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Research Article

Evaluation and Standardization of Green Synthesized Zinc Oxide Nanoparticles for Seed Priming in Sorghum (*Sorghum bicolor*)

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Abstract

Zinc oxide nanoparticles (Nps) was synthesized using Hibiscus leaf extract and Aspergillus niger and characterized by Particle size analyzer and Dynamic Light scattering method. The average particle size obtained was approximately <100 nm. Different concentrations of ZnO NPs were used for standardization of seed priming in Sorghum. The seeds were soaked for 10 hours in different concentrations of ZnO NPs synthesized from hibiscus leaf extract and Aspergillus niger (@ 500 ppm & 1000 ppm), along with chemically synthesized ZnO NPs (50nm and 100nm @ 500ppm & 1000 ppm each), recommended bulk ZnSO4 (500 ppm) and control (water soaking). Later it was shade dried and kept for germination in the germination paper for a period of 12 days. The observations were recorded such as germination rate, shoot length, root length, Root: shoot and seedling vigour index. It was observed that among all the treatments, the ZnO NPs synthesized from Aspergillus niger @ 1000ppm showed significantly highest germination rate (96.42%), root length (27.85cm), shoot length (20.60 cm), and seedling vigour index (4672) as compared to control [germination rate (89.28%), root length (25.05cm) and shoot length (17.55cm) and seedling vigour index (3803)] and it was on par with ZnO NPs@ 1000ppm from Hibiscus leaf extract. This study concludes that the green synthesized ZnO nanoparticles @ 1000 ppm is optimum for seed priming in Sorghum.

Keywords: *Green synthesis, Nanoparticles, Aspergillus niger, Sorghum, seed priming*

Introduction

Generally, the cereal grains are relatively poor in zinc content. It is estimated that about 40% of the Indian soils are deficient in zinc and when cereal crops are grown on these Zn deficient soils will further decreases the grain zinc concentration. This is due to the intensive cropping system, extensive application of inorganic fertilizer, less usage of organic manures and poor-quality irrigation water with high calcium carbonate content (Shukla and Tiwari, 2016). It has been reported that the Zn application on cereals, pulses, oilseeds, fibre crops and vegetables resulted in 9-12% increase in the yield at different locations in India. Application of Zinc not only increases the yield but also improves the grain quality (Shukla and Tiwari, 2016).

Among the cereal crops, Sorghum is one of the major staple food crops in India especially in rural and resource poor regions. It is cultivated in almost all regions by small and marginal farmers. Sorghum grains are considered as one of the components in poultry and livestock feed (Abdelhalim *et al.*, 2019).

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Although Sorghum is a C_4 species having high photosynthetic efficiency and high biomass yield potential but under adverse recurrent agro-environmental perturbations such as drought which is becoming more common as the climate continues to change, there is a clear need to improve the sustainability of crop production, so as to ensure global security of food, feed and agro-industrial production systems. The major challenge for global food and nutritional security is to feed the increasing world population with nutritious food even under adverse environmental conditions. Therefore, in the future, it is essential to increase not only production but also of high-quality food with the required level of nutrients and protein is the main challenge (Dimpka *et al*., 2019)
Nanotechnology is a multidisciplinary application used in various fields of science which includes medical science, pharmaceutical industries, electrical, electronic, tele communication, biotechnology and also in agriculture, food industries and other allied sectors. Metals exhibit different properties when gets reduced to nanosize level. The nanoparticles are studied extensively for their optical, catalytic, electronic, magnetic and other antimicrobial activities. In recent times, the nanoparticles synthesized from the plant or microbial extracts are much preferred than chemical

synthesis due to its simplicity, eco-friendliness and extensive antimicrobial activity, non-toxic by-products and large-scale synthesis (Vanaja *et al*., 2013).

Zinc oxide nanoparticles (NPs) are known to be one of the multifunctional inorganic metal oxide which enhances plant growth, probably due to increase in the level of endogenous auxin content which act as a cofactor for IAA biosynthesis. Another predicted reason might be the capacity of nano particle to penetrate through seed coat easily because of its smaller size and better absorption and utilization ability (Korishettar *et al.,* 2016).

Zinc also plays an important role in combating drought stress. A recent study highlights that during drought stress, it significantly reduced the grain Zn concentration in wheat by as much as 9.3% which was overcome by the foliar application of Zinc and thereby reduced the negative effects of drought stress. Also, similar study shows that appropriate Zinc application in crops would alleviate climate change impacts on crop yield and nutritional quality (Yavas and Unay, 2016). Therefore sustained research initiatives are needed to increase the zinc uptake through the development of innovative fertilizer sources. Recently, nanotechnology is coming into focus because nanoparticles (NPs) are smaller in size (<100 nm) having high surface area and reactivity. Recent studies revealed that powdered or nano sized particles are found to be effective in absorption and translocation. But, physiological aspects of nano zinc application and its accumulation in grains crops are less studied (Kon *et al.,* 2015). Hence, the present study was carried out to synthesize ZnO nanoparticles using Hibiscus leaf extract and from fungul extract (*Asperigillus niger*) and further to study its effect on germination by seed priming of Sorghum seeds with different concentration treatments.

Materials and Methods

Preparation of ZnO NPs using hibiscus leaf extract

Five grams of *Hibiscus* leaves were washed thoroughly with distilled water and both surfaces of leaves were sterilized using alcohol by gently rubbing. Then the leaves were heated for 30 min in 100 ml of distilled water at 50 °C. The extract was filtrated with Whattman filter paper no. 1 and further filtered using vacuum filter with pore size of 0.2 µm. The final filtrate was stored in cool dry place for further use (Bala N., 2015).

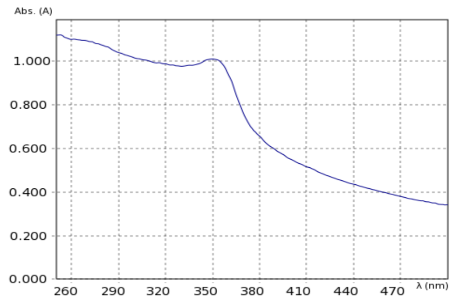
20 ml of the plant extract was heated at 50°C for 10 min and 50ml of 91 mM of zinc acetate solution (1 gm of zinc acetate was dissolved in 50 ml of distilled water) was added drop by drop with continues stirring until the reaction mixture became yellowish and cream colored precipitate of zinc hydroxide was formed. The reaction mixture was left for 30 min for complete reduction of zinc hydroxide to zinc oxide. Then the precipitate was collected by centrifugation at 16000 rpm for 10 min at 4°C. The precipitate was vacuum dried at 30°C and the sample was stored for further studies (Bala N., 2015).

Preparation of ZnO Nps using Aspergillus niger

The culture medium of fungus was comprised of the liquid (g/l) K_2HPO_4, 2.0; $(NH_4)_2SO_4$, 1.0; Yeast extract, 0.6; $MgSO_4 \bullet 7H_2O$, 0.1; KH_2PO_4, 7.0; and glucose, 10.0. The pH level was adjusted to 6.2 ± 0.2. The fungus was placed inside the flask and orbital shaker was used to incubate the fungus at 200 rpm and at the temperature of 37 °C. The culture of the fungi was filtered through Whatman no.1 filter paper. 91 mM Zinc acetate was added and kept in a shaking incubator at 200 rpm at 32 °C for 2 days. The white precipitate was produced at the bottom of the flask that indicates the reduction process. The white precipitate was centrifuged at 10,000 rpm for 10 min, and then it was vacuum dried at 30°C and stored for further studies (Kalpana *et al*., 2018).

Characterization of green synthesized ZnO NPs

Initially the synthesized ZnO NPs was confirmed using UV visible spectrophotometer which determines the optical property of the sample by measuring its maximum absorbance peak at 436nm (Fig1). Later the particle size was determined using particle size analyzer which focuses on particle size distribution based on the laser diffraction method (Fig 2). Further the ZnO NPs were characterized by Dynamic Light scattering technique which measures the intensity of the distribution of the particle (Fig 3). The Scanning electron microscopy (SEM) images were also taken to see the morphological features especially the size and shape of ZnO NPs (Fig 4).



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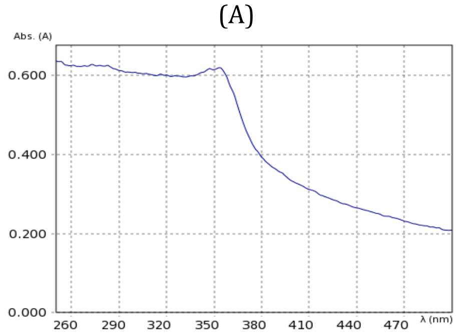

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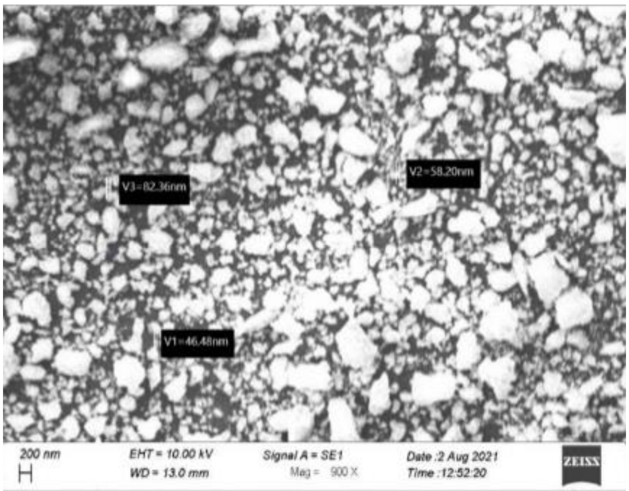
Figure1: Confirmation of Zn Nanaoparticle using UV-visible spectroscopy; A-Aspergillus niger, B- Hibiscus



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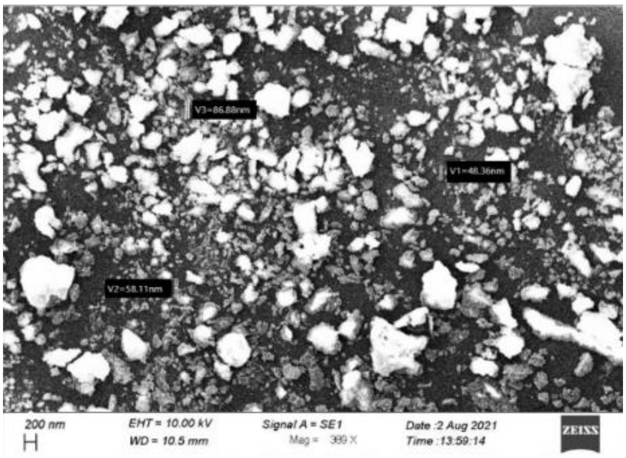

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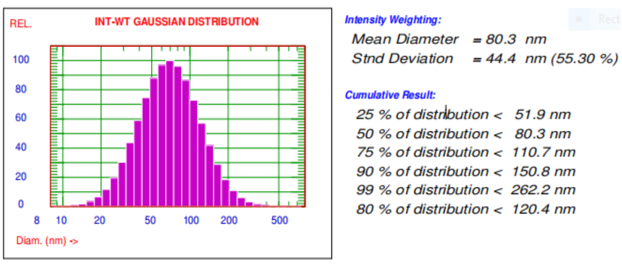
Figure 2: Size determination by Particle size Analyser; A- *Aspergillus niger*, B- Hibiscus



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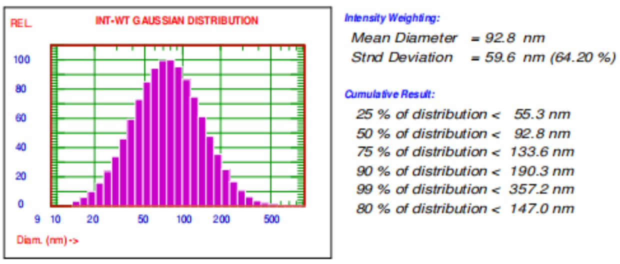
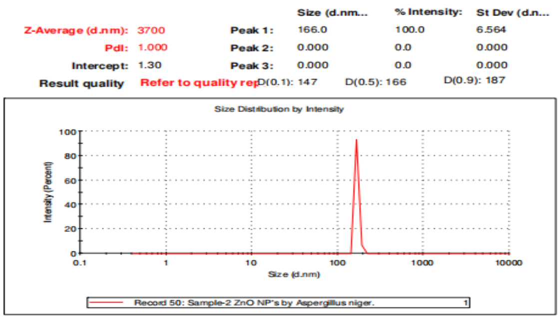


(B)

Figure 3: Particle size distribution by Dynamic light scaterring; A- Aspergillus niger, B-Hibiscus



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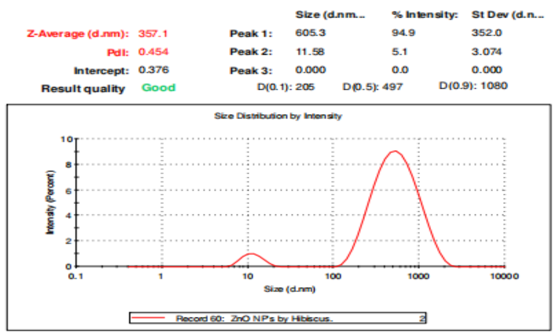

(B)

Figure 4: SEM image of ZnO nanoparticles in A Aspergillus niger and B: Hibiscus

Results and Discussion

Seed priming of different concentrations of ZnO nanoparticles (extracted from Hibiscus leaf extract and Aspergillus niger)

The lab experiment was set up with different concentrations of ZnO nanoparticles (500 ppm and 1000 ppm) extracted from Hibiscus leaf and *Aspergillus niger* along with the chemically synthesized ZnO (50nm and 100 nm) and bulk $ZnSO_4$ (500ppm) and control. The seeds were soaked in the different concentration treatments for 12 hours, later shade dried and kept for germination in a germination paper for a period of 10 days. The observations such as germination percentage (%), Root length (cm), Shoot length (cm), Root:Shoot, Seedling vigour index were recorded (Table 1).

Accordingly the highest germination rate was observed in sorghum seeds primed with 1000 ppm ZnO NPs synthesized both from Hibiscus leaf extract (96.42%) and *Aspergillus niger* (96.42%) as compared to control (89.28 %) and other treatments. Similar results were obtained by Jayarambabu *et al.*, 2014, with the application of nano zinc in mungbean. The increased germination may be due to better penetration of ZnO NPs inside the plant cell wall which in turn stimulates growth hormones such as auxin and gibberellins which promotes cell division and cell elongation process for enhancement of germination (Table 1).

Table 1: Invitro studies on the effect of seed priming with different concentrations of green synthesized ZnO NPs on sorghum seeds

Treatments		% Germination	Shoot length (cm)	Root length (cm)	Root:Shoot	SVI
T_1	Hibiscus extract 500 ppm	92.85	26.90	20.30	0.75	4383
T_2	Hibiscus extract 1000 ppm	96.42	27.30	20.35	0.74	4594
T_3	*Aspergillus niger* 500 ppm	92.85	26.20	19.80	0.75	4271
T_4	*Aspergillus niger* 1000 ppm	96.42	27.85	20.60	0.73	4672
T_5	Nano (<50nm) 500 ppm	89.28	25.20	17.75	0.70	3835
T_6	Nano (<50nm) 1000 ppm	92.85	25.40	18.48	0.72	4074
T_7	Nano (<100nm) 500 ppm	92.85	25.90	18.90	0.73	4160
T_8	Nano (<100nm) 1000 ppm	92.85	25.95	19.25	0.74	4197
T_9	Bulk $ZnSO_4$ 1000 ppm--	89.28	25.05	17.55	0.70	3803
T_{10}	Control	89.28	24.85	16.00	0.64	3647

The highest root length, shoot length and seedling vigor index (SVI) was observed in the sorghum seeds primed with 1000 ppm ZnO nanoparticles synthesized both from Hibiscus leaf extract (27.30 cm, 20.35 cm, 4594) and *Aspergillus niger* (27.85cm, 20.60, 4672) as compared to control (25.05cm, 17.55cm, 3803cm) respectively (Table 1). This can be attributed to increased mobilization of photometabolites within the seeds during soaking with ZnO NPs. Zn induces a range of physiological changes in the seed required for germination process, such as breaking of dormancy, hydrolysis or metabolization of inhibitors, imbibitions and enzyme activation (Harris *et al.*, 2007 and Samad *et al.,* 2014). Interestingly, in the present study there is much higher increase in the seedling vigour observed in nano ZnO treatments compared to bulk ZnSO4 which may be because of the efficient translocation and reactivity of nano zinc with other plant biomolecules (Pavithra *et al.,* 2017).

While the highest root: shoot ratio was observed in sorghum seeds primed with 500 ppm ZnO nanoparticles synthesized both from Hibiscus leaf extract (0.75) and *Aspergillus niger* (0.75) followed by 1000 ppm ZnO from Hibiscus leaf extract (0.74) and *Aspergillus niger* (0.73) as compared to control (0.64) (Table 1). The root is the most important and decisive plant part for water nutrient uptake and translocation from the soil to the above-ground parts. The root to shoot ratio is not only a primary indicator of physiological processes affecting crop growth and yield; it also aids in understanding the distribution of photosynthates and nutrients to above and below ground parts, as well as determining the source to sink ratio (Upadhyaya *et al.,*2015).

Conclusion

From this study we can conclude that zinc oxide (ZnO) nanoparticle at a concentration of 1000 ppm synthesized from *Aspergillus niger* and Hibiscus leaf extract was found to be most effective in seed priming of sorghum seeds which improves the overall plant growth as compared to bulk ZnSO4 fertilizer.

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Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

The authors have no conflicts of interest to declare that are relevant to the content of this article.

All authors certify that we have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

The authors have no financial or proprietary interests in any material discussed in this article.

Dr. Nethra.P, Nanditha. B.P, Dr. Ashwini. M and Dr. R.V. Koti declare that we do not have any conflict of Interest.

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