Synthesis and characterization of zinc oxide nano particles (ZnO NPs) and their effect on growth, Zn content and yield of rice (*Oryza sativa* L.)

Kuldeep Singh, Mukil Madhusudanan
Amity Centre for Soil Sciences
Amity University Uttar Pradesh
Noida, India
ksingh6@amity.edu, mmukil@amity.edu

Naleeni Ramawat
Amity Institute of Organic Agriculture
Amity University Uttar Pradesh
Noida, India
nramawat@amity.edu

Abstract— Zinc is the most crucial micronutrient when judged in respect of its deficiency in Indian soils. Nano particles (NPs) role on growth and development of cereals has scantly been reported. Laboratory scale and greenhouse experiments were carried out to examine the impact of zinc oxide NPs on rice growth traits and yield. Nano-zinc oxide and zinc sulfate were applied at various concentrations in suspension and in ionic form, respectively. A study was also conducted to characterize the shape and size of synthesized ZnO NPs. The ZnO NPs application improved seed germination, root/shoot growth, seedling vigor index, chlorophyll content, grain zinc concentration and yield. An increase in grain zinc concentration was observed on application of ZnO NPs over control (49.38%) and $ZnSO_4$ (8.49%). Grain yield was also improved by 8.84% and 3.89% respectively over control and $ZnSO_4$. Delivery of Zn nutrient through ZnO nanoparticles in right dose and of the right size could be effective and beneficial in enhancing the growth and yield attributes of rice crop. There is a possibility of reducing dose of Zn using nanostructured fertilizer like ZnO- NPs.

Keywords— NanoFertilizer, ZnO nanoparticles, Rice, Yield, Zinc Concentration

I. INTRODUCTION

Zinc (Zn) is one of the 17 indispensable elements which plays a key role for the normal growth and developments of the plants. Zinc is a key component of many enzymes and protein as it serves a significant role in a broad range of processes, such as growth hormone production [1] and internode elongation [2]. It is required in small amounts, but yet crucial to plant development. Zinc is the most important essential micronutrient that is required to enhance the productivity of the crop. Zinc is required for chlorophyll production, pollen function, fertilization and germination. Zinc is intermediate in its mobility or phloem export. Zn moves from leaves to roots, stem and developing grain and from one root to another. In industry nano-zinc oxide (ZnO) has been extensively used for several decades. But its application in agricultural field is still not emphasized and practiced so as to increase the nutrient use efficiency.

Zinc deficiency is a well-recognized global, micronutrient problem in agriculture soils resulting in sub-optimal production and also in human populations leading to poor human health. More than 90% of Zn in the soils occur as insoluble Zinc species and is unavailable to plants [3]. About 95% of rice crops are grown and consumed in Asia. Zn deficiency is the most widespread micronutrient disorder in rice [4]. In recent years, remarkable progress has been made in developing nanotechnology and it also provides the tool and the technological platforms to investigate its effect on biological systems. In addition to their small size, these particles are characterized by their large surface areas, high aspect ratios, and unusual surface properties [5]. Moreover nanotechnology is currently witnessing impressive advances in various directions such as synthesis of nano-particles (NPs), understanding their fundamental physical and chemical properties and organization of complex nano-scale matter using weak non-covalent interactions. Of late, engineered nano-materials have received a particular attention for their positive impact in improving agricultural productivity and are being increasingly produced for a wide range of applications. Nanoparticles of size below 100 nm fall in the transition zone between individual molecules and the corresponding bulk materials, which generate both positive and negative biological effects in living cell [6] [7]. Limited reports underline positive or no adverse effects of NPs on higher plants.

Rice (*Oryza sativa* L.) is widely cultivated in India and is produced more than any other grain crop. The importance of rice lies in its wide variety of applications besides serving as human food and animal feed. Nanoparticles with small size and large surface area are expected to be the ideal candidates for use as a Zn fertilizer in plants [8] [9]. Plant growth is hindered in low Zn soils and addition of Zn fertilizer to soil can improve plant growth. One role of nanoparticles in agriculture is stimulating crop growth [10]. Nanoparticles are estimated to be absorbed 15–20 times more than their bulk particles [11]. Shen *et al.* [12] reported that toxicity of ZnO NPs was higher in acidic soil than in neutral soil and that toxicity is lowest in alkaline soil. Strategies to address zinc deficiency in soil-animal-human chain are to apply Zn fertilizer to soil, seed, and foliar zinc application. Efficient use of Zn fertilizers assumed serious importance because their raw materials are fixed and insufficient availability is foreseen.

In the rural areas of India, rice and wheat contributes nearly 75% of the daily calorie intake [13]. These facts clearly point to an urgent need for a better Zn concentration of cereal grains in India. Before application, in-field characterization of nano-particles is essential to understand its behavior and reaction kinetics in soils and plants. According to research reviewed by Capaldi *et al.* [14], several possible mechanisms exist. Nanoparticles could decompose directly in the soil and produce ions, which may be incorporated in the plant system. Large NPs may first decompose in soil and produce smaller NPs, which may be incorporated in plant tissues. Alternatively, these smaller

NPs may further decompose forming ions, which can be incorporated in plant tissues. Exposure with plant tissue, NPs penetrate in to the cell wall and cell membrane of epidermis, cortex of root accompanied by a complex series of events to enter plant vascular bundle (xylem), and move to the stele. In recent years there has been mounting interest in management of fertilizers application in soils to obtain higher fertilizer use efficiency.

An attempt has been made in the present investigation to synthesize, characterize and study the effect of various concentrations of ZnO nano-particles in comparison to ZnSO4 application on growth, Zn content and yield of rice crop.

II. MATERIALS AND METHODS

A. *Synthesis and Characterisation of Zinc Oxide Nanoparticles*

Zinc oxide nanoparticles were prepared following the method of of Khizar Hayat *et al*. [15]. Further we have added 0.9 M sodium hydroxide [NaOH] to zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] solution drop wise at 55^oC under continuous high speed stirring. Further the solution was probe sonicated at higher amplitude for 30 min, then finally dried in a vacuum at 60^oC. ZnO NPs of size of 30-100 nm were used in the study.

The morphology and size of ZnO NPs was determined using scanning electron microscopy (SEM Zeiss MA EVO -18). The elemental composition of ZnO nanoparticles was confirmed by UV-Vis spectrophotometer (Shimadzu UV-1800) and energy dispersive analysis of X-rays (EDAX). Particle size distribution of nanoparticles was measured using dynamic light scattering (DLS Malvern Nano S 90).

B. *Preparation of Particle Suspensions and Zinc Ion Solution*

Using mechanical stirrer and ultrasonic vibration (100 W, 40 kHz) for 30 min, the synthesized ZnO nano-particles were added to double distilled water (DDW), suspended and dispersed. Zinc sulfate solution was prepared by dissolving zinc sulfate heptahydrate (ZnSO4.7H2O) in DDW.

C. *Seeds*

Rice seeds of variety 'PB 1121' were used having maturity with an average 140 days. Seeds were kept in a dry place in the dark under room temperature before use. The selected seeds were of uniform size to minimize errors in seeds germination and seedling vigor index.

D. *Seed germination*

Rice seeds were immersed in a 5% sodium hypochlorite solution for 15 min for sterilization. After rinsing three times with double distilled (DD) water, they were soaked in nano-ZnO suspensions and ZnSO4 at various concentrations (10, 50, 100, 500, 1000 ppm) and at various soaking periods (24, 48 and 72 h) in an incubator at ambient conditions (26±1°C) in the dark, DD water was used in the soaking process for a better control of the media (as per the recommendations of the International Seed Testing Association [16]. A piece of filter paper was put into each petri dish (100 mm × 20 mm), 5 ml of DD water, nanoparticle suspensions or $ZnSO_4$ solution were added and 20 seeds were then transferred onto each dish. Petri dishes were sealed and placed in an incubator. Following 8 days of treatment, following observations were recorded:

1) *Root and shoot length (cm)*

Root and shoot length of all the germinated seeds was recorded.

2) *Germination percentage (%)*

Germination percentage was calculated by taking the ratio of number of seeds sown to the number of seeds germinated in a petri plate.

3) *Seedling Vigor Index*

Seedling Vigor Index (SVI) was calculated by using the formula described by Abdul-Baki and Anderson [17].

Seed Vigor Index = Germination% × (root length + shoot length)

E. *Pot experiment*

A pot experiment was conducted on a Zn deficient soil of pH 8.4, EC 0.41dS/m and organic carbon 0.45%. The soil properties were determined by following standard procedures [18]: Four kg air dried soil was filled in polyethylene lined clay pots. Zinc was applied as $ZnSO_4$ @ 10ppm soil application, ZnO NPs (@ 2.5, 5, 10 and 20 ppm), ZnO NPs (@ 200ppm) seed priming and ZnO NPs (@ 100 ppm) foliar application. A basal dose of of N (100ppm), P (50ppm), K (50ppm), Cu (2.5ppm), Mn (5ppm) and Fe (10ppm) was applied through their AR grade salts. All the nutrients were thoroughly mixed with the soil before sowing of the crop. The pots were completely randomized and each treatment was replicated thrice. Four seedlings of rice (CV. PB 1121) were transplanted in each pot. The crop was grown up to maturity. The straw, root length, dry matter yield, grain and straw yield and Zn concentration in in grain and straw samples were measured. Chlorophyll content in the leaf samples was also determined.

F. *Chlorophyll Estimation*

Leaf sample were washed thoroughly with distilled water, 0.1g was measured for each sample and cut into small pieces. The leaves were put in 15ml test tubes and 7ml dimethyl sulfoxide (DMSO) was added to it. The test tubes were put in a water bath maintained at 60°C for 30 minutes. The tubes were then cooled at room temperature and the contents were filtered. Final volume was made up to 10ml using DMSO. The samples were then analyzed using UV-vis spectrophotometer at 645 and 663 nm wavelengths and the absorbance was recorded. Total chlorophyll was then calculated using Arnon's [19] equation:

Total Chl. = 20.2 (A645) + 8.02 (A663)

G. *Sample Preparation and Analysis of Grain & Straw*

Grain and straw samples were washed thoroughly with tap water, followed by distilled water and dried in a drying oven at temperature of 65°C to a constant weight. Dried unhusked seed samples and straw were ground and then, 1.0 g sample was placed in a 100 ml conical flask and kept overnight after adding a di-acid; nitric acid (HNO_3) plus perchloric acid ($HClO_4$) in 4:1 ratio [18] for pre-digestion (overnight). After 12 h, samples were digested on a hot plate at 150 to 290°C, until all the material was digested. It was then cooled and filtered through Whatman filter paper No. 42. After 3-4 washes in filter paper, filtrate was diluted to 100 ml by adding DD water then followed by readings taken on an atomic absorption spectrometer (AAS), make Analytik Jena, Model NovAA 350, Germany.

H. Statistical analysis

The results were presented as the mean and SE (standard error); n indicates the number of individual samples used for the experiment. Standard analysis of variance (ANOVA) procedures were used to calculate treatment means, standard errors and significant differences between treatments. The statistical significance was assessed with the F-test. A probability value of 0.05 or less (P 0.05) was taken to be statistically significant.

III. RESULTS AND DISCUSSION

A. Size and surface morphological studies of ZnO nanoparticles

Particle size of ZnO nanoparticles was analyzed by DLS using particle size analyzer (Fig. 1). Histogram of number distribution shows average particle size of 36.7nm. The polydispersity index (PDI) was 0.206 which indicates high mono dispersity of the particles. The size was further confirmed by SEM. The SEM measurements were used to determine the size, shape, and morphological study of zinc nanoparticles. SEM micrograph (Fig. 2) showed the nanoparticles were spherical with a diameter ~30nm. Elemental analysis (Fig. 3) confirms the purity of ZnO, which was carried out by EDAX attached with SEM and a peak at 250nm was observed using UV-vis spectrophotometer (Fig. 4)

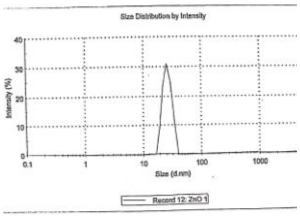

Fig.1. Intensity distribution of synthesized ZnO nanoparticles through dynamic light scattering

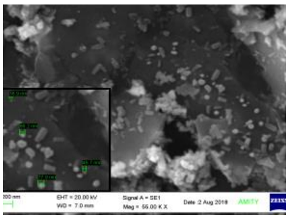

Fig.2. SEM image of synthesized ZnO nanoparticles

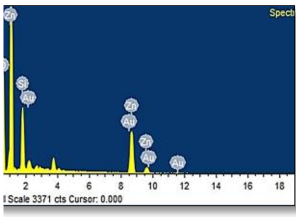

Fig.3. EDAX spectra of synthesized ZnO nanoparticles

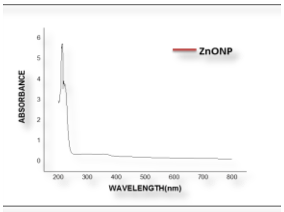

Fig.4. UV-vis. spectra of synthesized ZnO nanoparticles

B. Effect of ZnO nanoparticles on rice seed germination and seed vigor index

Rice seeds showed differential response to the treatment of various concentrations of both conventional $ZnSO_4$ and nanoscale ZnO particles. The highest germination percentage of 82% (Fig. 5) was observed when the seeds were treated with the nanoscale ZnO @100ppm and seedling vigor index (11093) (Fig. 6); and it was significantly more than that of control and $ZnSO_4$. Seeds treated with ZnO NPs (100 ppm) induce more growth (Fig. 7) which can be attributed to the fact that there is increase in production of auxin hormone which is responsible for shoot growth. Seed treated with $ZnSO_4$ showed less shoot growth as compared to seed treated with ZnO nanoparticles which can be inferred that ZnO naoparticles are absorbed by seeds to a larger extent unlike bulk ZnSO4.

Rice seeds showed differential growth in response to different concentration of ZnO NPs. At higher concentrations, inhibitory effect was observed. The effect of nanoparticles at lower dose is reported positive on the growth of plants. Among the different nanoscale ZnO concentrations, 100 ppm showed the maximum germination percentage. The results also proved that at higher concentration, the growth and seedling vigour is less as compared to bulk ZnSO4. Therefore concentration of ZnO NPs plays an important role in determining the growth and seedling vigour.

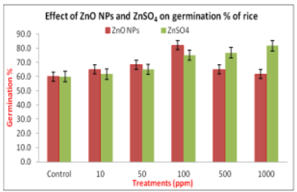

Fig.5. Effect of ZnO NPs and $ZnSO_4$ on seed germination % of rice.

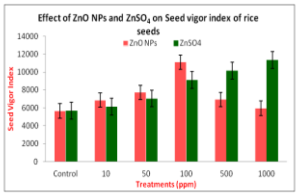

Fig.6. Effect of ZnO NPs and $ZnSO_4$ on seed vigor index of rice seeds.

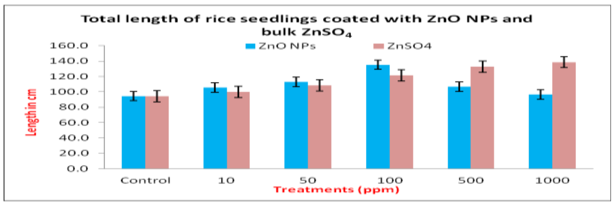

Fig.7. Total length of rice seedlings coated with ZnO NPs and $ZnSO_4$

C. Effect of ZnO nanoparticles on growth traits of rice

Results of pot study showed that the application of Zn nanoparticles at 10 ppm enhanced the growth of rice plant as compared to normal ZnSO4 (Fig. 8). Nano zinc oxide particles could enhance and maintain the growth of rice plant over conventional Zn fertilizer (as ZnSO4) and control (no Zn). The experimental results revealed that the rice plant showed response to Zn application. It was observed that application of Zn through ZnSO4 and nano ZnO increased the plant height. Experimental results depicted that plant height increased over control (57.3cm) and $ZnSO_4$ (66.57cm) on application of ZnO NPs (67.77cm) by 18.27% and 1.80% respectively (Fig. 9 a). The chlorophyll content of rice leaves treated with ZnO NPs (1.71µg/ml) showed improvement over control (1.50µg/ml)(13.99%) and normal $ZnSO_4$ (1.62µg/ml)(5.55%) (Fig. 9 b). The dry weight of shoot and root was increased due to application of Zn through ZnSO4, and nano zinc oxide (ZnO). The highest dry matter yield of shoot (14.96g) (Fig. 9 c) and root (3.08g) (Fig. 9 d) was recorded at 10ppm Zn through ZnO NPs. The grain Zn content in control, $ZnSO_4$ and ZnO NPs 10 ppm (soil) and 100 ppm (foliar) Zn through ZnO NPs was recorded as 19.4 ppm, 26.71 ppm, 29.12 ppm and 35.25 ppm respectively with an increase over control(49.38%) and bulk $ZnSO_4$ (8.49%) (Fig. 9 e). Highest grain yield was observed at ZnO NPs 10 ppm (1.60g) as compared to control (1.47g) (8.84% increase) and bulk $ZnSO_4$(1.54g) (3.89% increase) (Fig. 9 f). Similar findings were reported by Adhikari *et al.* [20] and Subbaiah *et al.* [21]. Results indicated that plant root might have the unique mechanism of assimilating nano-ZnO particles and using it for growth and development.

It was observed that ZnO nanoparticles are beneficial for plant growth and development. Zinc oxide NPs have the potential to boost the yield and growth of rice crop [22]. Small size of nanoparticles offers mobility which makes its transport easy to different parts of plants as compared to bulk $ZnSO_4$. Nanoparticles can also easily penetrate through the pores present in the membrane inside the cell due to its size which further increases its bio-availability. The large surface area of nanoparticles also makes its ability enhanced in terms of rapid uptake and bioavailability.

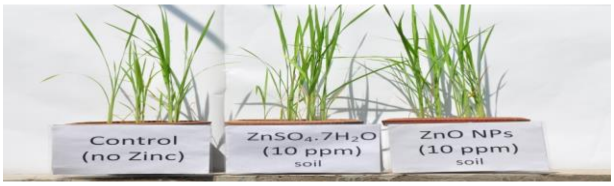

Fig. 8. Effect of soil application of $ZnSO_4$ and ZnO NPs on rice crop

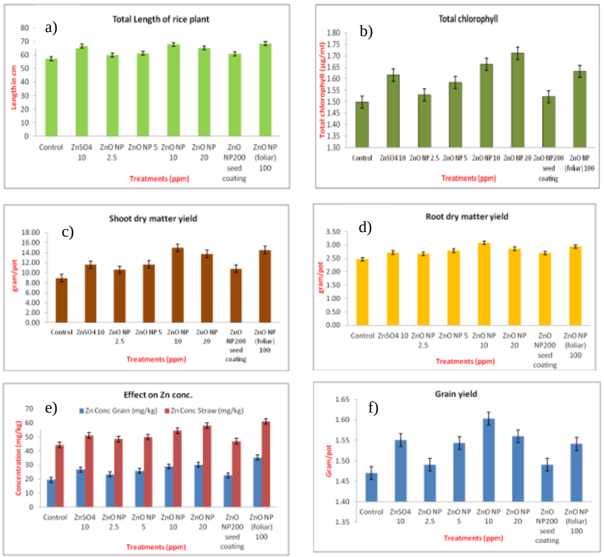

Fig. 9. Effect of bulk $ZnSO_4$ and ZnO NPs on a) total length b) total chlorophyll content c) shoot dry matter yield d) root dry matter yield e) Zn concentration and f) grain yield of rice plant

Nano-fertilizers can supply essential nutrients for plant growth, have higher use efficiency and can be delivered in a timely manner to a rhizospheric target or by foliar spray. More research is needed to determine the interaction between ZnO nanoparticles and plants. Porous nature of seed coats exhibit selective permeability, the interaction between particulate constituents and the plant may be limited until the radicles emerge and come into direct contact with the growth medium [23]. Lin and Xing [24] observed that ZnO nano-particle greatly adhered onto the root surface. It penetrated the root surface and entered into the plant cell. They also observed that individual ZnO nano particle was present in apoplast and protoplast of the root endodermis and stele, indicating that ZnO nano particle could be internalized by the plant cell.

IV. CONCLUSION

The delivery of Zn through ZnO NPs resulted in positive effect on seed germination, root and shoot growth, seedling vigour index, had higher chlorophyll content, Zn concentration and grain yield even at lower concentrations as compared to bulk $ZnSO_4$. Delivery of Zn nutrient through ZnO nanoparticles in right dose and of the right size could be effective and beneficial in enhancing the growth and yield attributes of rice crop. There is a possibility of reducing dose of Zn using nanostructured fertilizer like ZnO- NPs. New approaches for efficient fertilizer delivery using nanotechnology need to be explored, au courant of any potential risk to human health or the environment. More research is needed on the relationship between nano materials and plants and for its further application in the agricultural sector.

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