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Impact of nano ZnO on metabolic attributes and fluorescence kinetics of rice seedlings

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ABSTRACT

In this experimental analysis, application of nano-form of ZnO and their effects were compared with bulk form of ZnO by growing *Oryza sativa* (rice) plants in hydroponic condition. As, rice is a staple food in many countries like China, Japan, India and South Africa and has a great demand so, the assessment of their physiological response towards different concentration (50, 100, 500 and 1000 ppm) of both bulk and nano-form of ZnO is one of the important aspects. The result showed that growth was maximally increased in seedlings grown under 50 ppm, while after 100 ppm i.e. 500 and 1000 ppm, the ZnO nanoparticles exerted unfavorable effects on seedlings growth. The biomass accumulation is found to be higher in seedlings treated with nano- form of ZnO than that of its bulk-form. Higher biomass with nano-form of ZnO particularly at lower concentrations is linked with the reduction in the reactive oxygen species (ROS) generation that resulted in less production of malondialdehyde (MDA) and H_2O_2 contents. In-vivo visualization technique also supported the above statement i.e. reduction in the MDA and H_2O_2 contents at the lower concentration of nano ZnO. Further, these seedlings were also associated with prominent antioxidative enzymes (SOD, APX, DHAR, GST, CAT and POD). Along with this, the kinetics of PS II (Phi-E_0, Psi- E_0 and PI_{ABS}) showed higher values and energy fluxes parameters (ABC/RC, DI_0/RC, TR_0/RC and ET_0/RC) showed lower values in seedlings grown under lower concentration (50 and 100 ppm) of nano form of ZnO. Therefore, the present work has shown that only definite concentration of nano-form of ZnO would be able to improve the qualitative and quantitative characteristics of plants.

1. Introduction

The technology based upon the application of nano-material is one of the progressing fields of technology and has found application in almost all existing fields of science. Its advent in the agricultural system has improved the quality and productivity of food crops. In this line, application of nano-nutrient is also playing important role to increase the yield of plants. Among different nutrients such as phosphorus (P), nitrogen (N), and potassium (K), Zn is one of the necessary micro-nutrients for all living organism. In the higher plants, Zn is absorbed as a divalent cation (Zn^{2+}). It may either have a functional or structural role in the enzymatic reaction. Application of nutrients in nano form increases their availability and consequently quality of the plants (Fageria et al., 2002; Laware and Raskar, 2014; Peralta-Videa et al., 2014). Raliya and Tarafdar (2013) have observed that 10 ppm of nano-form of ZnO induced enhancement in the biomass, leaf area, synthesis of chlorophyll and protein, acid phosphatase and alkaline phosphatase activities of *Cyamopsis tetragonoloba*. Several studies showed that the beneficial range of nano form of ZnO varied with the species of plants in order to increase the plant growth and development. Ramesh et al. (2014) have used four concentration of nano ZnO i.e. 250, 500, 1000 and 2000 ppm in wheat plant and observed that lower concentration of ZnO NPs exhibited the beneficial effect on seed germination. However, higher dose of ZnONPs impaired seed germination. With the help of correlative light and scanning microscope, and inductive coupled plasma/atomic emission spectroscopy Mahajan et al. (2011) have observed the impact of ZnO nanoparticles at different concentration i.e. 0, 10, 20, 50, 100, 500, 1000, and 2000 ppm for *Vigna radiata* (mung seedlings) and 0, 1, 2, 5, 10, 20, 50, 100, 500, 1000, and 2000 ppm for *Cicer arietinum* (gram seedlings). The maximum beneficial effect was noticed at 20 ppm for mung seedlings and 1 ppm for gram seedlings. Thereafter, the growth rate was found to be reduced. So, the impact of nanoparticle on plants varied with the species, age, and characteristics of nanoparticles. Based on its role whether adverse or beneficial, it affects the biochemical characteristic and other physiological activities of plants. As it has been observed that antioxidant system perform crucial role in reducing the free radicals production that damages nucleic acids, proteins, and lipids leading to the induction of toxic

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products like malondialdehyde (MDA) (Foyer and Noctor, 2000; Apel and Hirt, 2004; Tripathi et al., 2015). In such an adverse condition, Zinc maintains the stability of biomembranes and protein by balancing ROS production and by regulating the physiological attributes of plants particularly the nanoform (Cakmak, 2000). Several studies show positive and negative impacts of nanoparticles on performance of higher plants but limited studies are available where whole physiology of plants are studied particularly in presence of nano ZnO. Among different agricultural crops, rice is one of the important staple food sources for many countries like China, Japan, India and South Africa. During the past three decades, the crop has seen consistent increases in demand and its growing importance is evident in the strategic food security planning policies of many countries. So, to know the physiological response of such an important crop towards different levels of nano-nutrient is one of the important aspects.

With the above context, the present piece of experiment has been performed to know more about the impact of nano ZnO over its bulk salts by analyzing the growth of the rice seedlings along with other physiological attributes and fluorescence kinetics. It will give the whole idea about the mechanism behind the regulation of physiological attributes with the application of nano nutrient.

2. Material and methods

2.1. Experimental chemical

Zinc oxide (ZnO; both nano and bulk form) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The characterization of nano form ZnO was done with the help of X-ray diffraction analysis with 2θ value 30–70°. The sharp intense peaks of ZnO confirms the good crystalline nature of ZnO and the peaks were obtained at 31.7°,34.5°, 36.2°, 47.7°, 56.6°, 62.2°, 68.4°. The peak of maximum height was obtained at 36.2° that matches with JCPDS file no. 04-005-5072 of ZnO (Fig. 1).

2.2. Plant material, treatment and growth conditions

For sterilization, the seed of *Oryza sativa* L. (rice) were soaked in 10% H_2O_2 solution for 20 min and thereafter washed thoroughly with double distilled water. For their germination, they were broadcast in the petriplate containing moistened filter paper and kept in dark. After 7 days, when the seeds were germinated they were transplanted to plastic cup, at a density of 10 seedlings per cup containing half strength Hoagland nutrient solution treated with different concentration (50,100, 500, 1000 ppm) of ZnO as bulk and in nano form based upon screening experiment. To increase the solubility of both form of ZnO, they were stirred (5 min) and sonicated for 30 min in an ice bath. For control treatment, Hoagland nutrient solution was used without the addition of ZnO. Rice seedlings were grown in the growth chamber (CDR model GRW-300 DGe, Athens) having 250 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR), with 16:8 h day- night regime and relative humidity was up to 65–70% with the temperature of

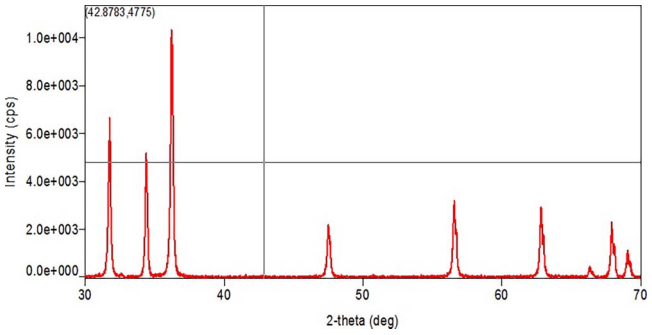

Fig. 1. X ray diffraction (XRD) pattern of the ZnO nanoparticle.

28 ± 2 °C. All the physiological and biochemical attributes in test seedlings were analyzed after 20 days of seed germination.

2.3. Measurement of fresh biomass

Fresh biomass was obtained by using electronic balance (Contech, CA 223, India) that gives the value of fresh weight (fw.) for root and shoot separately at the time of final sampling (20 days after seed germination)

2.4. Estimation of Zn in plants parts

For this, seedlings parts i.e. root and shoot (1 g) of were digested by adding tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio; v/v) at 80 °C (Allen et al., 1986). Thereafter, the samples were filtered using filter paper (Whatman no. 42) and thereafter with double distilled water, the volume of filtrate was maintained up to 50 ml. Atomic absorption spectrometer (iCE 3000 Series, Thermo Scientific, UK) is used to estimate the total concentration of Zn.

2.5. Measurements of chlorophyll a fluorescence

In a dark adapted leaves Chlorophyll *a* fluorescence was analyzed through hand held leaf fluorometer (FluorPen FP 100, Photon System Instrument, Czech Republic), different fluorescence parameters (quantum yield of primary photochemistry: φP_0 or Phi_P_0; yield of electron transport per trapped excitation:Ψ_0 or Psi_{0}; quantum yield of electron transport:φE_0 or Phi_E_0; and performance index of PS II: PI_{ABS} were estimated. Along with this the energy fluxes parameters (absorption of photon per active RC: ABS/RC; electron transport flux per active RC:ET_0/RC; energy dissipation flux per active RC:DI_0/RC and trapped energy flux per active RC:TR_0/RC) were also determined (Strasser et al., 2000).

2.6. Determination of reactive oxygen species and index of oxidative damage

Lipid peroxidation was estimated as malondialdehyde (MDA) content. 200 mg of test seedlings (root, shoot separately) were crushed in 5 ml of solution (ethanol and water- 80:20 v/v). Then centrifugation was performed at 3000 g for 10 min. The estimation of MDA content is done by the method given by Hodges et al. (1999). Superoxide radical (SOR) is estimated by following the procedure given by Elstner and Heupel (1976) with the help of standard curve of NO_2^-.

H_2O_2 concentration was estimated by following method given by Velikova et al. (2000). For this, absorbance of the reaction mixture (2 ml) containing tissue extract (500 µl), 10 mM potassium phosphate buffer (pH 7.0) and 1 M KI solution was read at 390 nm.

2.7. In vivo localization of reactive oxygen species

For the visualization of $O_2^{\cdot -}$, the method given by Castro-Mercado et al. (2009) was applied. For this, fresh leaves were kept in a solution made up of 0.1% nitro blue tetrazolium (NBT) dissolved in potassium phosphate buffer (pH 6.4) and 10 mM Na-azide, They were vacuum-infiltrated for 5 min. Then, after the appearance of dark blue spots, leaves were illuminated. Thereafter, leaves were bleached with boiling ethanol for 10 min. The leaf samples were photographed with a digital camera (Nikon, Coolpix S3100, Japan) to show the spot at which blue formazan is formed. Similarly, to visualize H_2O_2 localization, leaves from each treatment were kept in 1% solution of 3, 3′-diaminobenzidine (DAB) (Sigma Aldrich) and vacuum-infiltrated for 5 min. They were incubated at room temperature for 16 h in the absence of light (Thordal-Christensen et al., 1997). Then the leaves were illuminated till the appearance of brown spots. Further, to make the brown spot more clear, boiling water is used to bleach out chlorophyll content of leaves