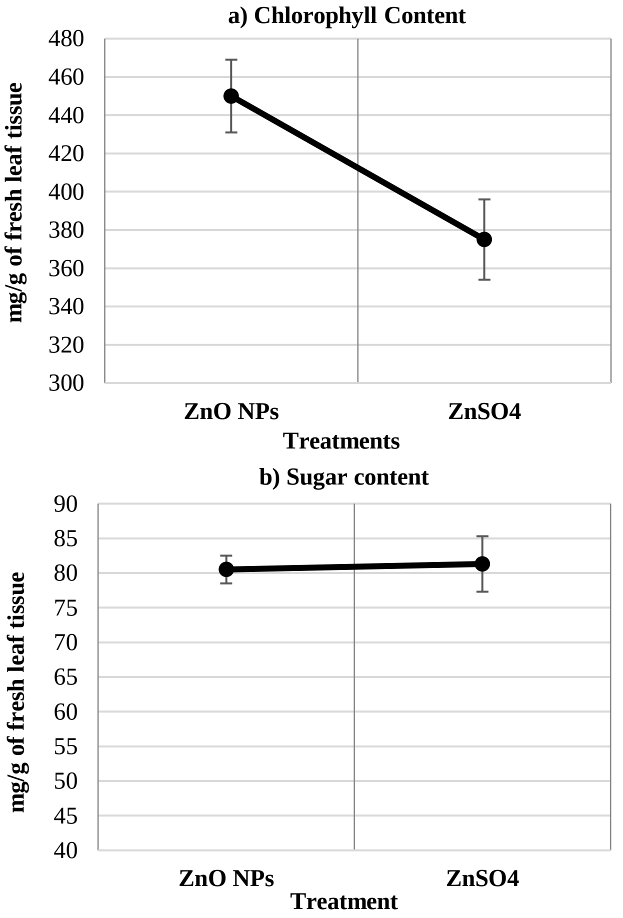

Fig. 6. Pot experiment biochemical parameters a) Chlorophyll content b) sugar content.

Comparison of ZnONPs and ionic zinc for *Zea mays* L. biochemical content: Chlorophyll content was also significantly higher in nanoparticles treated maize plants i.e., 451 mg per gram of leaf tissue as compared to control i.e., 378 mg/g of leaf tissue (Fig. 6 a) Whereas (Fig. 6b) shows the sugar content of leaf tissue of maize plants that is equivalent in both cases i.e., there is no significant difference between the two treatments, experimental and control both.

Stress study of ZnONPs and ionic zinc for *Zea mays* L.: Stress studies were also conducted for pot-grown maize plants by analyzing their phenolic, proline, and MDA content. These all studies showed that stress management by macro zinc nanoparticles was better as compared to zinc sulfate solution treatment. Fig. 7(a) shows that the phenolic content of nanoparticle treatment was 2% higher in nanoparticle-treated plants. Similarly, proline content was 36% higher in nanoparticle-treated plants whereas MDA content was 160% higher in salt-treated plants (Fig. 7b and c).

Study of grains of *Zea mays* L. for starch content: Figure 8a shows the grain starch content of maize grown under the treatment of zinc oxide nanoparticles and zinc salt treatments. Starch is the storage sugar of maize seedlings. It was found to be 209 mg per gram of dried seeds in nanoparticles-treated plants while it was 56 mg per gram of seeds in case of salt-treated plants.

Study of grains of *Zea mays* L. for zinc content: Zinc provided in nano and microform was presumed to be accumulated in the maize grains. Therefore, grain zinc content was also calculated. Figure 8b shows the grain zinc content of maize grown under the treatment of zinc oxide nanoparticles was 147% higher as compared to zinc salt treated plants. It was found to be 57mg/100 g of dried grains for zinc nanoparticles-treated plants and 23mg/100 g of dried grains for zinc salt-treated plants.

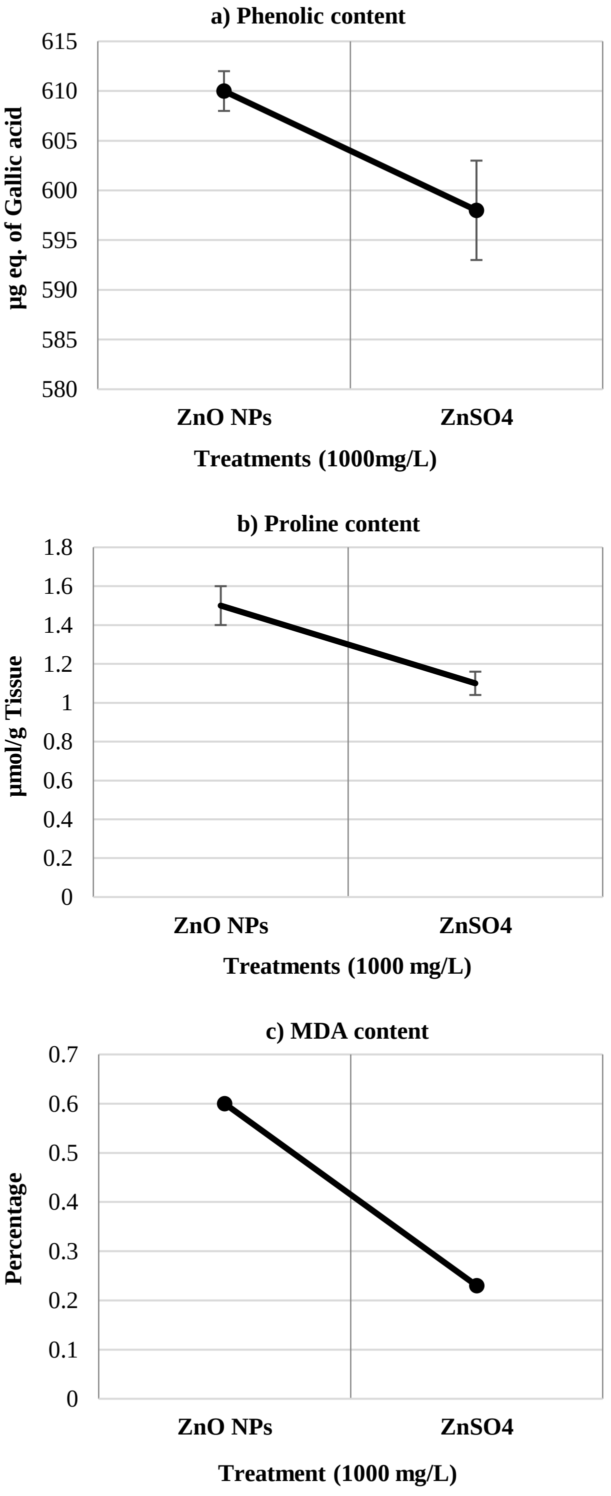

Fig. 7. Pot experiment biochemical parameters a) Phenolic content b) proline content c) MDA content.

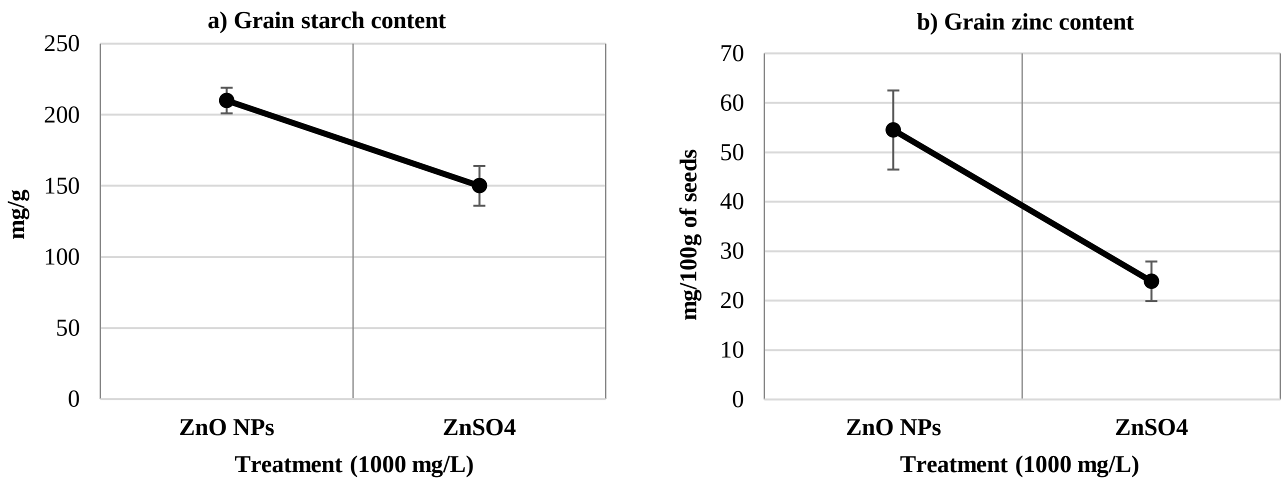

Fig. 8. Pot experiment biochemical parameters, a study of a) starch content b) Zn content.

Discussion

In the present research work, green synthesis of NPs was opted, i.e., using water extract of *Pisum sativum.* This type of synthesis can be carried out at a mild temperature and pressure without any toxic chemicals. Pea plants are known to have several secondary metabolites (Akula & Ravishankar, 2011). These secondary metabolites have a strong reducing potential that can react with metal ions to reduce them to metal nanoparticles (Marslin *et al.*, 2018). Furthermore, seeds of pea plants have high protein content that are strong stabilizing agents for newly synthesized nanoparticles. Thus giving rise to small and stable zinc oxide nanoparticles (Srivastava *et al.*, 2021; Tomoskozi *et al.*, 2001).

Nanoparticles formed in such a method can be initially tested for their formation by UV-Vis spectrophotometry. In the present study, UV-Vis analysis showed maximum absorption at 350nm. These results are in line with the results reported by Zak *et al.*, (2011) and Zheng *et al.*, (2015) showing the maximum absorption of zinc oxide nanoparticles at 370 nm and 386 nm respectively. Similarly, Hassan *et al.*, (2015) observed a significant peak of ZnO NPs between 340 to 390 nm. It has been noted that every element possesses its specific excitation energy and is excited upon absorbing those particular UV-vis radiations. Each type of metal and metal oxide nanoparticles shows a specific spectrum of absorption, therefore, a shift in absorption wavelength indicates the nanoparticles' synthesis (Nanocomposix, 2012). Furthermore, Former studies have also testified that ZnO nanoparticles retain high optical absorption in the long-wave (315-400 nm) and short-wave (280-315 nm) regions. Particle size analysis and SEM analysis have shown the formation of ZnO NPs 68-79.8 nm. Various studies have shown that above said techniques are reliable in confirming the synthesis of nanoparticles (Gajdosechova & Mester, 2019).

FTIR analysis shows the peaks of functional groups present on nanoparticles and FTIR analysis of ZnONPs of the present study has shown that the main compounds involved with nanoparticles were OH-containing phenolics. Other than phenolics, the metabolites with carbonyl, alkene, nitroxide containing compounds. Phytochemicals present in plant extract are responsible for capping and reducing metal ions into particles of nano-size (Javad *et al.*, 2017). Pea plants possess various health-assisting nutrients including fibers, proteins, carbohydrates, terpenoids, phenolics, phytoalexins, flavonoids, and isoflavonoids, etc. (Fahim *et al.*, 2019). These functional groups are the main reducing agents in the plant extract which can reduce Zn ions to Zn nanoparticles. Zak *et al.*, (2011) characterized ZnO nanoparticles with FTIR and found peaks at 375 cm^{-1}, 930 cm^{-1}, 1050 cm^{-1}, and 3400 cm^{-1}. Aminuzzaman *et al.*, (2018) observed in FTIR analysis of ZnO NPs by *Garciniam angostana* fruit extract significant bands related to O–H, C–H, C=O, C–C, C–OH, and C–O–C which is part of flavonoids and phenols. It is known that many secondary metabolites (ketones, alcohols, amines, terpenoids, and carboxylic acid) are part of plant extract, so these compounds cap and stabilize nanoparticles. These compounds help to form nanoparticles of small size range.

Nano fertilizers have the potential to alter the use of traditional fertilizers for the maintenance of sustainable agriculture. Controlled and on-demand delivery of nutrients in the soil at target sites can save from excessive fertilizer usage (Sabir *et al.*, 2014). Present research work reported a significant increase in the germination of corn seedlings under the effect of ZnO NPs (700-1000 mg/L). All other growth parameters studied were also significantly higher at 1000mg/L dose of ZnO NPs.

Previous work by Prasad *et al.*, (2012) reported the effects of ZnO NPs on peanut germination and growth factors. They applied ZnO NPs up to 1000 ppm which significantly improved seed germination, seed vigor, root and shoot length of the plant. Similarly, Slaton *et al.*, (2005) observed ZnO NPs' effect on *Oryza sativa* seeds which enhanced grain productivity; they also concluded that nanofertilizers are an effective and low-cost method as compared to traditional manures. It was also reported by Tarafdar *et al.*, (2014) that Zinc nanoparticles improve seed germination, root and shoot length, dry weight of the plant, and biochemical processes in *Pennisetum americanum.* The same effect is observed in the present study on maize seedlings.

It is suggested that zinc nanoparticles application during seed germination stages increases plant metabolism hence inducing early germination and flowering (Eichert *et* *al*., 2008). Zinc also plays a role in breaking dormancy and it is also known to be involved in the hydrolysis of inhibitors. Thus it positively controls and activates metabolism and enzymes involved in germination, finally enhancing germination percentage and germination rate (Kalal & Jajoo, 2021; Sedghi *et al*., 2013). Zinc has a vital role in cell membrane structural stability and protection (Welch *et al*., 1982). Researchers have noticed, the rapid water molecules uptake along with ZnO nanoparticles *via* cell wall of the seed coat into the seed kernel giving a signal for rapid germination of seed (Mahakham *et al*., 2017; Prerna *et al*., 2020).

The present study also produced positive effects on shoot length, root length, number of roots, fresh and dry seedling weight. Macro and micronutrients play their role in plant growth as a part of structural components and metabolic pathways. Zinc is one of the essential micronutrients which is part of photosynthetic machinery and is a cofactor of many enzymes (Kalal & Jajoo, 2021). Therefore, its sufficient and active supply to plants enhances their growth indicators, like shoot length, root length, and number of roots, etc. Zinc is also known for its role in protein and carbohydrates formation and cell elongation as well as tolerance to environmental stresses (Cakmak, 2000). Furthermore, α-amylase is the enzyme that converts stored seed sugars to active glucose molecules so that those may be used in active respiration. Zinc is also reported to play a role in enhancing the activity of this enzyme, thus providing more energy for growing seedlings (Kalal & Jajoo, 2021). These all factors support our results. An increase in seedling weight has resulted from higher germination, increased shoot, and root length. All these enhanced activities cause an increase in the overall increase in seedling fresh and dry biomass/ weight.

We reported here, higher chlorophyll content in ZnONPs treated plants as compared to control. The higher chlorophyll content is a strong indicator of the higher rate of photosynthesis, giving higher biomass accumulation. So results of the present section of this study can be positively related to the results of the previous section 4.2.1. Higher carotenoid content assures more photoprotection of plants. It has also been reported that zinc is involved in the biosynthesis of chlorophyll while protecting its SH groups (Cakmak, 2008). It is also evident from previous research work that zinc takes part in chloroplast development and maintaining the function of photosystem II (Kalal & Jajoo, 2021).

Our work is in line with Hajra and Mondal (2017) who reported higher chlorophyll and carotenoid content in zinc oxide nanoparticles treated *Cicer arietinum* plants. Similarly, Prasad *et al*., (2012) reported the highest chlorophyll content in treated plants at 1000 mg/L of zinc nanoparticles. It was proposed by them that higher chlorophyll accumulation may be due to the complementary effect of other inherent nutrients like magnesium, iron, and sulfur as well. ZnONPs treatment also enhanced the sugar content of growing seedlings of corn in the present study. It may be related to the role of zinc in enhancing the activity of α-amylase which in turn increases the soluble sugars for growing plants. It is another positive attribute of ZnONPs treatment which increases the respiratory activity of plant cells, thus adding to growth. Our results can be correlated with those of Hajra & Mondal (2017), where the higher level of sugar (1.8 mg g^{-1} fw) was recorded at the lower dose ZnO nanoparticles treated plants. Rawashdeh *et al*., (2020) explained that NPs treatment induced early carbohydrate mobilization that resulted in the reduction of the carbohydrate content. That is why sugar content was reported to decrease in the present study in ZnO NPs dose where seedling growth was high.

Zinc is an important nutrient for protein and carbohydrate production. It increases the cation transfer capability of roots which causes efficient nutrient absorbance mostly nitrogen and in consequence protein content of a plant is improved. Zinc nanoparticles when applied at earlier stages of plants, support seed maturity, protein formation and many other physiological processes (Yilmaz *et al*., 1987; Laware & Raskar, 2014).

In the present study, ZnONPs treatment enhanced the proline content of corn seedlings as well. Proline plays an important role in various stages and processes of plant development. However, there are few reports on the effect of endogenous proline accumulation on germination and seedling vigor under suitable conditions of germination and development. Proline synthesis can also play an important role in promoting germination. An increase in free proline was observed before germination in Arabidopsis seeds (Abrantes *et al*., 2018).

In the present experiment, when the results of growth and yield parameters of ZnONPs and macro zinc (ionic form) were compared, zinc oxide nanoparticles treated particles have predominantly higher germination percentage (19%), shoot length (55%), root length (37.5%), leaf area (33%), cobs per plant (33%). This increase in growth is well explained in earlier sections that how zinc can be influential for plant growth. But now the question is that ZnONPs are showing their higher efficiency for promoting corn growth as compared to its ionic form. This can be well correlated with the fact that the bioavailability of zinc in nano-form is greatly enhanced due to its small size and larger surface area (Naseer *et al*., 2023). Nano-form of zinc is absorbed at higher rates by plants as compared to the low solubility of macro zinc in soil solution (Prasad *et al*., 2012).

The present study showed a significantly higher chlorophyll, starch, and zinc content in NP-treated plants as compared to salt-treated plants. While sugar content was same for both treatments. Zinc content is usually lower in zinc-treated plants due to the lower mobility of such ions in plants. This lower mobility is caused by the hindrance of negative ions present in apoplasts of cells (Rossi *et al*., 2019). Zinc in nano-form is not positively charged, so may be moving faster through the tissues. In this way bio-fortification of grains, vegetables and fruits can be done with zinc to decrease the deficiency of zinc in common people. Such crops grown under the effect of ZnO NPs can be used as functional foods. Whereas higher chloroplast, and starch content can be interrelated. Higher pigment content may be attributed to the role of zinc in chlorophyll synthesis. It causes a higher rate of photosynthesis causing the higher storage sugar content i.e., starch. Soluble sugar content is equal in both treatments showing that surplus sugars may be actively changing into starch in NP-treated plants. ZnO NPs have a higher surface area to react with other enzymes, molecules, and proteins to react with and give results in significantly higher growth and biochemical responses (Arsalan & Younus, 2018).

Higher proline and phenolic content with reduced MDA content in NP-treated plant as compared to the inverse case of salt-treated plants show that NP treatment significantly improves the defense system of plants controlling the production of defense chemicals like proline and phenolics that ultimately reduces the MDA content. MDA content in higher levels shows a structural loss of cells that is higher in salt treated plants. This study can be related to the study reported by Saleh *et al*., (2021), Firoozi *et al*., (2016), and Ahmad *et al*., (2021). Stress induction by NPs may cause the accumulation of phenolics in treated plants that may fight with ROS stress, thus leading to the lesser loss of cell protein or lipids (MDA content). While zinc salt particles are in macro form, they are not having such active surfaces to induct such action-reaction systems in the plant body. Therefore, an optimized dose of ZnO NPs is necessary to control the growth, biochemical, and stress parameters in plants.

Conclusion

It is concluded that bio-synthesized zinc oxide nanoparticles enhanced the growth and zinc content of *Z. mays*. However, in the future, more research work needs to be done, particularly at the field level, to see the long-term effects of nanoparticles in plants and humans. Zinc oxide nanoparticles can be used as nano-fertilizers to increase plant growth and can overcome nutrient deficiencies in soils and plants.

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