Zinc Nanoparticles for Contributing Salinity Stress Resilience in Wheat (Triticum aestivum L.)

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Abstract

Wheat is the most widely grown food crop in the world. The world is seeing much of its wheat yield decline due to salt stress. This study aimed to evaluate the effectiveness of zinc nanoparticles in improving resilience to salt stress in wheat (*Triticum aestivum* L.). ZnNP was biosynthesized using *Crassula multicava* leaf extract as reducing agent and capping agent. The synthesized nanoparticles were characterized to evaluate structural and biochemical aspects. The ZnNPs were found to be irregular in shape at 100nm magnification by TEM analysis. The average size of the biosynthesized ZnNPs was 64.6 nm as evidenced by DLS analysis. The experiment was laid out with a randomized complete block designed and arranged with eight replications in the pots with 5 rows having one row of control and the other 4 rows of different salinity levels such as 100mM, 75mM, 50mM, and 25mM. The crop was subjected to a total 3 treatments; The first NaCl stress was given on emergence of the Tillering stage (30 DAS) and after 2 days of salinity stress period the wheat plant pots were treated with 2mM zinc nanoparticles, second at the Booting stage (58 DAS) and third at Flowering stage. Plants that were not stressed with NaCl and not treated with NPs were considered as controls. Plant growth parameters were recorded. The results obtained showed that the application of ZnNP had a positive effect on all morphological and yield characteristics of wheat plants. The best results were recorded in potted plants subjected to 25 mM NaCl stress and treated with ZnNPs and showed a significant effect compared to the control at $p < 0.05$. The study concluded that ZnNPs can contribute to enhancing the resilience of wheat to salt stress.

1. Introduction

In many regions of the world, wheat production is significant. It is commonly known as the king of cereals. It belongs to the Poaceae family and genus *Triticum*. It has a hexaploidy (2n = 6x = 42) genome, with a size estimated at ~ 17 Gigabase (Dvorak et al., 2012).

Wheat (*Triticum aestivum* L.) is the most common cereal crop farmed worldwide and a food staple (Carte., 2002). Most of these cultivars are varieties of two broad groups: common wheat and durum wheat (Shukla et al., 2022). Bread wheat (*T. aestivum*) accounts for about 95% of total consumed wheat worldwide (Hirst, K. Kris., 2021).

More than 36% of people worldwide use wheat as a staple meal. Nearly 55% of the food's carbohydrates and 20% of food calories come from wheat (Wiese, 1977; Breiman and Graur, 1995). In India, wheat crop is grown in about 30 million hectares with grain yield of approximately 90 million tonnes. Wheat contributes roughly 34% of the nation's overall production of food grains, with a yield of 31.25 quintals per hectare (Yadav et al., 2014; Shukla et al., 2022). Uttar Pradesh is the highest wheat producing state in India (Singh et al., 2021).

In the context of agriculture, Salt stress induces osmotic stress and ion toxicity, through the increased assimilation of the Na^+/K^+ ratio (Arif et al., 2020). Salinity impairs seedling establishment, slows plant growth, leads to poor yield development and ultimately reduces crop yield (Turan et al., 2009). Currently, more than 20% of agricultural land of world is affected by salinity, which includes about 950 million hectares of the total agricultural land of the world (Hafeez et al.,2021; Zaman et al.,2018; Munns et al.,2008).

Nanotechnology can play an important role in increasing production and improving the quality of food produced by farmers (Mousvi and Rezaei, 2011). In modern agriculture, Nanotechnology is one of the most effective methods to mitigate salinity stress as compared to conventional methods used to manage salinity stress (Shukla et al., 2020). It is the field that involves using tiny particles called nanoparticles to improve the performance of biological systems. Nanobiotechnology could involve using nanoparticles to enhance the salt tolerance of plants or to improve the efficiency of irrigation systems (Shukla et al., 2017). This method can be more sustainable and cost-effective in the long run as compared to conventional methods. Nanomaterials are now being explored for crop improvement aspects (Khan et al., 2017). It has been reported that application of nano-ZnO to wheat plants under salinity stress resulted in the increased photosynthetic activity (Wang et al., 2020; Shukla et al., 2022).

The phytogenic approach for the production of metal oxide nanoparticles is an eco-friendly approach (Kuppusamy P et al., 2014; Shukla et al., 2020). Biosynthesis uses bacteria, plant extracts, fungi, etc. as a capping and reducing agent along with the precursors to produce nanoparticles instead of conventional chemicals for bioreduction and agricultural applications for crop improvement (Hasan S., 2015). In the present study phytogenic zinc nanoparticles (ZnNPs) were evaluated for their efficacy in incorporating salinity stress resilience in wheat.

2. Methodology

2.1. Preparation of plant extract and synthesis of ZnNPs

The methodology was adopted from Rani et al. (2022) with slight modifications. Zinc nanoparticles were successfully synthesized using aqueous extract of *Crassula multicava* (Fairy crassula) plant leaves. The fresh leaves were collected of *Crassula multicava* from a home garden, in Meerut, UP, India. After the collection of leaves they were washed with tap water several times to remove the dust particles were kept on blotting paper so that the water would get soaked and then the leaves were allowed to dry in a hot air oven at 50°C for about 3 days. The dried leaves were then crushed into fine powder. The leaf-powder was mixed with distilled water in 1:10 ratio and was boiled at for 15 to 20 min. Further, it was filtered with Whatman No. 1 filter paper and stored at 4°C. A 2mM solution of ZnSO was prepared. The plant extract and salt solution were mixed in 1:4 ratio (test) and monitored for color change against control (plant extract and distilled water in 1:4 ratio. The flasks were placed in a shaking incubator at 200 rpm.

2.2. Characterization of biosynthesized nanoparticles

The nanoparticles were preliminary characterized through UV-Vis spectrophotometer. Further characterization was done through Field emission scanning electron microscopy (FESEM) and Dynamic

Light Scattering (DLS) to determine the size, shape and topographic parameters of NPs. Energy Dispersive X-Ray (EDX) was used to determine the elemental composition of synthesized nanoparticles. Fourier Transform Infrared Spectroscopy (FTIR) was used to establish idea about the molecular interactions between NPs and plant extract.

2.3. Pot experiment

Late sown genotype of wheat namely PBW 226 was chosen and obtained from Hindustan Beej Bhandar XMGW + MPQ, Kotla Bazar, Meerut, Uttar Pradesh 250002, India. Pot Experiment was set up at the Department of Biotechnology of Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India; during Rabi season 2022–2023. The experiment was laid out with a randomized complete block designed and arranged with eight replications in the pots with 5 rows having one row of control and the other 4 rows of different salinity levels such as 100mM, 75mM, 50mM, and 25mM. Each pot was 16 cm long and 12 cm wide. The experiment aimed at growing the wheat variety under different levels of salinity stress to assess the effect of the zinc nanoparticle on the wheat crop. The Wheat plant pots which did not have salinity stress and were also not treated with zinc nanoparticles were considered controls. The plant pots other than the control had different levels of salinity stress and were also treated with zinc nanoparticles. The First NaCl stress was given on the emergence of the Tillering stage after 30 DAS of sowing. Then after 2 days of salinity stress period, the wheat plant pots were treated with zinc nanoparticles by spraying them on the leaves of the wheat crop, second at the Booting stage after 58 DAS of sowing and third at the Flowering stage occurs about 3–5 days after the full head emergence. All the other agronomic practices were kept normal and uniform. The plants were harvested after 121 days of sowing. Data on germination, growth and yield parameters were recorded. The recorded experimental data was then analyzed by ANOVA using Statistical Package for Social Sciences (SPSS) software 16.0.

3. Results

3.1. Biosynthesis of ZnNPs using *Crassula multicava* leaf extract

The Zinc nanoparticle reaction mixture was incubated for about 24 hours. The reduction of zinc ions to zinc nanoparticles was monitored by a color change of the reaction mixture, which was observed after incubation (Paul et al., 2016). This indicated the synthesis of zinc nanoparticles (Figs. 1 & 2). Noorjahan et al., (2015) used Neem (*Azadirachta indica*) leaf extracts for the synthesis of zinc nanoparticles. Due to the Surface Plasmon Resonance phenomenon, a color change from pale white to brown happened as a result of exposure to leaf extracts, which allowed researchers to detect the reduction of zinc ions into zinc nanoparticles. The metal nanoparticles have free electrons, which helps in the formation of the Surface Plasmon Resonance absorption band, which is due to the united vibration of the electrons of metal nanoparticles in resonance with light waves. Using plant extract to synthesize metal oxide is of significant advantage due to the production of functional molecules that reduce metal ions (Rai & Ingle., 2012). Phenols, terpenoids, ketones, aldehydes, and amides are the primary phytochemicals involved in nanoparticle synthesis (Sundrarajan et al., 2011).

3.2. UV-Vis Spectrophotometry Analysis of Nanoparticles

The UV-Vis spectra showed peaks at 300 nm, 340nm and 360nm which are corresponding to ZnO nanoparticles. For ZnO nanoparticles, the absorbance peak is reported between 310 nm and 360 nm of wavelength (Ghamsari et al., 2017). After the addition of aqueous salt (ZnSO) into leaves extract (*Crassula multicava*), the spectra were taken at a different time interval (0 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs) between 200 nm to 800 nm (Fig. 3). The peak demonstrates the presence of ZnO Green Nps in the reaction mixture (Santhoshkumar et al., 2017; Jayachandran et al., 2021).

3.3. FESEM Analysis of Nanoparticles

The surface morphology of the biosynthesized zinc nanoparticles was principally characterized using FESEM and the resulting images are displayed in Fig. 4. The FESEM and EDX facility was accessed from the University Science Instrumentation Centre (USIC) near the Department of Physics &Astrophysics University of Delhi (North campus) Delhi-110007, India. The FESEM report depicted that the nanoparticles were fine-sized and the agglomerated form of nanoparticles. The nanoparticles are known to attach to one another over time, which leads to aggregation or agglomeration (Sundrarajan et al., 2015). Ecological considerations have a significant impact on the stability of NPs and agglomeration. As a result, as the nanoparticles are being formed, they cling to one another and spontaneously form asymmetrical clusters (Agarwal et al., 2017).

3.4. EDX Analysis of Nanoparticles

The elemental composition analyses of the ZnNPs from the EDX plot of the FESEM images are shown in Fig. 5, where the X-axis shows energy in KeV and the Y-axis depicts intensity count. From the EDX spectrum, the presence of Zn elements was determined from the ZnK peak at ~ 1.2 keV which confirmed the existence of ZnNPs in the sample. The carbon, oxygen, calcium, magnesium, chlorine and potassium could be the elements from the proteins or compounds present in the plant extracts, whereas the presence of sulphur could be due to the use of ZnSO as the precursor for the synthesis of ZnNPs (Singh et al., 2022). The EDX Analysis of synthesized ZnNPs revealed that the elemental and quantitive weight composition of nanoparticles constituted are carbon 36.20%, oxygen 39.05%, calcium 10.34%, sulfur 6.99, zinc 3.58, magnesium 2.83, chlorine 1.01% (Table 1).

Table 1
Results of elemental analysis by EDX

Element	Wight percentage	Atomic percentage	Net Intensity
Zn	3.58	0.89	30.38
O	39.05	39.8	1197.90
Na	0.00	0.00	0.11
Mg	2.83	1.90	253.43
S	6.99	3.56	632.62
K	0.00	0.00	0.00
Cl	1.01	0.47	78.17
Ca	10.34	4.21	533.59

3.5. DLS Analysis of Nanoparticles

Dynamic Light Scattering (DLS) principle shows the particle size distribution and average size of zinc nanoparticles obtained through a particle size analyzer. The size distribution intensity graph of zinc nanoparticles is presented in Figs. 6. The result revealed that the average size of the biosynthesized ZnNPs was 64.6 nm with Polydispersity Index is 0.586. Chaudhuri et al., (2017) synthesized the ZnONPs using leaf extract of *Calotropis gigantea* as a reducing agent and the average diameter of nanoparticles was 11 nm. Yedurkar et al., (2016) reported the 145.1 nm average size of ZnNPs prepared with *Ixora Coccinea* leaf extract.

3.6. FTIR Analysis of Nanoparticles

FTIR Spectroscopy was used to determine the functional groups that are present in the sample and to study the surface interaction of synthesized nanoparticles with other molecules that are involved in synthesizing and stabilizing the nanoparticles. The IR peaks of the zinc nanoparticles were observed at 3264.30 cm^{-1}, 1555.56 cm^{-1}, 1394.99 cm^{-1}, and 1083.69 cm^{-1} (Table 2). Maher et al. (2023) reported FTIR spectral peaks of ZnONPs to be found at 3442, 2921, 2353, 1398, 419, 3414, 2345, 1391, and 436 cm^{-1}. In a report, a large peak was visible at 3414 and 3442 cm^{-1} due to substantial O-H stretching of the hydroxyl moiety of phenols and alcoholic functionality in the highest energy state (Al-Trad et al., 2019). According to Agarwal et al. (2020), the characteristic peaks between 400 and 600 cm^{-1} are attributed to the Zn-O stretching vibration.

Table 2
Various peaks and corresponding functional groups as inferred from FTIR spectral report of zinc nanoparticles.

Absorption peak (cm^{-1})	Functional group
3264.30 cm^{-1}	Corresponds to thestreching vibration of the hydroxyl (—OH) functional group.
1555.56 cm^{-1}	Associated with the stretching vibration of the carbonyl (C = O) functional group.
1394.99 cm^{-1}	Corresponds to the bending vibration of the methyl (CH3) functional group.
1083.69 cm^{-1}	Corresponds to the stretching vibration of the C-O bond in esters.

3.7. Effect of ZnNPs treatments on wheat crops

The variety PBW 226 chosen for the experiment was late sown variety. The variety was widely adopted in the area of NWPZ (North Western Plain Zone) and released in 1989. This variety is suitable for late sown and irrigation conditions. The crop variety PBW was harvested after 121 DAS respectively. The impact of ZnNPs on morphological growth under salinity stress was measured in terms of Germination percentage (GP), Days of 50% heading (50% DH), Days of 50% anthesis (50% DA), Plant height (PH), No. of effective tillers per plant (TPP), Spike length (SL), Grain yield per plant (GYPP) and the number of grains per spike (GPS). The graphical representation of various growth and yield parameters of control and treatments is shown in Fig. 10).

Wheat crop plants treated with ZnNPs (2mM) and Zn ions significantly accumulated Zn that was measured by using an Atomic Absorption Spectrophotometer (AAS). After 1 week of ZnNPs treatment, the wheat leaves were collected from the control row and the other 4 rows of treatment and washed with distilled water. The wheat leaves were digested as per the method given by Homaee & Ehsanpour (2016) and analysed through AAS. This facility was accessed from Advance Research and Analytical Services, Ghaziabad, Uttar Pradesh, India. The results indicate that the concentration of Zn ions in the sample of control wheat leaves was found to be 0.72 mg/L because control wheat leaves that must have been naturally accumulated through soil. The concentration of Zn ions and ZnNPs in 100 mM, 75mM, 50mM, and 25mM salt stressed wheat plant leaves was found to be 0.69 mg/L, 1.08 mg/L, 1.03 mg/L and 1.11mg/L respectively. The 25mM salt stressed plants showed maximum accumulation.

3.7.1 Effect on GP

The count of germinated seeds was taken after 20 days of sowing. Germination percentage (GP) was counted after 20 days of sowing as per the following formula:

GP = seeds germinated × 100

Total seeds

The result of ANOVA with LSD at $p \leq 0.05$ showed that the highest GP was observed in the T1 plants, whereas the lowest was recorded in the T4 plants. The LSD analysis depicted that the GP in control was insignificantly different with T1, T2, T3, and T4 plants. The T1 plants showed significantly higher GP as compared to T4, but there was no significant difference in the GP of T2 and T3. T4 plants showed significantly lower GP as compared to T1. The overall analysis showed that the GP significantly improved due to the application of different treatments except T4.

3.7.2 Effect on days to 50% heading

The result of ANOVA with LSD at $p \leq 0.05$ showed that the earliest days to 50% heading was observed in the T4 plants, whereas the latest was recorded in control plants. The LSD analysis depicted that the days to 50% heading in control was significantly later than T1, T2, T3, and T4 plants. The T1 plants showed significantly later days to 50% headings compared to T3 and T4, but there was no significant difference in the days to 50% heading of T2 plants. T2 plants showed significantly later days to 50% heading as compared to T3 and T4. T3 and T4 plants showed significantly earlier days to 50% heading as compared to T1 and T2 plants. The overall analysis showed that the days to 50% heading significantly improved due to the application of different treatments except for control.

3.7.3 Effect on days to 50% anthesis

The result of ANOVA with LSD at $p \leq 0.05$ showed that the earliest days to 50% anthesis was observed in the T4 plants, whereas the latest was recorded in control plants. The LSD analysis depicted that the days to 50% anthesis in control was significantly late than in T1, T2, T3, and T4 plants. The T1 and T2 plants showed significantly different days to 50% anthesisas compared to T3 and T4 plants, but there was no significant difference in the days to 50% anthesis of T1 and T2 plants. The T4 plants showed significantly early 50% anthesis as compared to T1, T2, T3, and T4. The overall analysis showed that the days to 50% anthesis significantly improved due to the application of ZnNPs.

3.7.4 Effect on plant height (PH)

The result of ANOVA with LSD at $p \leq 0.05$ showed that the highest PH was observed in the T4 plants, whereas the lowest was recorded in the control plants. The LSD analysis depicted that the PH in control was significantly lower than T1, T3, and T4; however, it showed an insignificant difference with the T2 plant. The T1 plants showed significantly higher PH as compared to T2, but there was no significant difference in the PH of T1, T3, and T4. The T3 plants showed significantly lower PH as compared to T2. The overall analysis showed that the PH significantly improved in all the treatments except for T2.

3.7.5 Effect on no. of effective tillers per plant

The result of ANOVA with LSD at $p \leq 0.05$ showed that the highest number of effective tillers per plant was observed in the T4 plants, whereas the lowest was recorded in the T1 plants. The LSD analysis depicted that the number of effective tillers per plant in control was significantly lower than T4; however, it showed insignificant differences with T1, T2, and T3 plants. The T1 plants showed a significantly lower no. of effective tillers as compared to T4, but there was no significant difference in the no. of effective tillers of T1, T2, and T3 plants. The T4 plants showed a significantly higher number of effective tillers per plant as compared to T1. The overall analysis showed that the number of effective tillers per plant significantly improved due to the application of different treatments except for T1.

3.7.6 Effect on Spike Length

The result of ANOVA with LSD at $p \leq 0.05$ showed that the highest SL was observed in the T2 plants, whereas the lowest was recorded in the Control plants. The LSD analysis depicted that the SL in control was significantly lower than in T1, T2, T3, & T4 plants. The T1 plants showed significantly higher SL as compared to the control, but there was no significant difference in the SL of T2, T3 & T4. The overall analysis showed that the SL significantly improved due to the application of different treatments except for control.

3.7.7Effect on No. of Grains per Spike

The result of ANOVA with LSD at $p \leq 0.05$ showed that the highest number of grains per spike was observed in the T4 plants, whereas the lowest was recorded in the Control plants. The LSD analysis depicted that the number of grains per spikein control was significantly lower than T3 and T4, however, it showed insignificant differences with T1 and T2 plants. The T1 plants showed significantly lower no. of grains per spike as compared to T3 and T4, but there was no significant difference in the no. of grains per spike of T2. The T2 plants showed a significantly lower number of grains per spikeas compared to T2. T4 plants showed significantly higher no. of grains per spikeas compared to T1 and T2. The overall analysis showed that the number of grains per spike significantly improved due to the application of different treatments except for control.

3.7.8 Effect on Grain Yield per Plant

The result of ANOVA with LSD at $p \leq 0.05$ showed that the highest GYPP was observed in the T4 plants, whereas the lowest was recorded in control plants. The LSD analysis depicted that the GYPP in control was significantly lower than the T1, T2, T3, and T4 plants. The T1 plants showed significantly lower GYPP compared to T2, T3, and T4. The T4 plants showed significantly higher GYPP compared to the T1, T2, and T3 plants. The overall analysis showed that the GYPP significantly improved in treatments.

Various researchers reported that salinity stress can dramatically reduce the wheat plant growth and yield attributes due to the compromised water potential and osmoregulation (Sabagh et al., 2021). In this study, it was observed that a 100 mM salt stress imposed high salinity stress in wheat variety PBR 226. Results showed positive impact of ZnNPs on wheat plants with imposed salinity stress. According to Sturikova et al. (2018) ZnNPs can improve the Zn-uptake by plants under salinity stress which improves the growth and yield parameters. Nanoparticles have a higher ability and dynamism to be absorbed and accumulated in plants as attributed by their fine-size (Khalid et al., 2022).

5. Conclusion and Future Prospects

In conclusion, this study delved into the pressing issue of declining wheat yields worldwide due to salt stress, a challenge exacerbated by changing environmental conditions. The research specifically focused on assessing the potential of zinc nanoparticles (ZnNPs) synthesized from *Crassula multicava* leaf extract to enhance wheat resilience under salt stress conditions. Through a meticulously designed experiment, we subjected wheat plants to varying salinity levels and applied ZnNPs at different growth stages. The findings of this study shed light on the remarkable positive impact of ZnNPs on wheat plants facing salt stress. The careful characterization of the synthesized nanoparticles revealed their structural and biochemical properties. Notably, the application of ZnNPs resulted in significant improvements in various morphological and yield-related characteristics of wheat plants. The most promising outcomes were observed in plants subjected to 25 mM NaCl stress and treated with ZnNPs, demonstrating a substantial and statistically significant effect compared to the control group. These results underscore the potential of ZnNPs as a viable solution to mitigate the detrimental effects of salt stress on wheat crops. By enhancing the resilience of wheat plants, these nanoparticles offer a promising avenue to address the challenges posed by salinity, thereby contributing significantly to ensuring global food security. As the world grapples with the imperative need to sustainably increase food production, the findings of this study provide valuable insights and open new doors for further research and application of nanotechnology in agriculture.

Declarations

Author Contributions

G.S.: Conceptualization, methodology, validation, manuscript writing; A.S.: Data analysis, Co-wrote the manuscript; A.T.: Performed experiments and co-wrote manuscript; S.S.G.: review and editing. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

All authors declare no conflict of interest.

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Ethics Approval

Not Applicable.

Consent to Participate

Not Applicable.

Consent to Publish

Not Applicable.

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Figures

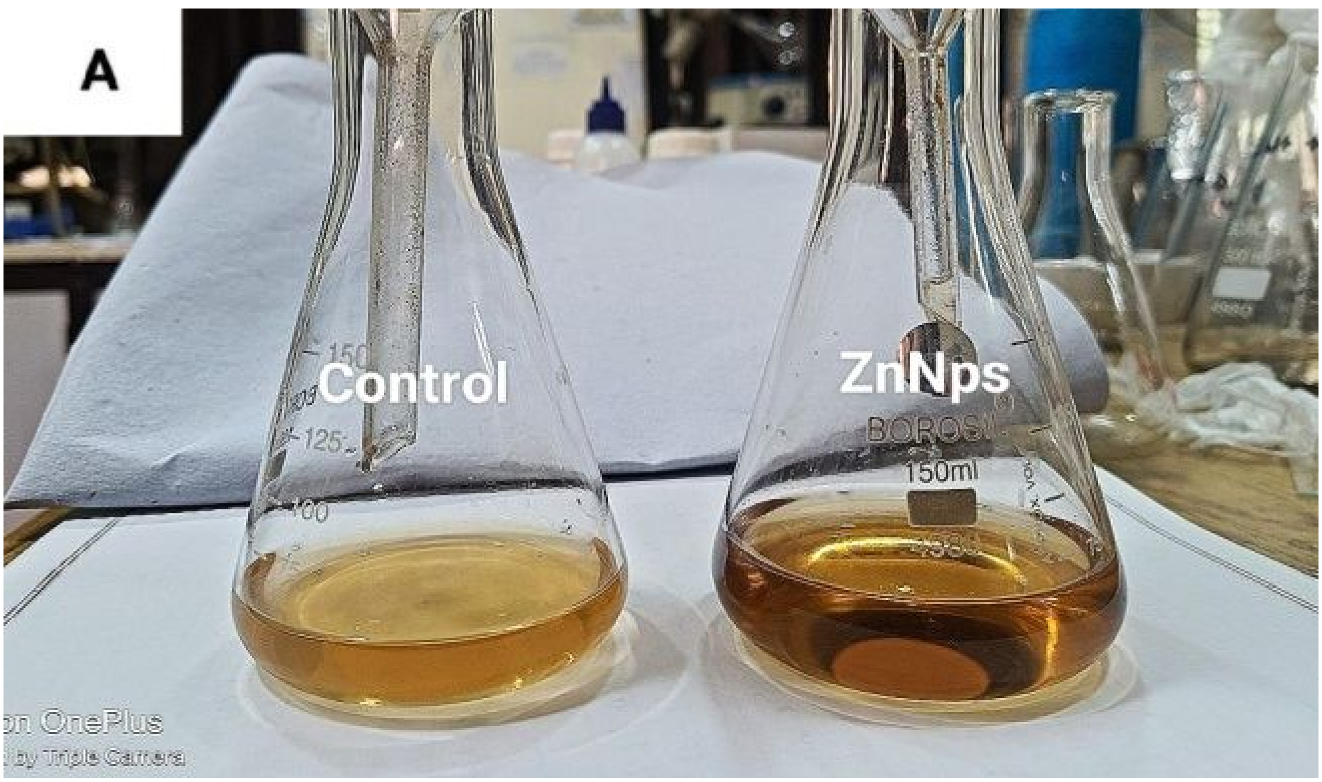

Figure 1

Colour variation in Control and ZnNPs flasks after 24 hrs.

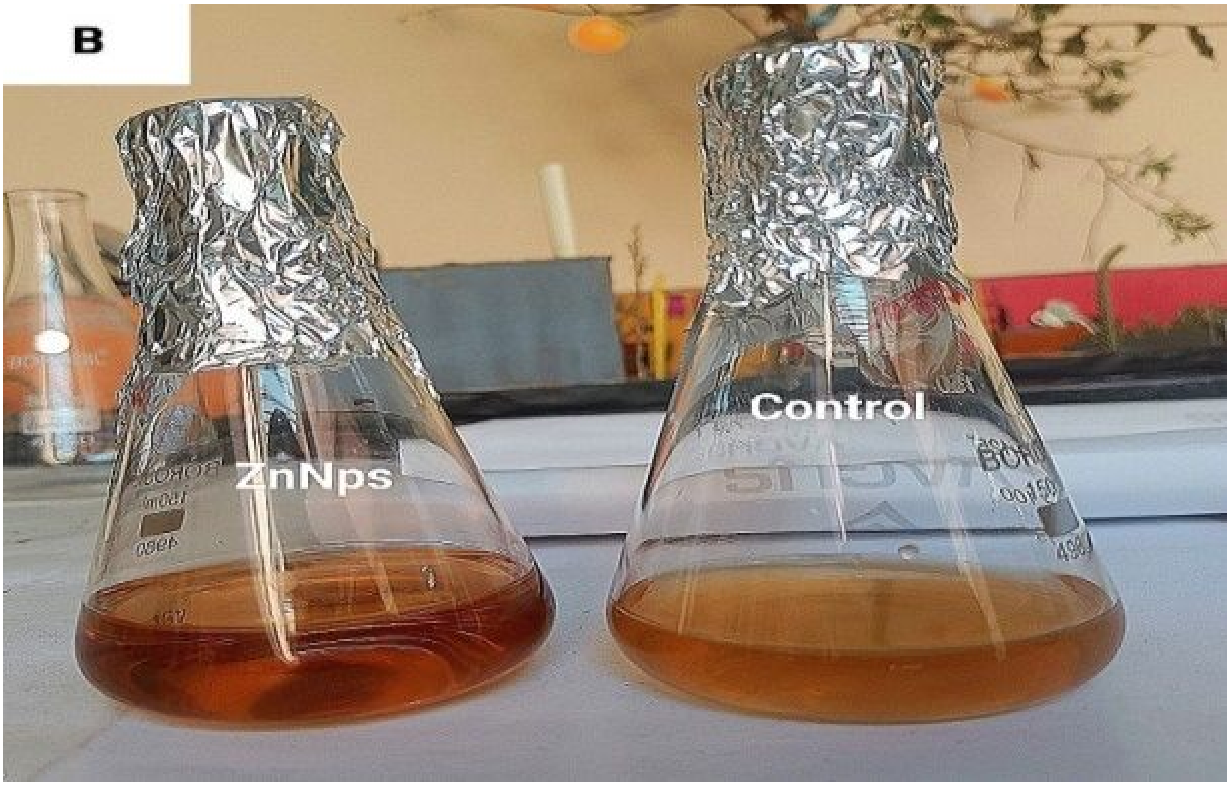

Figure 2

Colour variation in Control and ZnNPs flasks after 48 hrs.

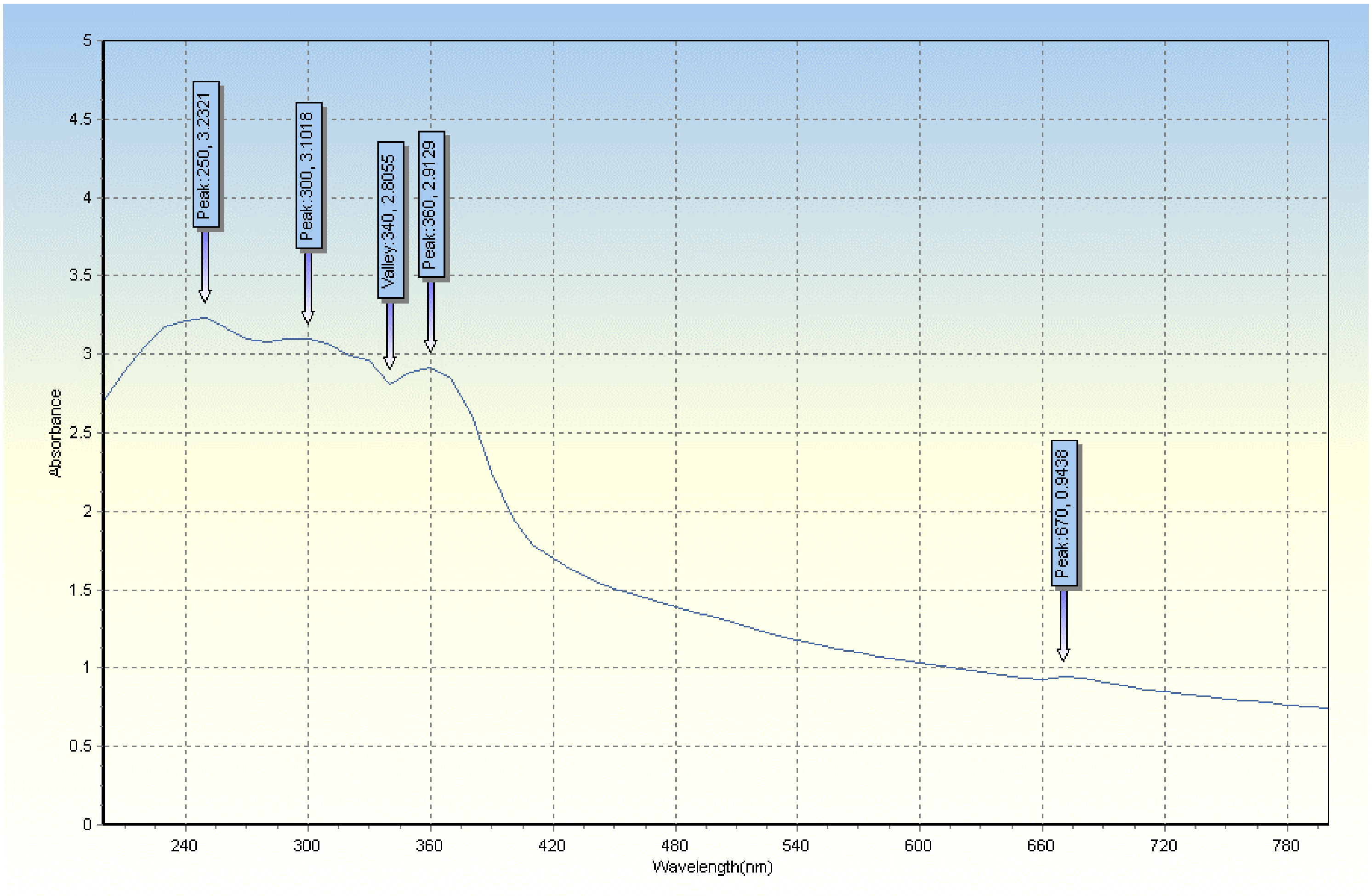

Figure 3

Spectral report of UV-Vis analysis of ZnNPs at 120 hrs

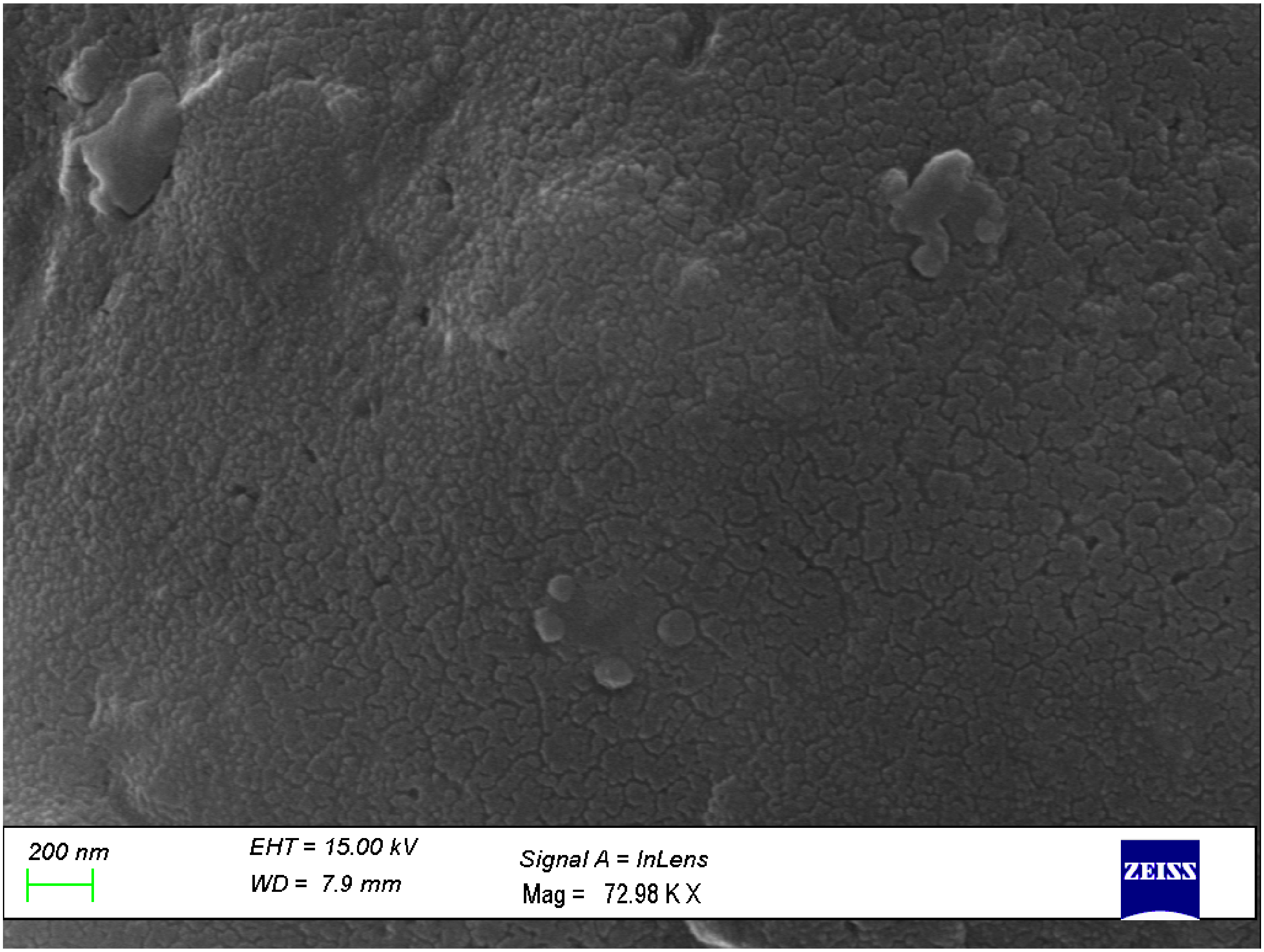

Figure 4

FESEM Image of Zinc nanoparticle.

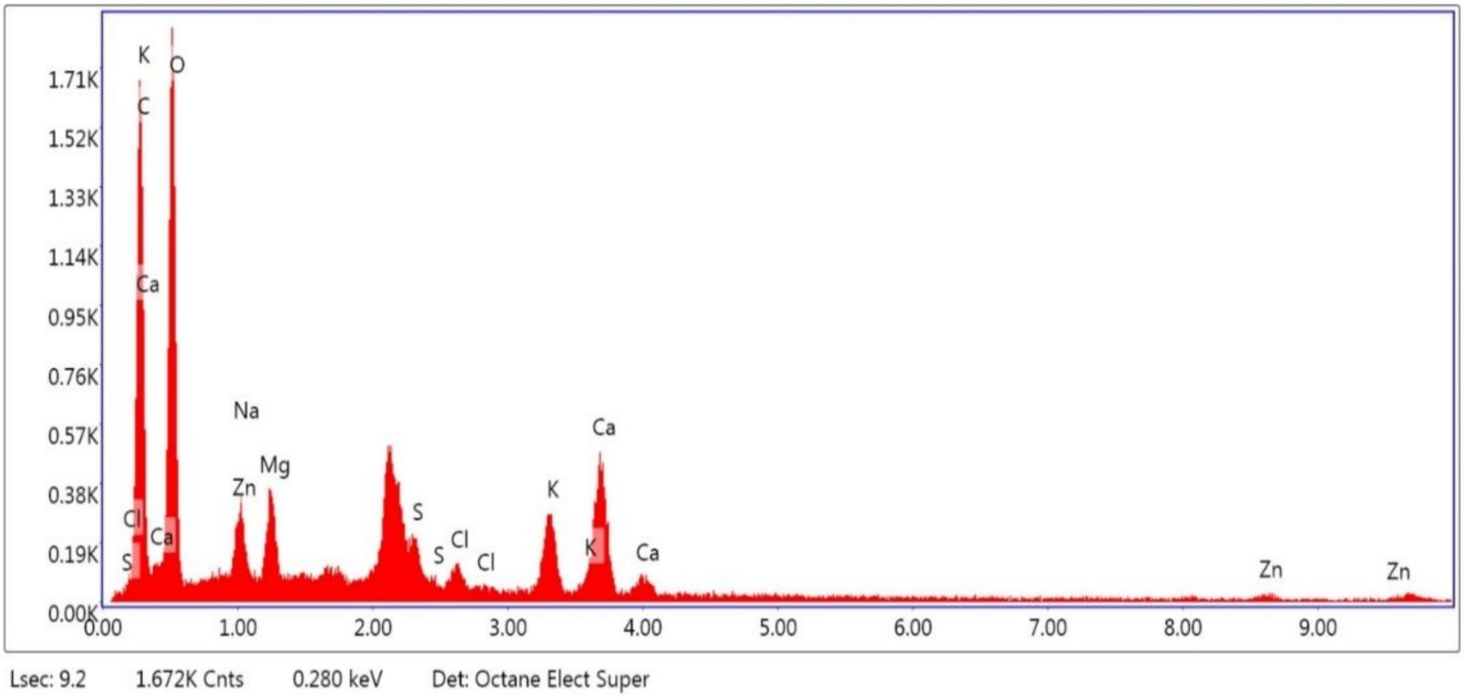

Figure 5

EDX report of Zinc nanoparticles

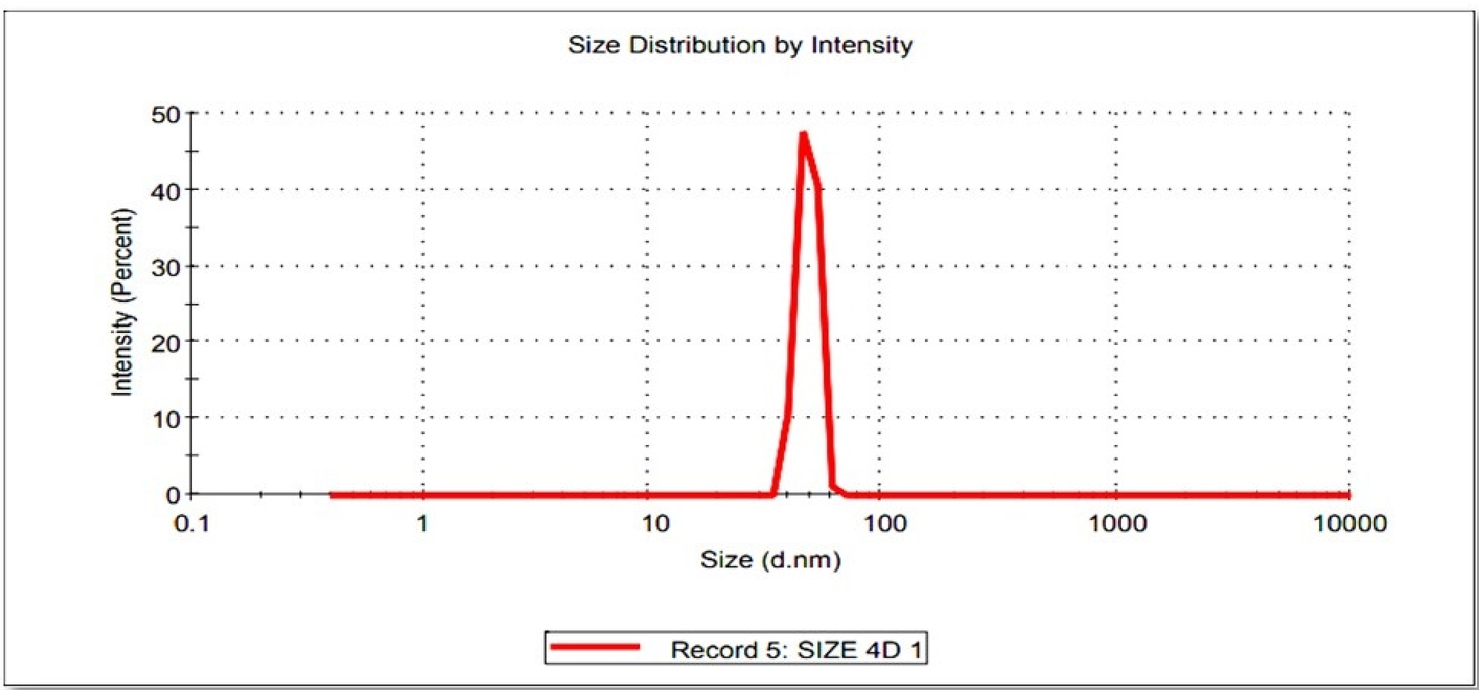

Figure 6

Size distribution intensity graph of zinc nanoparticle as revealed by DLS

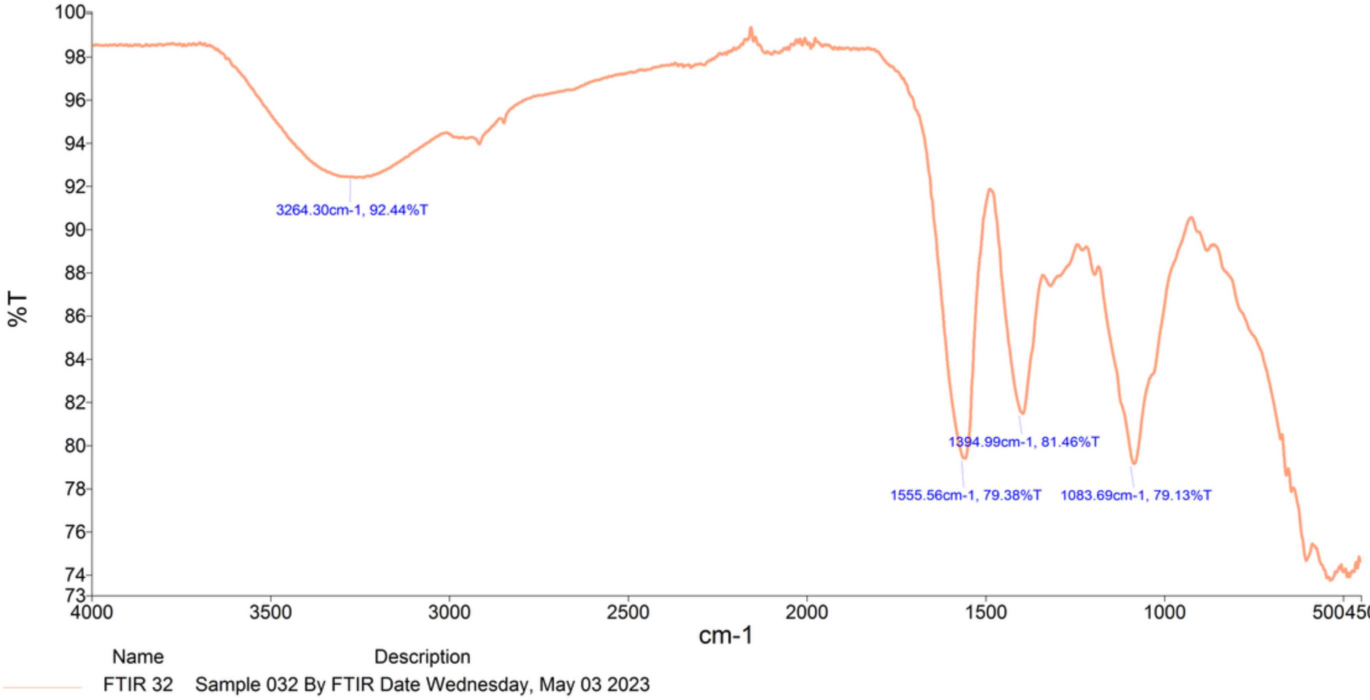

Figure 7

FTIR spectral report of zinc nanoparticle

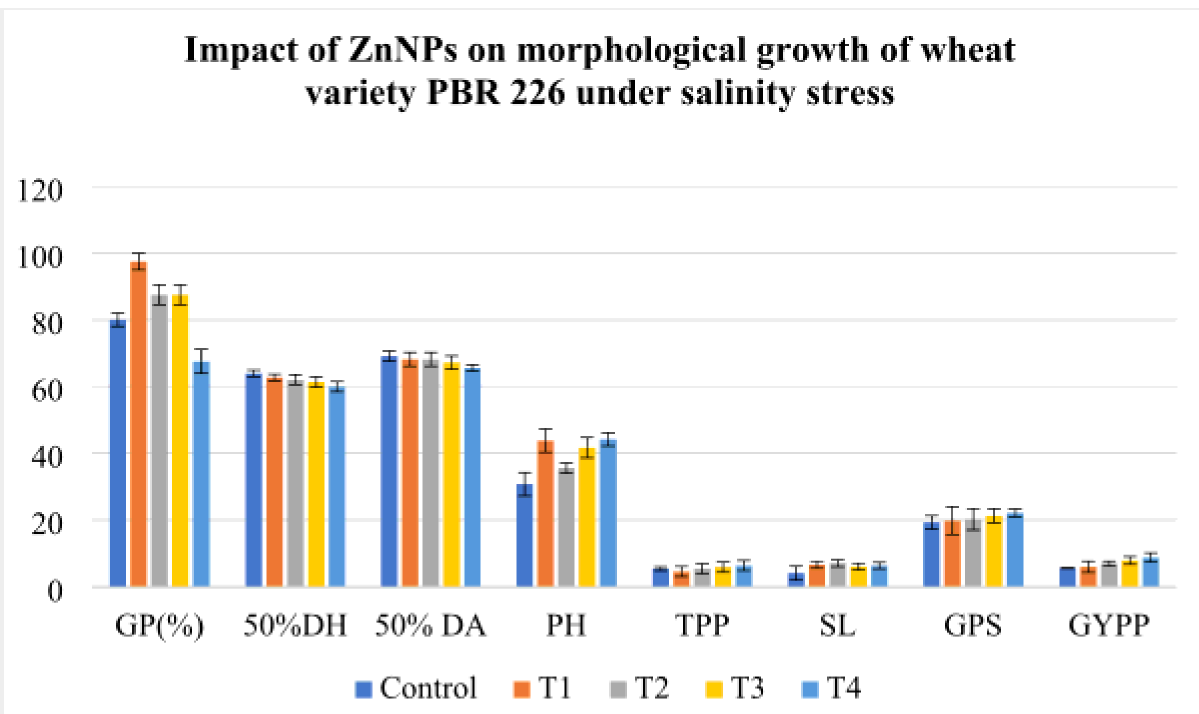

Figure 8

Graphical representation of growth and yield attributes of control and treated wheat plants under salinity stress.