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Efficacy assessment of ZnO nanoparticles on yield attributes and yield of rice

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Abstract
A pot culture experiment was conducted in *Kharif*, 2017 in the glass house of College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The experiment consisted of 2 levels of modes of application, 2 levels of source of ZnO nanoparticles and 4 levels of concentration was laid out in 3 Factorial experiments in Completely Randomized Design with 3 replications. The experiment shows that application of ZnO nanoparticles can significantly improve growth, yield attributes and yield of rice significantly. Foliar application of 50 ppm of nano ZnO was found to be the best treatment for improving yield of rice.

Keywords: nanoparticle, ZnO, rice, yield

Introduction
Zinc is an essential micronutrient and plays a wide array of roles in plant metabolism. A number of researchers have reported the essentiality of zinc and its role in plant growth and yield (Brown *et al.*, 1993; Marschner, 1993 and Fageria *et al.*, 2002) [4, 10, 6]. It is the only metal that is found in all six enzyme classes (oxidoreductase, transferase, hydrolases, isomerases, lyases and ligases) (Auld, 2001) [3]. Zinc is required for the production of chlorophyll, functioning of pollen, fertilization and germination (Kaya and Higgs, 2002; Pandey *et al.*, 2006 and Cakmak, 2008) [8, 14, 5]. It has a key role in controlling the production and toxicity of free radicals which damages the membrane lipids (Alloway, 2008) [1]. It also plays a crucial role in biomass production of crops (Kaya and Higgs, 2002) [8], photosynthesis, cell division, protein synthesis, maintaining integrity of the membrane structure (Marschner, 1995) [11], provides resistance against the pathogen infection and improve sexual reproduction by affecting the production and shape of pollen and changes in the stigma structure. With the adoption of nanofertilization techniques, problems like low nutrient use efficiency, environmental pollution, soil health deterioration can be avoided as well as yield levels can also be elevated or sustained. Application of nano based technologies in agriculture including fertilizer development is regarded as one of the promising approach to increase crop production and meet out the hunger of human civilizations (Lal, 2008) [9]. In addition to these, the smart delivery system of nano fertilizer makes it an excellent choice for agronomic application to crops. However, research on these fertilizer is still scarce and a lot of research work need to be done. In India, crops grown on most of the soils suffer from one or more micronutrient deficiency, even though their content in soils are in adequate amount of respective elements. Analysis of soil and plant samples has indicated that 49% of soils of India are potentially deficient in Zn (Singh, 2008) [15]. Zinc deficiency is mostly associated with calcareous soil found in rice-wheat cropping region in the *tarai* soils of Indo-gangetic plains (Singh *et al.*, 2005). Periodic assessment of soil test data also suggests that zinc deficiency in Indian soils is likely to increase from 49 to 63% by the year 2025 as most of the marginal soils brought under cultivation are showing zinc deficiency (Singh, 2008) [15].
Taking all these into consideration a pot experiment was planned to see the effect of nano-ZnO on growth, yield attributes and yield of rice.

Materials and Method
A pot experiment was planned in *Kharif*, 2017 in the glass house of College of Agriculture of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand

The experiment consisted of 2 levels of modes of application, 2 levels of source of ZnO nanoparticles and 4 levels of concentration was laid out in 3 Factorial experiments in Completely Randomized Design with 3 replications. The treatments details are explained as follows:

Factor A: Mode of application
- M_1 : Seed soaking/ seedling root dip
- M_2 : Foliar spray

Factor B: Source of ZnO nanoparticles
- S_1 : Green synthesized ZnO nanoparticle (ZnO_G)
- S_2 : Chemically synthesized ZnO nanoparticle (ZnO_C)

Factor C: Concentration of ZnO nanoparticles
- C_1 : 0 ppm
- C_2 : 10 ppm
- C_3 : 20 ppm
- C_4 : 50 ppm

Nano-ZnO was synthesized following procedure given by Singh *et al*. (2016) [11]. Nano ZnO thus synthesized was characterized following Scanning Electron Microscopy (SEM) and UV- Visible Spectroscopy. In rice root dipping was done where, 30 seedlings of 25 days old were uprooted and dipped in desired concentrations of ZnO nanoparticles. Three foliar spray were also done in the desired treatment. Each time 50 ml of suspended ZnO NPs were sprayed per pot. Hand sprayer was used for the foliar application with spray volume of 50 ml per pot. During life span of crops, the total number of foliar sprays were three.

The maturity of the crops was determined visually when 80% of the panicles matured and harvesting was done manually.

Results and Discussion

Scanning Electron Microscopy: Scanning electron microscopy images confirmed the size and shape of the ZnO nanoparticles. The size and shape of the green synthesized ZnO nanoparticles, studied under SEM clearly revealed the particle size found to range from 74 to 148 nm with an average of 111 nm when observed at different resolutions and triangular to spheroidal in shape. Whereas, in case of chemical ZnO nanoparticles, the particle size found to range from 180 to 250 nm with an average of 215 nm when observed at different resolutions and spherical in shape. The nanoparticles were found in clumps as well as individual particles depending on the effect of ultrasonication on the dispersion of the agglomerated or clumped nanoparticles.

UV-vis spectroscopy: The absorbance $peak_{max}$ was observed at 368 nm for green synthesized ZnO and 371 nm for chemically synthesized ZnO nanoparticles respectively, which was typical feature of ZnO nanoparticles. The present findings are in accordance with the findings of Singh *et al.* (2011).

Effect of mode of application, source and concentration of ZnO nanoparticles on plant height of rice at various crop growth stages: Results showed that mode of application varied significantly at all the stages of plant height (Table 1). Seedling root dip had significantly more effect on plant height than foliar spray at 30 DAT while the reverse trend was observed in later stages (60 DAT and 90 DAT). The response of seedling root dip was found to be 3.4% higher than foliar spray at 30 DAT, whereas reverse trend of response was observed with the advancement of growth stages. It was 5.3% and 6.3% higher with foliar spray than seedling root dip at 60 and 90 DAT, respectively. Among the sources of ZnO nanoparticles, ZnO_G was numerically better at all the stages than ZnO_C, but not found to reach at the level of significance. The nanoparticles concentrations @ 0, 10, 20 and 50 ppm varied significantly at all the stages of plant growth *i.e.* 30, 60 and 90 DAT, respectively. Maximum plant height (97.4 cm) was observed with 50 ppm concentration. The response varied from 10 to 21% at 30 DAT and 13 to 26% at 60 DAT and 12 to 24% at 90 DAT, respectively. This increase in plant height might be due to the zinc-induced auxin synthesis in the plant. Auxin being a plant hormone has been reported to enhance cell elongation and thus improves shoot length. Similar results were reported by Tarafdar *et al*. (2014) [19]. They also suggested that foliar application of ZnO nanoparticles at 10 ppm concentration on pearlmillet significantly increased the plant height up to 10.8% over control.

Effective tillers per plant: Mode of application *viz.,* foliar spray significantly increased the effective tillers per plant over seedling root dip (Table 2). Foliar spray was recorded to give a greater number of effective tillers, the response was found to be 9.1% superior to seedling root dip. Zinc oxide nanoparticles applied either as ZnO_G or ZnO_C influenced the number of effective tillers. Both sources exhibited comparable response. The varying concentrations of ZnO nanoparticles had also significant effect on the effective tillers and the highest number of effective tillers (7.3) was recorded at 50 ppm, which was 37.8% higher over control. The interaction effect of mode of application and concentration (M×C) was also found significant (Table 2.1). The foliar spray at 10 and 20 ppm was superior to 20 and 50 ppm of seedling root dip, respectively. 50 ppm of foliar spray was found to produce maximum number of effective tillers (7.9) per plant. The favorable effect of ZnO nanoparticles may be attributed to the influence of zinc on metabolism and biological activities stimulating enzymatic activities and other processes which in turn encourage the vegetative growth.

Grains per panicle: The mode of application had significant effect on number of grains per panicle (Table 2). Foliar spray was superior to seedling root dip by 4.5%. Among the sources, both showed comparable effects. The zinc oxide nanoparticle at varying level of concentration significantly increased grains per panicle. Maximum number of grains (79.0) was recorded at 20 ppm which was 20% over control followed by 50 ppm (77.9) and 10 ppm (76.0), respectively. The interaction effect of mode of application and concentration (M×C) was found significant (Table 2.2). Foliar spray of 10 ppm and seedling root dip at 50 ppm showed comparable effects. 20 ppm foliar spray was found to produce maximum number of grains (82.6) per panicle.

1000-grain weight: Mode of application significantly influenced the 1000-grain weight (test weight) of rice grains (Table 2). Out of two, foliar spray was found to be slightly superior over seedling root dip. The sources of ZnO nanoparticles were not found to increase the 1000-grain weight of rice, significantly. The 1000-grain weight was increased significantly with increase in concentrations of ZnO nanoparticles at varying level *viz.,* 0, 10, 20 and 50 ppm. Maximum test weight (23.01 g) was recorded at 50 ppm which was 5.6% higher over control. Increment in 1000-grain weight might be attributed to increasing in the assimilatory surface which can be traced back to the increase in the number of effective tillers in the corresponding treatments

Grain yield: Mode of application *viz.*, foliar spray significantly increased the grain yield of rice and highest yield of 10.86 g plant^{-1} was recorded, which was 13.2% higher to seedling root dip (Table 3). Out of the sources of nanoparticles, ZnO_G was found superior, the response being 2.3% higher over ZnO_C but was not found to reach up to the level of significance. The zinc oxide nanoparticles at varying level of concentrations from 0, 10, 20 and 50 ppm increased the grain yield, significantly. The highest yield (11.97 g plant^{-1}) was recorded at 50 ppm concentration which was 56.9% followed by 47.2 and 31.8% at 20 and 10 ppm, respectively, over control. The interaction effect of mode of application and concentration (M×C) was found significant (Table 3.1). Foliar spray at 20 and 50 ppm showed comparable effects and foliar spray at 10 ppm was slightly superior to 20 ppm of seedling root dip. Similarly, 20 ppm of foliar spray was superior to 50 ppm of seedling root dip mode. Maximum grain yield (12.90 g plant^{-1}) was found at 50 ppm of foliar spray. Increase in grain yield was due to increase in the number of effective tillers per plant, number of grains per panicle and 1000-grain weight of the grains. These results are in conformity with those reported by Tarafdar *et al*. (2014) [19], indicating that foliar spray of ZnO nanoparticles @ 10 mg L^{-1} increased the grain yield by 29.5% over control in pearl millet. Armin *et al*. (2014) [2] suggested foliar application of nano-Fe at 4% increases grain yield of wheat up to 22.09% over control.

Straw yield: In between the mode of applications, foliar spray had significantly more effective in increasing the straw yield of rice up to 14.63 g plant^{-1} which was10.7% higher over seedling root dip (Table 3). Out of two sources of ZnO nanoparticles, both were at par with respect to straw yield. The effect of concentrations was found significant. The highest straw yield (15.50 g plant^{-1}) was recorded at 50 ppm and the response was 30% over control. The interaction effect of mode and concentration (M×C) had a significant impact on straw yield (Table 3.2). Foliar spray at 20 ppm was superior to seedling root dip at all the concentrations *viz.*, 10, 20 and 50 ppm, however, maximum straw yield (16.66 g plant^{-1}) was observed at 50 ppm foliar spray. Similar results were reported by Narendhran *et al*. (2016) [13] that significantly higher shoot biomass in nano-ZnO treated sesamum plants over control.

Biological yield: The mode of application differed significantly and foliar spray had resulted 11.8% superior in increasing the biological yield than seedling root dip (Table 3). However, both the sources of ZnO nanoparticles gave comparable yield. Biological yield was significantly increased at all ZnO concentrations *i.e.* 0, 10, 20 and 50 ppm. With the increase in concentration, biological yield was increased and the highest biological (27.47 g plant^{-1}) was recorded at 50 ppm which was 40.4% over control. The interaction effect of mode of application and concentration (M×C) was found significant. 10 ppm of foliar spray was comparable with 50 ppm of seedling root dip, whereas 20 and 50 ppm in foliar spray was far superior to seedling root dip. 50 ppm foliar spray was found to produce maximum biological yield (29.57 g plant^{-1}). The favorable effect of ZnO nanoparticles may be attributed to the influence of zinc on metabolism and biological activities stimulating enzymatic activities and other processes which in turn encourage the vegetative growth. In addition to it, the increase in grain weight enhances the biological yield altogether. Ghafari and Razmjoo (2013) [7] reported foliar spray of iron nanoparticles at 200 ppm increased the yield attributes and yield in wheat. Torabian *et al*. (2017) [20] found foliar spray of nano $FeSO_4$ increased the biological of sunflower.

Table 1: Effect of mode of application, source and concentration of ZnO nanoparticles on plant height of rice at various crop growth stages

Treatments	30 DAT (cm)		60 DAT (cm)		90 DAT (cm)	
Mode of application (M)						
Seedling root dip (M_1)	53.5		74.5		86.2	
Foliar spray (M_2)	51.7		78.4		91.6	
SEm ±	0.4		0.8		1.1	
CD (*P*=0.05)	1.1		2.3		3.0	
Source of ZnO nanoparticles (S)						
ZnO_G (S_1)	52.9		76.9		89.6	
ZnO_C (S_2)	52.3		75.9		88.2	
SEm ±	0.4		0.8		1.1	
CD (*P*=0.05)	NS		NS		NS	
Concentration of ZnO nanoparticles (C)						
0 ppm (C_1)	47.2		66.8		78.6	
10 ppm (C_2)	52.0		75.7		88.0	
20 ppm (C_3)	54.3		78.8		91.6	
50 ppm (C_4)	56.9		84.4		97.4	
SEm ±	0.5		1.1		1.5	
CD (*P*=0.05)	1.5		3.3		4.3	
Interactions	SEm ±	CD (*P*=0.05)	SEm ±	CD (*P*=0.05)	SEm ±	CD (*P*=0.05)
M×S	0.5	NS	1.1	NS	1.5	NS
M×C	0.7	NS	1.6	NS	2.1	NS
S×C	0.7	NS	1.6	NS	2.1	NS
M×S×C	1.0	NS	2.3	NS	3.0	NS

Table 2: Effect of mode of application, source and concentration of ZnO nanoparticles on yield attributing characters of rice

Treatments	Effective tillers plant^{-1}	Grains panicle^{-1}	1000-grain weight (g)
Mode of application (M)			
Seedling root dip (M_1)	6.17	73.04	22.47
Foliar spray (M_2)	6.73	76.33	22.68
SEm ±	0.04	0.42	0.07
CD (*P*=0.05)	0.12	1.22	0.20
Source of ZnO nanoparticles (S)			
ZnO_G (S_1)	6.49	75.08	22.59
ZnO_C (S_2)	6.41	74.29	22.55
SEm ±	0.04	0.42	0.07
CD (*P*=0.05)	NS	NS	NS
Concentration of ZnO nanoparticles (C)			
0 ppm (C_1)	5.31	65.83	21.85
10 ppm (C_2)	6.37	76.00	22.62

20 ppm (C_3)	6.79		79.00		22.74	
50 ppm (C_4)	7.32		77.92		23.08	
SEm ±	0.06		0.60		0.10	
CD (*P*=0.05)	0.17		1.73		0.28	
Interactions	SEm ±	CD (*P*=0.05)	SEm ±	CD (*P*=0.05)	SEm ±	CD (*P*=0.05)
M×S	0.06	NS	0.60	NS	0.10	NS
M×C	0.08	0.24	0.85	2.45	0.14	NS
S×C	0.08	NS	0.85	NS	0.14	NS
M×S×C	0.12	NS	1.20	NS	0.20	NS

Table 2.1: Interaction effect of mode of application and concentration of ZnO nanoparticles on number of effective tillers per plant in rice

Concentration of ZnO nanoparticles (C)	Mode of application (M)	
	Seedling root dip (M_1)	Foliar spray (M_2)
0 ppm (C_1)	5.27	5.35
10 ppm (C_2)	6.22	6.53
20 ppm (C_3)	6.47	7.12
50 ppm (C_4)	6.73	7.92

Table 2.2: Interaction effect of mode of application and concentration of ZnO nanoparticles on number of grains per panicle in rice

Concentration of ZnO nanoparticles (C)	Mode of application (M)	
	Seedling root dip (M_1)	Foliar spray (M_2)
0 ppm (C_1)	65.50	66.17
10 ppm (C_2)	73.00	79.00
20 ppm (C_3)	75.67	82.33
50 ppm (C_4)	78.00	77.83

Table 3: Effect of mode of application, source and concentration of ZnO nanoparticles on grain, straw and biological yield of rice

Treatments	Grain yield (g plant^{-1})		Straw yield (g plant^{-1})		Biological yield (g plant^{-1})	
Mode of application (M)						
Seedling root dip (M_1)	9.59		13.22		22.81	
Foliar spray (M_2)	10.86		14.64		25.50	
SEm ±	0.16		0.21		0.36	
CD (*P*=0.05)	0.46		0.60		1.04	
Source of ZnO nanoparticles (S)						
ZnO_G (S_1)	10.34		14.05		24.39	
ZnO_C (S_2)	10.11		13.81		23.92	
SEm ±	0.16		0.21		0.36	
CD (*P*=0.05)	NS		NS		NS	
Concentration of ZnO nanoparticles (C)						
0 ppm (C_1)	7.63		11.92		19.56	
10 ppm (C_2)	10.06		13.69		23.75	
20 ppm (C_3)	11.23		14.60		25.83	
50 ppm (C_4)	11.97		15.50		27.47	
SEm ±	0.22		0.29		0.51	
CD (*P*=0.05)	0.65		0.85		1.46	
Interactions	SEm ±	CD (*P*=0.05)	SEm ±	CD (*P*=0.05)	SEm ±	CD (*P*=0.05)
M×S	0.22	NS	0.29	NS	0.51	NS
M×C	0.32	0.91	0.42	1.20	0.72	2.07
S×C	0.32	NS	0.42	NS	0.72	NS
M×S×C	0.45	NS	0.59	NS	1.02	NS

Table 3.1: Interaction effect of mode of application and concentration of ZnO nanoparticles on grain yield (g plant^{-1}) of rice

Concentration of ZnO nanoparticles (C)	Mode of application (M)	
	Seedling root dip (M_1)	Foliar spray (M_2)
0 ppm (C_1)	7.60	7.67
10 ppm (C_2)	9.44	10.69
20 ppm (C_3)	10.26	12.20
50 ppm (C_4)	11.04	12.90

Table 3.2: Interaction effect of mode of application and concentration of ZnO nanoparticles on straw yield (g plant^{-1}) of rice

Concentration of ZnO nanoparticles (C)	Mode of application (M)	
	Seedling root dip (M_1)	Foliar spray (M_2)
0 ppm (C_1)	11.84	12.01
10 ppm (C_2)	13.25	14.13
20 ppm (C_3)	13.47	15.73
50 ppm (C_4)	14.33	16.67

Table 3.3: Interaction effect of mode of application and concentration of ZnO nanoparticles on biological yield (g plant^{-1}) of rice

Concentration of ZnO nanoparticles (C)	Mode of application (M)	
	Seedling root dip (M_1)	Foliar spray (M_2)
0 ppm (C_1)	19.44	19.67
10 ppm (C_2)	22.69	24.82
20 ppm (C_3)	23.73	27.94
50 ppm (C_4)	25.37	29.57

Conclusion

From the experiment, it can be concluded that application of 50 ppm nano ZnO through foliar application can improve growth parameters and yield of Rice. However to confirm the result more experiments especially, field scale experiments need to be done.

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