



Effect of ZnO Nanoparticles on Chlorophyll Content of Wheat Plants (*Triticum Aestivum* L.)

J. A. Bagawade, S. S. Jagtap

Abstract: Nanoparticles have achieved novel applications in biotechnology and agricultural industries. Nanoparticles on plants may cover a new insight to the ecosystems. There is a need for advance study of the possible effects of the nanoparticles on plant growth and development. In the present investigation, zinc oxide nanoparticles have been prepared by the simple chemical route and were authorized by UV-vis spectrophotometer and X-ray diffraction (XRD) analysis alongwith transmission electron microscope (TEM). The consequence of various concentrations of synthesized zinc oxide nanoparticles on wheat seeds (variety: lok-1) was studied by soaking approach and follow its effect on seedling growth of wheat (at 5 days). The seed germination, plant growth & chlorophyll content characteristics were measured by using standard biophysical techniques and studied. Results showed enhancement in germination and growth characteristics in five days grown wheat seedlings for control upto thousand ppm. Above thousand ppm, the considerable drop off was observed in these parameters upto two thousand ppm. Also, the chlorophyll content in the control sample is greater than the samples treated with the various concentrations of zinc oxide nanoparticles. There was a noticeable effect that employing suitable concentration of ZnO nanoparticles could support the seed germination of wheat in contrast to untreated control.

Keywords: Zinc oxide nanoparticles, Wheat, Chlorophyll, Growth

I. INTRODUCTION

Nanotechnology is one of the rapidly developing discipline and revolutionary fields in science and technology and has found several applications in various areas such as electronics, cosmetics, pharmaceuticals, textiles, crop production, protection and improvement, smart delivery systems of fertilizers, herbicides, pesticides, fertilizer and irrigation management, food and agricultural production etc. [1, 2]. It has a promising potential to solve many of the agricultural-related problems with remarkable improvement. Effect of different nanoparticles on germination, growth characteristics, biomass and physiological activities, chlorophyll content has also been reported [3, 4]. A number of investigators have also reported the crucial role of zinc for plant yield and development [5-8]. Some Plant species, i.e. rape, corn, lettuce, radish, ryegrass, cucumber and wheat [8-10] are sensitive toward ZnO nanoparticles.

Presence of ZnO nanoparticles in surrounding environment affects plant architecture, physiology and biochemistry [10,11]. It is important to examine their effects on the quality and productivity of the major cereal crop like wheat with almost 75% of its 600 million tons annual production used in food supply [12]. Thus, this growth depends upon concentrations of nanoparticles which may be different in different plants. However some few studies have been reported on investigating the potential of high concentration of nanoparticles in wheat growth. Our goal of the present study aimed to assess the effects of various concentrations of zinc oxide nanoparticles on germination, percentage, seedling growth and especially chlorophyll content in Wheat (*Triticum aestivum* L.).

II. MATERIALS AND METHODS

Basically, zinc oxide (ZnO) NPs are prepared by using zinc chloride and sodium hydroxide as precursors (Aldrich 99%). Zinc oxide nanoparticles were synthesized by dissolving 20 ml of zinc chloride (0.1M), 100 ml of sodium hydroxide (0.1 M NaOH) and thioglycerol (TG) in methanol [13]. The synthesized TG capped zinc oxide NPs suspended in water were then used for treatment. The experimental treatments included five concentrations of nano ZnO (10000, 8000, 6000, 4000 and 2000 ppm) and untreated control. The healthy Wheat (*Triticum Aestivum*.) seeds (variety Lok-1) with uniform size were surface sterilized in 5% sodium hypochloride solution for half an hour and then rinsed with distilled water for several times to remove excess of chemical. The treatment of nano ZnO to elected seeds was similar to our our previous paper and seeds soaked in distilled water were acted as control as reported earlier [10]. In this study, agar gel used as growing medium or a substrate to reduce the errors. The germination rate (%) was calculated as the proportion of the germinated grains to total number of grains. The seedling surfacing was observed periodically, shoot, root length, and fresh weight (FW) were measured [10]. The numbers of seeds germinated of each sample after twenty four hours of sowing were measured. We used the germination parameters: Weighted germination index (WGI), Final percentage germination (GP) for each treatment was calculated after seven days. Similarly, Vigor index for each sample was calculated by using the Vigor index formula, Seedling vigor was computed based on the formula [14] After an incubation period of days, plumule and radical length of seedlings were measured using a ruler. The chlorophyll content in leaves was measured as an indicator of the plants photosynthetic performance.

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Fresh 0.02 gm shoots of each sample were cut from seed and dissolved in 80 % acetone solution. After 24 hours, absorption spectra were recorded for each chlorophyll solution by using UV-Visible spectroscopy.

Final chlorophyll contents chlorophyll-a, chlorophyll-b and total chlorophyll were estimated by using Arnon's formula. [15], $C_a = 0.0127 D_{663} - 0.00269 D_{645}$, $C_b = 0.0229 D_{645} - 0.00468 D_{663}$, and $C_{total} = C_a + C_b = 0.0202 D_{645} + 0.00802 D_{663}$ [16]. Where, C_a and C_b are chlorophyll a and b, respectively and D is density value at the respective wavelengths.

III. RESULTS & DISCUSSIONS

The size and morphology of zinc oxide nanoparticles were examined using TEM and XRD. TEM revealed the presence of spherical monodispersed zinc oxide nanoparticles with ~3-5nm size as reported in our previous study [13]. XRD analyses indicated single phase ZnO Wurtzite crystal structure. Particle size calculated from the XRD and that from the TEM is comparable to each other. [13]. In this study, we assessed the impact of zinc oxide nanoparticles on mainly responsive phases of plant progress, i.e. seed germination and seedling growth. Among wheat germination indices, seed germination rate was affected by practical treatments. The percentage seed germination decreased for various concentrations of nanoparticles as shown in Figure 1 [10].

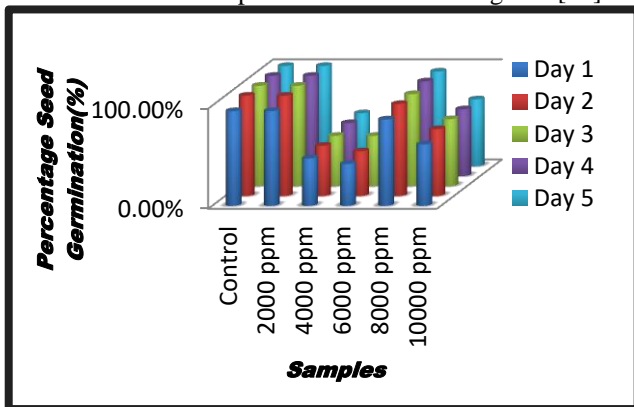


Fig. 1 Percentage Seed Germination

The seed germination was remained 100% in 2000 pm. sample which was similar to control. However, the percentage seed germination of nanoparticles treated wheat seedlings was reduced as compared to control sample from 4000 ppm to 10000 ppm as except small increase at 8000 ppm shown in figure 1. The growths of nanoparticle treated seedlings reduced with enhance in concentration and follow the similar trend as that of percentage seed germination (data not shown). The shoot length and root lengths are decreased with increase in concentrations of nanoparticles. However, no significant change was observed in number of secondary roots. Alike tendency was observed for shoot weight and root weights. Also, there is a decrease in vigor index in nanoparticles treated seedlings compared control [10]. The absorption spectra of chlorophyll are shown in figure 2.

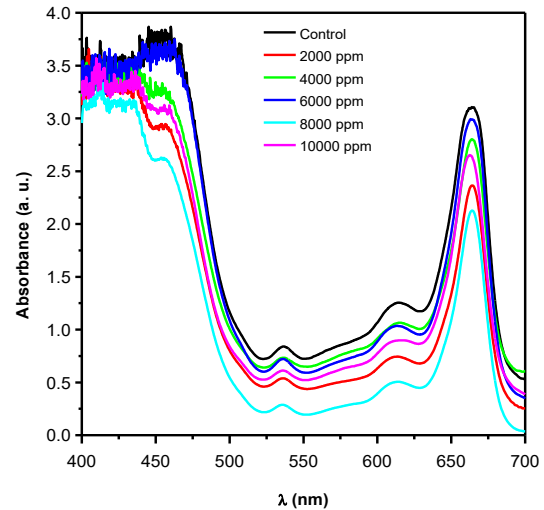


Fig. 2 Absorption Spectra of Chlorophyll

The chlorophyll contents viz., chlorophyll-a, chlorophyll-b and total chlorophyll were calculated from the absorption spectra by using Arnon's formula. From figure 2, it is seen that the absorbance in control sample is higher than the samples treated with the different concentrations of ZnO NPs. Also, the chlorophyll content in the control sample is greater than the samples treated with the different concentrations of ZnO NPs as shown in table 1.

Table I Chlorophyll Content calculated by using Arnon's formula

Samples	C_a (mg/l)	C_b (mg/l)	C_{total} (mg/l)
Control	34.87	26.11	60.98
2000 ppm	27.1	13.75	40.85
4000 ppm	31.56	20.58	52.14
6000 ppm	33.82	22.98	56.8
8000 ppm	24.68	9.3	33.98
10000 ppm	30.09	18.82	48.91

All treatments prove that zinc oxide nanoparticles did not negatively affect wheat seed germination. A decrease in the root length has been detected with further increasing the concentration of zinc with longer soaking time tempting inhibition of root development. The toxicity of zinc oxide nanoparticles toward number of wheat roots and its root length is noticeable for different concentrations. Several previous studies showed that the zinc oxide nanoparticles increase plant development for different concentrations of zinc oxide nanoparticles.e.g. 400 ppm and 1000 ppm concentration give higher yield in peanut [17] ; 500 ppm and 1000 ppm in soya bean [18]; 10 ppm to 40 ppm in onion,

studied by Raskar et al. [19]; & 1.5 ppm in chickpea, Uday Burman [20] reported that lesser applications of zinc oxide nanoparticles proved positive effect on seed germination. Also same results were obtained in radish, rape, corn, lettuce and cucumber to [8] and these results were consistent with Arnab Mukherjee [15] in green pea plants (*Pisum sativum* L.). Thus, the effect of nanoparticles on germination depends on concentrations of NPs and may vary from plants to plants. Seed germination is beginning of a physiological process that needs water imbibitions. The toxic effect is more uttered in the roots. Yang and Watts [21] reported the same results for alumina nanoparticles (nano- Al_2O_3). However, in our case, the toxicity of zinc oxide nanoparticles was found to be greater with respect to its concentration. This physical or chemical toxicity is due to the size, shape, chemical composition, surface energy of nanoparticles and mainly plant species.

IV. CONCLUSIONS

In summary, zinc oxide nanoparticles affect responsive phases of wheat plant development, the seed germination and growth characteristics & chlorophyll content of wheat plant. The chlorophyll content in the control sample is greater than the samples treated with the different concentrations of ZnO NPs. Results of our previous and present study showed development in germination, growth & chlorophyll content in 5 days grown wheat seedlings for control up to 1000 ppm and above 1000 ppm, the considerable decrease was observed in these factors upto 2000 ppm. In addition, for various concentrations of zinc oxide nanoparticles the root, dry matters shoot and seedling lengths were influenced in wheat plants. i.e. employing suitable concentration of zinc oxide NPs could support the seed germination of wheat in comparison to untreated control or else high concentrations had inhibitory or whichever effects on wheat. Further study should be carried out to determine impacts of zinc oxide nanoparticles on new agricultural crops as well.

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