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Evaluation of nano-metal oxides for increased fodder production in barley (*Hordeum vulgare* L.)

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Abstract
A study was conducted in a net house to evaluate the effect of seed treatment with nano zinc oxide (250, 500, 750 and 1000 µg ml^{-1}) and nano copper oxide (50, 100, 200 and 400 µg ml^{-1}) on seed germination and growth of barley under pot conditions. The results were compared with macro forms ($ZnSO_4$, ZnO, $CuSO_4$ and CuO). It was found that 500 µg ml^{-1} of nZnO enhanced germination percentage and shoot length as compared to other treatments while other plant growth attributes were similar in all the treatments except the control. In case of nCuO, highest germination percentage was recorded with 100 µg ml^{-1} nCuO. Spikelet number and shoot fresh weight were found higher at 200 µg ml^{-1} nCuO, while stem dry weight, shoot length and root fresh weight were highest in the treatment having macro $CuSO_4$. However all the plant growth attributes were significantly higher in all the treatments than control. Our study indicated that micronutrients requirement can be fulfilled using nano-metal oxides through seed treatment to increase the growth and yield in barley.

Keywords: Nano metal oxides, barley, germination, growth

Introduction

Barley (*Hordeum vulgare* L.) has been traditionally used as dual purpose crop viz. for grain purpose for human consumption and green fodder as animal feed in India (Kharub *et al*., 2013) [4]. It is an important *rabi* cereal crop mainly grown in the drier regions of India (Rajasthan, Southern Haryana, South-West Punjab and Western U.P). With the development of high yielding dual purpose varieties it can serve as an alternative fodder source to the livestock along with the satisfactory grain yield from regenerated crop (Singh *et al*. 2017) [12, 13]. To meet the nutritional requirement of livestock, production of quality feed and fodder is very important. Barley is mainly grown in drought affected and salinity stress prone areas. It is needed to improve the tolerance of the crop plants to these stresses for improved quality and yield of green fodder.

Micronutrients are capable to induce tolerance of plants to various environmental stresses (Baybordi *et al*., 2006). These trace elements play an important role in various physiological processes in the plant. Among micronutrients, zinc (Zn) and copper (Cu) are significant in countering the susceptibility of plants to the environmental stresses. About 200 enzymes require Zn as a functional component. Zn plays very important role either as a metal component or as a functional, regulatory or structural cofactor of various enzymes. Apart from this, it is also an important element essential for protein and carbohydrate synthesis and also participates in metabolism regulation of lipids, nucleic acid and saccharides (Ghasemian and Ghalavand, 2010) [3]. Similarly Cu proteins are important in physicochemical processes such as photosynthesis, respiration, detoxification of super oxide radicals, and lignification (Römheld and Marschner, 1991) [11]

Several researchers reported the improvement in plant growth due to Zn and Cu micronutrients fertilization (Kobraee *et al*., 2011; Mondal *et al*., 2011; Zain *et al*., 2015; Aske *et al*. 2017) [5, 8, 14, 1]. Particle size may affect agronomic efficiency of micronutrient fertilizers. Reduced particle size results in increased number of particles per unit weight of applied nutrient. Reduction in particle size also increases the specific surface area of a fertilizer, which leads to increase the dissolution rate of fertilizer with low solubility in water such as zinc oxide (ZnO) (Prasad *et al*., 2012) [9].

Farmers are using both sulfates and chelated Zn and Cu (with ethylene diamine tetra acetic acid, EDTA) for soil and foliar applications; however, the efficacy is low. Nanoparticles are expected to be the ideal candidates for use as a Zn and Cu fertilizer in plants. The present study was conducted to investigate the promotory effects of various concentrations of ZnO and CuO nanoparticles on germination, growth and yield parameters, and chlorophyll content of barley (*Hordeum vulgare* L.) and to generate new information on the efficacy of nanoscale ZnO and CuO on the growth and development of barley.

Materials and Methods

Experimental details

The experiment was conducted at Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh, India. Treatments consisted of different concentrations of nZnO (250, 500, 750 and 1000 µg ml^{-1}) and nCuO (50, 100, 200 and 400 µg ml^{-1}), recommended dose (RDF) of macro salts of same nutrients ($ZnSO_4$ and $CuSO_4$ at a rate of 20 kg ha^{-1}) and control (without any nano or macro salts). All the treatments were arranged in a completely randomized design with three replications. The nano zinc oxide (nZnO) and nano copper oxide (nCuO) having 30 nm and 40 nm with 99.9 and 99% purity respectively were purchased from Sisco Research Laboratories Pvt. Ltd Mumbai, India. Seed treatment was carried out by soaking seeds in a solution of respective concentration of nano particles (NPs) and macro salts (treatment wise) in distilled water for 6 hours followed by air drying. One hundred treated seeds per replication were placed in moist filter paper in 11 cm diameter petri plates in three replications.

Germination percentage

The seed germination was counted at 7 days after treatments n (ISTA, 1995) and the seed germination count was expressed in percentage using the following equation-

$$\text{Germination Percentage (GP)} = \frac{\text{Number of seeds germinated}}{\text{number of total seeds}} \times 100$$

Growth attributes

Treated seeds were sown in pots (30 cm diameter). Plants were harvested at two different intervals [60 and 120 days after showing (DAS)]. The plant growth parameters such as shoot length (cm), root length (cm), number of tillers, stem weight (g) and root weight (g) were recorded after the harvest of Barley.

Data analysis

All the data from the experiment were subjected to normality test using PROC UNIVARIATE analysis in SAS 9.3. Non-normal data were subjected to square root transformation. ANOVA for all the variables were performed using PROC GLM procedure in SAS 9.3. While performing ANOVA treatments were kept as fixed effect and replication as random effect. Post-hoc test for all the significant data were performed using Fishers Least Square Significance (LSD) test and significance were tested at α 0.05.

Results and Discussion

Germination percentage

Barley seeds germination was effected by Zn and Cu seed treatments compared to control (Figures 1 and 2). The highest germination (85%) was recorded in nZnO at 500 µg ml^{-1} concentration compared to other treatments, though the data is not significant (p 0.15). Furthermore the germination was declined with increase in the concentration of nZnO (Figure 1). Similarly, Cu treatments did not significantly influenced (p 0.06) the barley seed germination (Figure 2). The nCuO treatment at 100 µg ml^{-1} recorded the highest germination percentage (87.5%), whereas the lowest was recorded in control (60%). Similar to nZnO the relationship between nCuO concentration and germination was inversely propionate (Figure 2). Raskar *et al*. (2014) reported a decrease in onion (*Allium cepa* L.) germination with increase in the concentration of nZnO above 20 µg ml^{-1}. Likewise, Srinivasan *et al*. (2017) [13] observed a reduction in cowpea germination with increase in the concentration of nCuO above 25 µg ml^{-1} and four NPs *viz*. ZnO, TiO_2, CuO and Ag improved germination percentage significantly as compared to control in oat and berseem (Maity *et al*. 2018) [7].

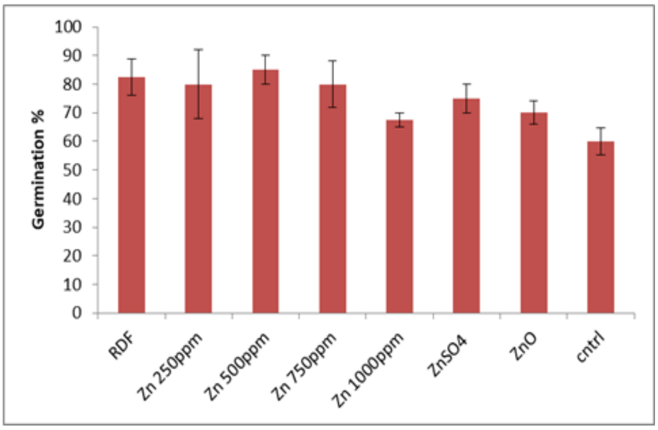

Fig 1: Effect of nano and macro zinc particles on germination of dual purpose barley

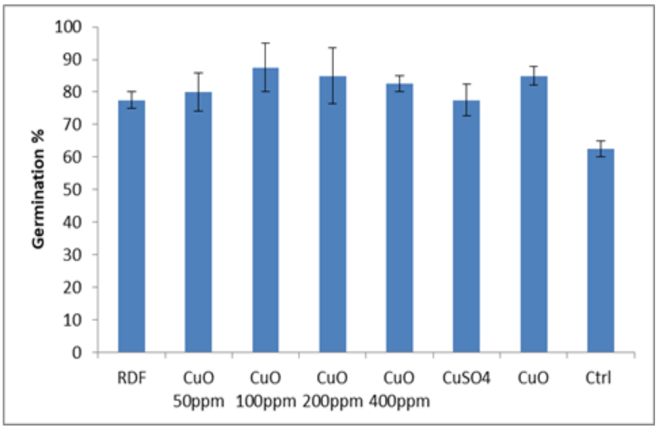

Fig 2: Effect of nano and macro copper particles on germination of dual purpose barley

Growth attributes of fodder barley

Nano ZnO treatments had significantly ($p<0.05$) influenced the root and shoot length, root and shoot fresh weight, and number of tillers per plant (Table 1). The nZnO concentrations 250 µg ml^{-1} was recorded the highest root length (22.63 cm), followed by 500 µg ml^{-1} of nZnO (19.57 cm), 750 µg ml^{-1} of nZnO (19.37 cm) and $ZnSO_4$ (18.40 cm). Whereas, the shoot length was highest at 500 µg ml^{-1} of nZnO (53.04 cm), followed by 750 µg ml^{-1} of nZnO (51.98 cm), 1000 µg ml^{-1} of nZnO (50 cm) and $ZnSO_4$ (48.33 cm) (Table 1). Significant increase in root and shoot fresh weight was recorded in all the treatments as compared to control (1.88 cm and 8.13 cm, respectively). Similar results were also reported by Prasad *et al*. (2012) [9] who have observed a significant increase in root volume and root dry weight after nZnO at 100 µg ml^{-1} treatment in peanut. Numbers of tillers per plant were also significantly increased in all the treatments having nZnO (5.38 to 5.88) as compared to RDF (4.88), macro $ZnSO_4$ (4.75), ZnO (3.75) and control (3.13) treatments. All other growth attributes (spikelet numbers and weight, grain weight and stem dry weight) found no significant ($p<0.05$) differences among all the treatments but significantly higher as compared to control treatment. According to Lin and Xing, 2007 the increase in concentration of nZnO above a certain level would reduce both the plant growth in radish, rape, lettuce and cucumber, the similar kind of trend was also observed in this study.

Table 1: Effect of Zn micronutrient on plant growth parameters of dual purpose barley

Treatment	Spikelet (No.)	Spikelet weight (g)	Grain weight (g)	Stem dry Weight (g)	Root Length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Tillers/ plant
RDF	5.54(±1.1)	29.17(±6.2)	24.25(±4.9)	18.15(±1.9)	21.53(±0.1)ab	49.33(±2.2)ba	5.13(±0.5)a	26.00(±1.6)a	4.88(±0.4)ab
Zn 250µg ml-1	4.19(±1.0)	24.23(±8.1)	17.04(±7.2)	13.49(±3.4)	22.63(±2.0)a	44.84(±2.8)b	5.50(±0.2)a	27.68(±1.6)a	5.63(±0.4)a
Zn 500µg ml-1	5.77(±1.9)	30.35(±6.8)	20.91(±3.6)	17.55(±4.0)	19.57(±2.5)abc	53.04(±2.1)a	5.13(±0.5)a	26.00(±0.9)a	5.38(±0.4)a
Zn 750µg ml-1	5.46(±0.8)	32.54(±6.0)	22.64(±3.1)	23.51(±2.8)	19.37(±0.2)abc	51.98(±3.4)ab	5.00(±0.4)a	26.25(±1.4)a	5.88(±0.7)a
Zn 1000µg ml-1	3.90(±0.3)	28.10(±1.6)	19.78(±1.3)	16.54(±2.9)	18.00(±0.3)bc	50.00(±2.6)ab	4.88(±0.7)a	25.13(±3.1)a	5.63(±0.5)a
$ZnSO_4$	6.42(±0.9)	23.15(±10.3)	17.02(±7.9)	18.70(±5.5)	18.40(±2.2)abc	48.33(±1.0)ab	5.13(±0.4)a	25.00(±0.3)a	4.75(±0.6)ab
ZnO	4.88(±1.1)	33.67(±8.4)	22.63(±6.0)	19.78(±4.6)	13.57(±0.6)d	44.96(±3.9)b	4.88(±0.5)a	23.00(±1.5)a	3.75(±7.5)bc
cntrl	2.93 (±0.4)	6.6(±1.7)	4.6(±1.9)	11.3(±2.3)	15.9(±1.4)dc	28.2 (±1.4)c	1.88(±0.1)b	8.13(±1.0)b	3.13(±0.4)c
P value	0.34	0.17	0.20	0.41	0.01	<.0001	0.0003	<.0001	0.01

Table 2: Effect of Cu micronutrient on plant growth parameters of dual purpose barley

Treatment	Spikelet (No.)	Spikelet weight (g)	Grain weight (g)	Stem dry weight (g)	Root Length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Tillers/ plant
RDF	5.00(±0.8)ab	25.85(±7.0)a	22.28(±6.5)	11.81(±2.9)bc	18.97(±0.4)	43.23(±3.1)ab	5.08(±0.5)ab	25.25(±3.3)a	4.63(±0.6)
CuO 50µg ml-1	5.19(±0.6)ab	30.06(±3.5)a	32.41(±11.7)	16.92(±2.8)ab	17.93(±3.1)	46.58(±1.2)a	4.10(±0.2)bc	21.50(±0.1)ab	3.50(±0.5)
CuO 100µg ml-1	3.63(±0.1)bc	25.58(±1.3)a	21.87(±1.4)	14.23(±4.5)b	18.23(±2.5)	45.58(±1.9)a	3.88(±0.4)c	22.75(±0.3)ab	4.00(±0.2)
CuO 200µg ml-1	5.92(±0.8)a	30.41(±3.8)a	22.43(±3.0)	15.82(±4.1)ab	18.53(±0.2)	49.10(±1.00)a	3.63(±0.4)c	24.50(±0.8)a	4.63(±1.0)
CuO 400µg ml-1	4.85(±0.9)ab	26.78(±3.5)a	22.17(±3.5)	16.51(±1.4)ab	17.43(±0.8)	48.98(±1.5)a	3.13(±0.5)c	20.25(±1.4)b	4.75(±0.6)
CuSO4	4.98(±0.3)ab	30.00(±5.0)a	22.69(±3.5)	22.77(±2.1)a	16.57(±0.1)	49.63(±2.4)a	5.38(±0.4)a	23.13(±1.8)ab	4.75(±0.8)
CuO	5.00(±0.4)ab	30.94(±3.7)a	27.19(±2.8)	14.81(±2.4)ab	16.28(±0.1)	36.03(±4.8)bc	3.75(±0.3)c	13.50(±0.1)c	2.75(±0.3)
Ctrl	2.17(±0.3)c	8.67(±1.6)b	7.13(±1.7)	5.34(±0.9c	13.73(±0.1)	34.68(±3.3)c	1.50(±0.2)d	11.13(±0.4)c	4.13(±0.6)
P value	0.003	0.01	0.13	0.02	0.28	0.001	<.0001	<.0001	0.24

Similarly nCuO treatment having 200 µg ml^{-1} showed the highest number of spikelets (5.92) as compared to RDF (5.00), macro $CuSO_4$ (4.98), CuO (5.00) and control (2.17) treatments (Table 2). The maximum shoot fresh weight of barley was recorded in RDF (25.25 g) and nCuO at 200 µg ml^{-1} (24.50 g) compared to $CuSO_4$ (23.13 g), CuO (13.50g) and control (11.13 g) treatments. Root fresh weight was significantly higher with $CuSO_4$ (5.38g) and RDF (5.08 g) compared to other treatments (Table 2). Shoot length was recorded significantly higher in the nCuO (from 45.58 cm to 49.10 cm) and macro $CuSO_4$ (49.63 cm) treatments as compared to RDF (43.23 cm) followed by CuO (36.03 cm) and control (34.68 cm) treatment. Stem dry weight was significantly (p 0.02) higher in $CuSO_4$ (22.77g), followed by 50 µg ml^{-1} of nCuO (16.92 g). Spikelet weight was found at par among all the treatments (from 25.85 to 30.94 g) which is significantly higher than the control (8.67 g) treatment. There was no significant difference in number of tillers per plant among all the treatments. Grain weight and root length was recorded from 21.87 g to 32.41 g and 16.28 to 18.97 g respectively among all the treatments which is significantly higher than the control (7.13 g and 13.73 g, respectively) treatments. Hafeez *et al*. (2015) reported that there was a significant increase in growth and yield of wheat when Cu nano particles were applied in the pot soil. Srinivasan *et al*. (2017) [13] reported a significant higher plant growth attributes in the treatments of nCuO as compared to RDF and control.

Conclusion

In order to explore the potential of nanotechnology in agriculture for efficient nutrient use with lower doses it is important to analyse the effects of nano nutrients on plant growth parameters. Size of nanoparticles would play a very important role in behaviour, in reactivity, in toxicity etc. Considering these aspects the effect of nano metal oxides on seed germination and plant growth attributes were observed in barley. The results revealed that micronutrient Zn and Cu can be supplied through nZnO and nCuO. Both the nano metal oxides enhanced seed germination, spikelets number and weight, grain weight, stem dry and fresh weight, root length and fresh weight, shoot length and number of tillers per plant. Results revealed that nano-nutrients can be supplied to crop plants through seed treatment to get the beneficial effects on plant growth. Further detailed study on the accumulation, behaviour and mechanism of action is required to explore the nano-nutrients in crop production.

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