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Biogenic Synthesis of Zinc Nanoparticles using Wheat Root extract and endophytic Bacterium and their Effect on Wheat Germination and Seedling Vigour Index

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ABSTRACT: Nanoparticles are now used much more frequently in consumer goods as a result of the development of nanotechnology. This raises questions about how they may affect the health of plants. In present study, Zinc Oxide nanoparticles (ZnO NPs) were synthesized from wheat root extract and endophytic bacterium by green synthesis method. The synthesized nanoparticles were characterized by UV-visible spectrophotometry which showed an absorbance peak at 362 nm and 365 nm and particle size analysis showed that an average size of zinc oxide nanoparticles synthesized from wheat root extract was 19.66 nm and zinc oxide nanoparticles synthesized from endophytic bacterium was 37.3 nm, respectively. Different concentrations ZnO NPs (250, 500, 750 and 1000 ppm) were used to treat wheat seeds in order to assess their impact on seed germination. Significant effects on germination percentage, root length, shoot length, seedling length, root dry weight, shoot dry weight and seed vigour index (I and II) were seen at 500 ppm. Moreover, a decrease in all parameters that determined the negative effect of nanoparticles at higher concentrations was observed as ZnO NP concentration increased (to 750 ppm). Consequently, the findings of this study contributed that wheat root extract and endophytic bacterium may be employed as suitable sources for the green synthesis of ZnO NPs and that ZnO NP treatment had a substantial impact on seedling growth and Seed vigour index.

Keywords: Wheat root extract, Endophytic bacterium, ZnO NPs, SVI.

INTRODUCTION

Numerous plant enzymatic activities, metabolic processes, oxidation-reduction reactions, processes, and the maintenance of regulatory proteins all need zinc as a key structural co-factor s (Maret, 2009). Zn not only activates crucial enzymes like Alcohol Dehydrogenase (ADH), Carbonic Anhydrase (CA), and structural Zn-finger domains facilitating DNA binding of transcription factors (TFs), but it also makes protein-protein interactions easier (Sriram and Lonchyna 2009). These enzymes play numerous roles in fundamental biochemical and physiological processes, including catalysis of enzymes, carbohydrate metabolism, protein synthesis, preservation of the integrity of cellular membranes, control of auxin biosynthesis, pollen formation, and chlorophyll synthesis (Marschner, 2012). As a result, plants with Zn deficiencies develop more slowly, exhibit leaf nacrosis, have smaller leaves and produce fewer seeds (Elhaj and Unrine 2018). In addition to these effects, earlier literature has shown that Zn plays a substantial role in seed germination and the establishment of seedlings in the field. (Yilmaz *et al.*, 1998; Rengel and Graham 1995; Cakmak, 2000; Marschner, 1995). In India, soil Zn deficiency is currently 36.5% and is projected to rise to around 63% by 2025 (http://zinc.org.in/zinc-uses/zinc-in-crops/) if the current Zn loss trend continues.

In particular, wheat, a cereal crop that makes up a large portion of the diet of the Indian population, has a naturally low Zn concentration. This concentration is further decreased when the crop is grown on soil that is deficient in Zn (Das *et al.*, 2019), which intern leads to human Zn deficiency. In humans, Zn deficiency results in diarrhoea, stunted growth and immunological disorders because Zn plays a vital role in reproduction, development and immunity (Deshpande *et al.*, 2017). According to estimate 26% of Indians are Zn deficient. (http://zinc.org.in/zinc-uses/zinc-in-crops/). More than 2 billion people around the world lack zinc and this leads to 800,000 deaths per year. (http://zinc.org.in/sustainability/).

Several methods are employed to cure wheat plants from Zn deficiency. Zn fertiliser applied to the soil is a straight forward and efficient strategy that has been widely utilised to eliminate soil Zn shortage (Kutman *et al.*, 2010). It is connected to several issues, though. Several soil characteristics, such as salinity, high pH

and the calcareous composition of the soil, affect the bioavailability of zinc in soil or the absorption of zinc by plants from the soil. Due to the unfavourable soil characteristics (high pH, salinity and calcareous composition of the soil), insoluble Zn salt forms, rendering the metal unavailable to plants (Alloway, 2009). Micronutrients are needed in very small amounts and are also only absorbed by plants in extremely little amounts, so using fertilisers excessively wastes money, raises crop production costs and depletes the soil's fertility. Also, the excessive use of fertiliser pollutes the environment by washing out during rainstorms and contaminating rivers and groundwater (Deshpande *et al.,* 2017). Thus, environmental contamination, excessive Zn fertiliser use and soil bioavailability are still issues that require a more effective approach to address. Zinc oxide nanoparticles (ZnO NPs) are currently being employed widely in agricultural applications to address issues with conventional chemical fertilisers. Due to their tunable physical, chemical and biological properties, ZnO NPs have been shown to have a good impact on plant development and yield in numerous prior research (Munir *et al.,* 2018; Mahajan *et al.,* 2011; Rameshraddy *et al.,* 2017; Du *et al.,* 2011).

In present study ZnO NPs has been synthesized from wheat root extract and endophytic bacterium by using green synthesis method. Because it doesn't require any toxic chemicals, high temperatures or pressure and doesn't produce hazardous byproducts, which are frequently produced by physical and chemical methods (Savithramma *et al.,* 2011; Bhumi and Savithramma, 2014), the nanoparticles synthesized using this method are less toxic, more affordable and uniform in size. Further, wheat root extract act as a reducing agent, hence that secondary metabolites may used as a capping agent for synthesizing zinc oxide nanoparticles Therefore, the root extract of this plant can be used as a reducing material and surface stabilizing agent to synthesize ZnO NPs (Bhumi and Savithramma 2014). The synthesized nanoparticles were analyzed by UVspectroscopy and Particle size analysis and further studied to evaluate the effect on seed germination and seedling growth.

Plant material and Endophytic Bacterium. The plant material i.e Wheat variety PDKV Sardar (AKAW 4210-6) used for the synthesis of nanoparticles in the present investigation was collected from Wheat Research Unit, Dr. PDKV, Akola and Bacterial endophyte culture was isolated from wheat root.

Chemicals used during the investigation

Various chemicals used for the synthesis, characterization studies of Nanoparticles as detailed in the specific protocol were purchased from Himedia and SRL.

Preparation of wheat root extract. The 5 g fresh roots of selected plant material was weighed and washed with tap water followed by distilled water to remove the dust particles. The roots were cut into small pieces and then ground by mortar and pestle using 500 ml of de-ionized water until a fine paste is formed. The homogenous mixture was boiled at 60-70ºC for 30 min. After cooling the slurry was filtered using filter paper. The filtrate was centrifuged at 10000 rpm for 30 min to remove the heavy biomass and only the supernatant was taken. The supernatant aqueous solution was again filtered by using Whatman filter paper No. 1 and stored in the refrigerator for further analysis.

Green synthesis of nanoparticles (ZnO-NPs) from wheat root extract

Nanoparticles were prepared by green synthesis as per the protocol of Bagheri *et al.* (2019) with some modifications. The 15 mM solution of salt (Zinc acetate) was prepared in sterile double distilled water. In the Erlenmeyer flask the 0.5% root extract was added to 100 ml of 15 mM metal ion solutions at 90ºC for 45 min on a magnetic stirrer. After 45 min 3% 1N NaOH solution was added into it. The precipitate obtained after the addition of 3 % 1 N NaOH solution was centrifuged at 10,000 rpm for 6-8 min and washed twice with sterile double distilled water to purify them. Then obtained precipitate was dried at 60ºC for 45 min in vaccum concentrator. After drying the precipitate, it was stored at room temperature.

Green synthesis of (ZnO-NPs) nanoparticles from endophytic bacterium

Bacterial strain and culture conditions. The fresh bacterial strain *P. guariconensis* was maintained on nutrient agar medium at 37 ºC for 24 h. Further, the culture was subcultured into nutrient broth medium at continuous orbital shaking at 150 rpm, at 37 °C for 24 h. The supernatant was collected after centrifugation at 5000 rpm for 5 min in overnight bacterial culture and it is used for synthesis of ZnO NPs.

Synthesis of zinc oxide nanoparticles. Nanoparticles were prepared by green synthesis as per the protocol of Neethipathi *et al.,* (2017) with some modifications. The 100 ml of cell free supernatant and 100 ml of Zinc nitrate solution (1 mM) was taken in 500 ml beaker. The beaker containing mixture solution was placed on magnetic stirrer at room temperature for 24 hrs and it was dried at 120 °C.

Characterization of Nanoparticles

UV Visible spectrophotometer. It is the main approach for characterising metal nanoparticle production, stability and molar absorptivity. Free flowing electrons exist in the closely spaced conduction and valence bands. Due to the collective oscillation of these electrons in resonance with light, these electrons cause SPR (Surface Plasmon Resonance), which produces an absorption band. Consider the UV absorption peak in the UV-A and UV-B area of the spectrum that would provide UV protection.

Particle size analysis. The most crucial properties of systems containing nanoparticles are particle size and size distribution. It controls how nanoparticles are distributed *in-vivo*, their biological fate, and their toxicity and targeting capabilities. Furthermore, it may affect the stability and surface area of nanoparticles. Photon-correlation spectroscopy or dynamic light scattering are now the quicker and most common ways to estimate particle size. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

Germination experiment

Seed Treatment. 100 g of Wheat variety PDKV Sardar (AKAW 4210-6) seeds were weighed and were placed them into a container bottle. Different concentrations viz, 250 ppm, 500 ppm, 750 ppm, 1000 ppm solutions of nanoparticles were added into a container bottle and closed the lid tightly. Samples were gently inverted for 2-3 min. After treatment, seeds were spread on a clean canvas or wax paper using a tray. Seeds were dried at room temperature for 24 hrs. Seed germination assay was recorded by paper towel method given by Moshtaghi *et al.* (2015). Three replicates of 50 seeds were placed between moist paper towels and germinated in a germinator at 28ºC for 8 days. Germination (protrusion of radicle by 2 mm) was recorded in daily intervals. The germination rate for each treatment was calculated. Three replicates of 50 seeds were used in the standard germination test (temperature of 28ºC and incubation time of 8 days). At the end of the germination test, lengths of normal seedlings were measured and then they dried in an oven at 80ºC for 24 hours. The dried seedlings were weighed and the mean seedling length and dry weight for each treatment at each replicate were determined. Vigour index was calculated as the product of germination percentage by seedling length. The experiments were performed in triplicate and Completely Randomized Design (CRD) was used to analyze the results. The following parameters were recorded after germination experiments.

Germination percentage (%). Subsequently, seed germination was evaluated and normal seedlings were counted on the 8th day of incubation and considered as standard germination.

Root length (cm), Shoot length (cm), **Seedling length (cm).** From each replication, the random selection of five normal seedlings was done on the 8th day from the start of the germination test. The length of the radicle (in cm) was measured and the mean root length was calculated for each replication.

On the 8th day from the start of germination, randomly five normal seedlings were selected from each replication and shoot length (in cm) was measured for each treatment and the mean of shoot length was calculated.

Seedling length (in cm) was determined by adding shoot and root length.

Fresh weight (g), Dry weight (g). The five randomly selected seedlings were placed on the electronic balance and weight of selected seedlings were measured in gram.

The five randomly selected seedlings were placed in the oven at 80°C for 24 hrs to remove the moisture from seedlings and the weight of dried seedlings were recorded by an electronic balance and measured in gram.

Seedling vigour index-I and Seedling vigour index-II. The seedling vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973).

Seedling Vigor Index (SVI) I = (root length + shoot length) × Germination (%)

Seedling Vigour Index-II = Standard germination (%) × Seedling dry weight (g).

Statistical analysis. Statistical analysis was done by employing standard statistical methods as stated by Panse and Sukhatme (1967) using software OPSTAT and correlation analysis was done as per the formula suggested by Karl Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Green synthesis of metal nanoparticles. Green synthesis of metal nanoparticles was carried out by Wheat root extract and Endophytic bacteria by the protocol of Bagheri *et al.* (2019); Neethipathi *et al.* (2017), respectively with little modifications.

Synthesis of ZnO NPs from wheat root extract: Green synthesis of metal nanoparticles was carried out by Wheat root extract by the protocol of Bagheri *et al.* (2019) with little modifications. Zinc oxide nanoparticles were synthesized by using 15 mM Zinc acetate solution which is the metal precursor for the synthesis of zinc oxide nanoparticles, its colorless solution was changed into white precipitation after the addition of root extract of wheat and NaOH indicating the synthesis of zinc oxide nanoparticles as shown in (Fig. 1). Wheat root extract act as a reducing as well as stabilizing agent, hence that secondary metabolites may used as a capping agent for synthesizing zinc oxide nanoparticles.

Synthesis of ZnO NPs from endophytic bacterium. Green synthesis of metal nanoparticles was carried out by cell free extract of endophytic bacteria by the protocol of Neetipathi *et al.* (2017) with little modifications. Zinc oxide nanoparticles were synthesized by using 15 mM Zinc acetate solution which is the metal precursor for the synthesis of zinc oxide nanoparticles. Before reaction, Zinc acetate solution was colorless but its color was changed into brown after reacted with *P. guariconensis* culture supernatant as depicted in (Fig. 2). This result indicates the synthesis of zinc oxide nanoparticles

Characterization of synthesized ZnO NPs

UV Visible spectrophotometer analysis: The synthesis of ZnO-NPs synthesized from wheat root extract and endophytic bacteria were confirmed by the UV-Visible spectrophotometer. The absorbance peak at 362 nm and 365 nm were observed for ZnO-NPs synthesized from wheat root extract and endophytic bacteria, respectively. The absorbance recorded for ZnO NPs synthesized from wheat root extract was 1.6 on the spectrophotometer. A typical pick is observed as shown in (Fig. 3). Similar results were obtained by Bagheri *et al.* (2019). They synthesized the Zinc oxide nanoparticles from *S. baicalensis* root extract and the absorbance peak of UV-Visible spectrophotometer ranges from 250-550 nm and this indicates the formation of zinc oxide nanoparticles. The absorbance recorded for ZnO NPs synthesized from endophytic bacteria was 0.8 on the spectrophotometer. A typical pick is observed as shown in (Fig. 4). Similar results were obtained by Neethipathi *et al.* (2017). They synthesized the zinc oxide nanoparticles from the cell free supernatant of endophytic bacteria and the absorbance peak of UV-Visible spectrophotometer

ranges from 300 - 900 nm.

Particle size analysis: The particle size distribution was found by NTA (Nanoparticles Tracking Analysis). The particle size distribution curve and particle intensity/size showed that the average size of Zinc oxide nanoparticles from wheat root extract was 19.66 nm and Zinc oxide nanoparticles from endophytic bacteria was 37.3 nm as shown in **(**Fig. 5 and 6). The size of the ZnO nanoparticles synthesized from wheat root extract and endophytic bacteria were found to be below 100 nm as per the definition of the nanoparticles. Sangeetha (2011) reported that the synthesis of nano structured zinc oxide particles by both chemical and biological methods. Highly stable and spherical zinc oxide nanoparticles were produced by using zinc nitrate and Aloe vera leaf extract. The average size was found in between 25-40 nm. Rajendran *et al.* (2021) prepared ZnO NPs using *Rubus fairholmianus* root extract (RE) as an efficient reducing agent. The average particle size calculated from the Debye-Scherrer equation is 11.34 nm. SEM analysis showed that the RE-ZnO NPs spherical in shape with clusters (1-100 nm).

***In-vitro* seed germination:** In the present study effect of ZnO-NPs from Wheat root extract and ZnO-NPs from endophytic bacterium was studied on seed germination of wheat seeds by using the roll paper towel method as shown in (Fig. 7). Seed germination is an important trait of planting value of seed is a process in which radicle and plumule emerge out from the seed coat. The present findings confirmed significant improvement in seed vigor after treatment with nanoparticles when compared with control. Seeds were disinfected with 0.1% (w/v) $HgCl_2$ solution for 2-3 min and washed with sterile distilled water thoroughly (Zafar *et al*., 2016). Seeds were coated with different concentrations of ZnO-NPs synthesized from wheat root extract and endophytic bacteria separately for 4-5 mins, then dried overnight and used for experiments. Untreated seeds were used as a control. *In vitro* study involved seed germination on germination paper as per paper towel method (Phaneendranath, 1980; modified from Masangwa *et al*., 2017). They were watered as required and germinated seeds were recorded after every 2 days. After 8 days of germination, seedlings were harvested and radicle length (RL), plumule length (PL), seedling length (SL), fresh weight, dry weight, SVI I and SVI II was recorded.

Germination percentage (%): The germination percentage of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The germination percentage ranged from 86.53 % to 98.33 %. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest germination percent (98.33 %). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (97.47 %), treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (96.80 %), treatment of 750 ppm (T_7) nanoparticles synthesized from endophytic bacterium (94.73 %), treatment of 1000 ppm (T_4) nanoparticles synthesized from wheat root extract (93.87 %) and treatment of 1000 ppm (T_8) nanoparticles synthesized from endophytic bacterium (93.13 %) as showed in the (Table 1). The obtained results showing the harmony with the findings given by Solanki and Laura (2018); Raja *et al.* (2019) revealed that seeds soaked with 600 mg/l zinc oxide NPs had maximum germination as compared to the control seeds.

Shoot length (cm): The shoot length of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The shoot length ranged from 10.23 cm to 13.92 cm. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest shoot length (13.92 cm). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (13.22 cm), treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (12.61 cm), treatment of 750 ppm (T_7) nanoparticles synthesized from endophytic bacterium (12.11 cm), treatment of 1000 ppm (T_4) nanoparticles synthesized from wheat root extract (11.57 cm) and treatment of 1000 ppm (T_8) nanoparticles synthesized from endophytic bacterium (11.32 cm) as depicted in the (Table 1). According to Prasad *et al.* (2012) at higher concentration of ZnO NPs inhibitory effect was well observed. Similar inhibition was also observed by Lin and Xing (2007).

Root length (cm): The root length of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The root length ranged from 9.42 cm to 12.33 cm. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest root length (12.33 cm). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (12.14 cm), treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (11.93 cm), treatment of 750 ppm (T_7) nanoparticles synthesized from endophytic bacterium (11.81 cm), treatment of 1000 ppm (T_4) nanoparticles synthesized from wheat root extract (10.82 cm), treatment of 1000 ppm (T_8) nanoparticles synthesized from endophytic bacterium (10.66 cm) and treatment of 250 ppm (T_1) nanoparticles synthesized from wheat root extract (10.26 cm) as showed in the (Table 1). Solanki and Laura (2018) revealed that highest root length was observed with ZnO NPs at a concentration of 500 ppm and lowest is recorded with the control.

Seedling length (cm): The seedling length of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The seedling length ranged from 19.65 cm to 26.10 cm. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest seedling length (26.10 cm). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (25.30 cm), treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (24.54 cm), treatment of 750 ppm (T_7) nanoparticles synthesized from endophytic bacterium (23.92 cm) and treatment of 1000 ppm (T_4) nanoparticles synthesized from wheat root extract (22.30 cm) as showed in the

(Table 1). The outcomes are in accordance with the findings of Solanki and Laura (2018) stated that higher concentration of Zn causes significant reduction in root as well as shoots length, hence the seedling length.

Fresh weight (g): The fresh weight of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The fresh weight ranged from 0.25 g to 0.36 g. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest fresh weight (0.36 g). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (0.34 g), treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (0.32 g), treatment of 750 ppm (T_7) nanoparticles synthesized from endophytic bacterium (0.30 g), treatment of 1000 ppm (T_4) nanoparticles synthesized from wheat root extract (0.31 g) and treatment of 1000 ppm (T_8) nanoparticles synthesized from endophytic bacterium (0.29 g) as depicted in the (Table 1). Nano-ZnO (500 ppm) has the highest fresh weight of 0.34g. However, the lowest fresh was observed to be of control. The fresh weight of the seedling decreases with increasing concentration of ZnO NPs was recorded by Solanki and Laura (2018).

Dry weight (g): The dry weight of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The dry weight ranged from 0.13 g to 0.22 g. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest dry weight (0.22 g). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (0.21 g) and treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (0.19 g) as showed in the (Table 1). According to Solanki and Laura (2018) the Nano-ZnO (500 ppm) has the highest dry weight. However, the lowest fresh was observed to be of control. The dry weight of the seedling decreases with increasing concentration of ZnO NPs was recorded

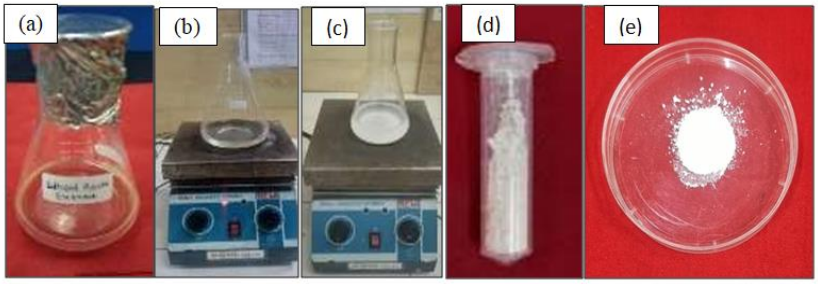
Seedling vigor index-I: The seedling vigor index I of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The seedling vigor index I ranged from 1699 to 2568. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest seedling vigor index I (2568). However, this treatment was found to be statistically at par with the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (2465), treatment of 750 ppm (T_3) nanoparticles synthesized from wheat root extract (2375) and treatment of 750 ppm (T_7) nanoparticles synthesized from endophytic bacterium (2267) as showed in the Table 1. Similar results of ZnO NPs were depicted by Rawat *et al.* (2018) in case of seedling vigor index I.

Seedling vigor index -II: The standard germination of wheat variety PDKV Sardar (AKAW 4210-6) was significantly affected by different treatments. The seedling vigor index II ranged from 11.24 to 21.63. Treatment of 500 ppm (T_2) nanoparticles synthesized from wheat root extract recorded highest seedling vigor index II (21.64) followed by the treatment of 500 ppm (T_6) nanoparticles synthesized from endophytic bacterium (20.47) as showed in the (Table 1). Similar results of ZnO NPs were obtained by Mamun *et al.* (2018) in case of seedling vigor index II.

Table 1: Effect of seed treatments of nanoparticles synthesized from wheat root extract and endophytic bacterium on seedling growth under laboratory conditions.

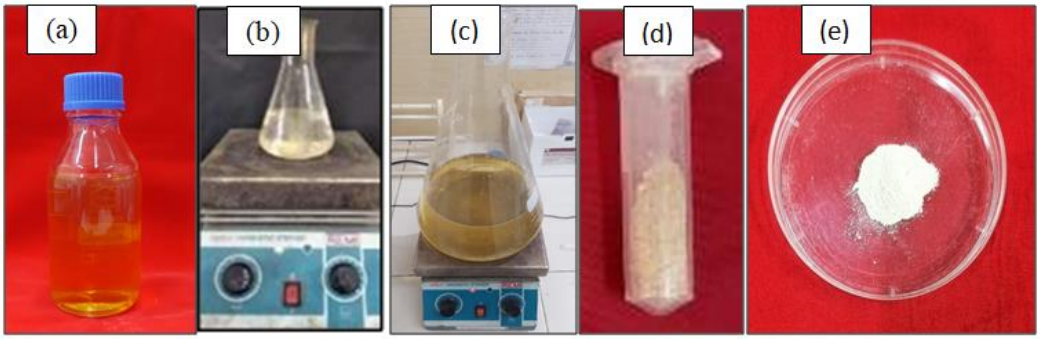
Treatments	Germination percentage (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Fresh weight (g)	Dry weight (g)	Seedling Vigor Index I	Seedling Vigor Index II
T_0 (Control (Water))	86.53	10.23	9.42	19.65	0.25	0.13	1699	11.24
T_1 ZnO NPs - WRE (250 ppm)	92.24	11.11	10.26	21.37	0.28	0.16	1970	14.75
T_2 ZnO NPs - WRE (500 ppm)	98.33	13.92	12.33	26.10	0.36	0.22	2568	21.64
T_3 ZnO NPs - WRE (750 ppm)	96.80	12.61	11.93	24.54	0.32	0.19	2375	18.39
T_4 ZnO NPs - WRE (1000ppm)	93.87	11.57	10.82	22.30	0.31	0.18	2093	16.89
T_5 ZnO NPs - ENB (250 ppm)	91.67	10.99	9.47	20.43	0.26	0.15	1874	13.76
T_6 ZnO NPs - ENB (500 ppm)	97.47	13.22	12.14	25.30	0.34	0.21	2465	20.47
T_7 ZnO NPs - ENB (750 ppm)	94.73	12.11	11.81	23.92	0.30	0.18	2267	17.10
T_8 ZnO NPs - ENB (1000ppm)	93.13	11.32	10.66	21.90	0.29	0.17	2040	15.84
SEM±	**1.49**	**0.66**	**0.66**	**0.99**	**0.017**	**0.006**	**105.58**	**0.74**
C.D. @ 1 %	**6.07**	**2.70**	**2.71**	**4.03**	**0.073**	**0.028**	**429.78**	**3.01**

WRE : Wheat root extract; ENB : Endophytic bacterium



(a) Filtrate of root extract; (b) 15 mM Zinc Acetate + 0.5 % root extract + boiling at 90°C; (c) Precipitation after addition of 3 % NaOH; (d) After centrifugation nanoparticles dried in vacuum concentrator; (e) Nanoparticles in powder form.

Fig. 1. Synthesis of ZnO NPs from wheat root extract.



(a) Cell free supernatant of endophytic bacterium; (b) 15 mM Zinc Acetate + 0.5 % cell free supernatant + boiling at 90°C; (c) Precipitation after addition of 3 % NaOH; (d) After centrifugation nanoparticles dried in vacuum concentrator; (e) Nanoparticles in powder form.

Fig. 2 Synthesis of ZnO NPs from endophytic bacterium.

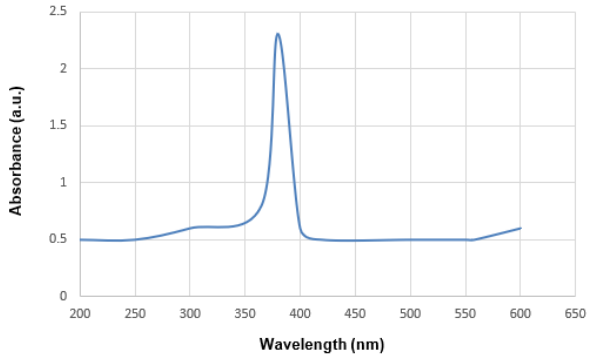


Fig. 3. UV-visible spectrometry of ZnO NPs synthesized from wheat root extract.

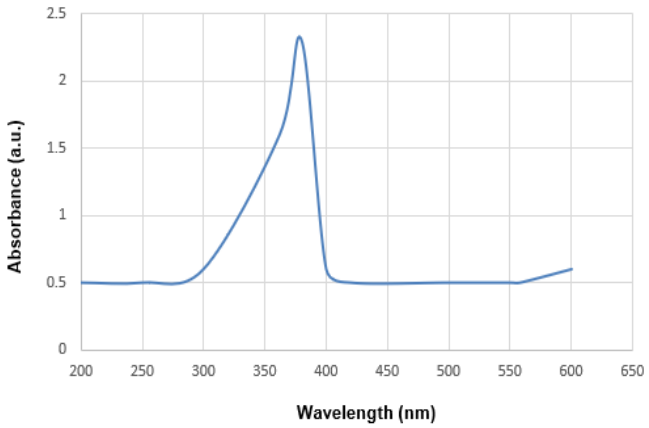


Fig. 4. UV-visible spectrometry of ZnO NPs synthesized from endophytic bacterium.

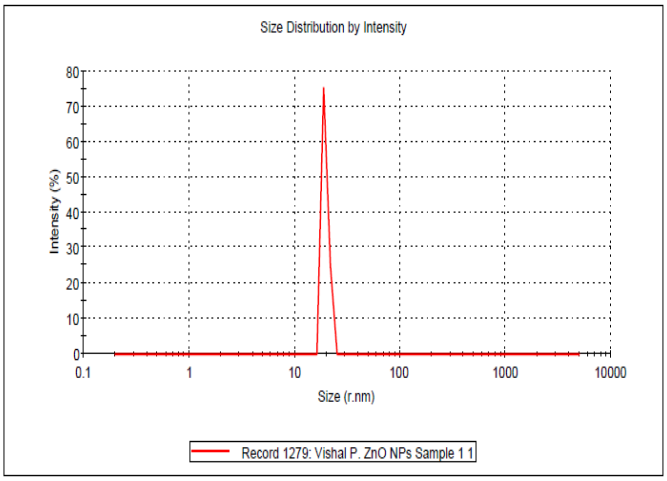


Fig. 5. Particle size analysis of ZnO NPs synthesized from wheat root extract.

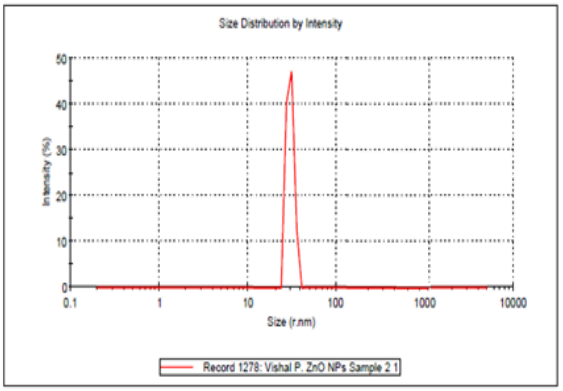


Fig. 6. Particle size analysis of ZnO NPs synthesized from endophytic bacterium.

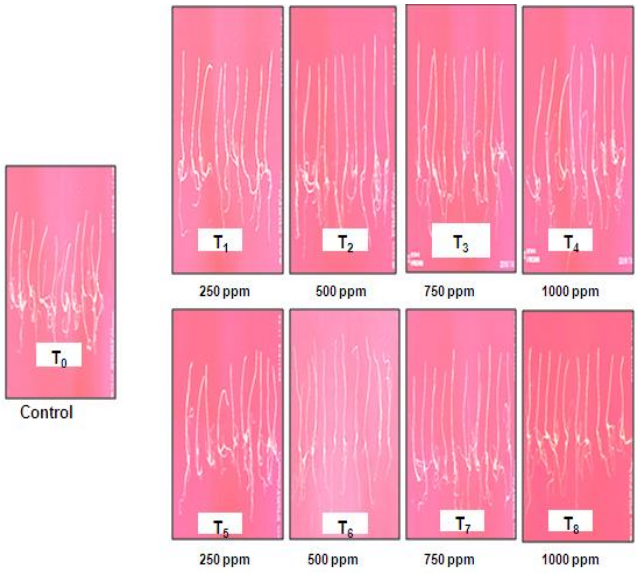


Fig. 7. Effect of seed treatment on various seed growth attributes of PDKV Sardar (AKAW 4210-6) under the laboratory conditions.

CONCLUSIONS

Present study concluded, ZnO NPs can be synthesized by using wheat root extract and endophytic bacterium as a reducing agent. UV-visible spectroscopy and Particle size analysis indicated the synthesis of ZnO NPs. The significant increase was observed in germination percentage, root length, shoot length, seedling length, root dry weight, shoot dry weight, seed vigour index I and seed vigour index II at different concentrations of ZnO NPs treatment. The maximum increase in all parameters was exhibited at 500 ppm concentration. Further, increase in concentration (750 ppm) showed a reduction in all parameters that was possible due to the toxicity of nanoparticles at higher concentration.

FUTURE SCOPE

The optimized concentration i.e (500 ppm) of ZnO Nanoparticles can be exploited in future for the commercial investigation.

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Conflicts of Interest. None.

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