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Potential of Copper Nanoparticles to Increase Growth and Yield of Wheat

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Abstract

Rapidly expanding use of nanoparticles in every discipline of life necessitates finding their potential to increase food production to match with burgeoning population. This study was conducted first time in Pakistan to determine the potential of copper nanoparticles (Cu-NPs) for enhancing growth and yield of wheat cultivar Millat-2011. Several experiments were conducted for this purpose. Seed germination was not affect with 0.2 to 0.8 ppm but decreased significantly at 1.0ppm of Cu-NPs. In solution culture concentration of Cu-NPs higher than 2ppm proved deleterious to wheat plants. Whereas MS medium blended with low concentrations of Cu-NPs (0.2, 0.4, 0.6, and 0.8 and 1.0ppm) significantly increased leaf area, chlorophyll content, fresh and dry weight, and root dry weight as compared to control plants. When applied to soil in pots Cu-NPs (10, 20, 30, 40 and 50ppm) significantly increased growth and yield of wheat as compared with control. However, 30ppm Cu-NPs produced significantly higher chlorophyll content, leaf area, number of spikes/pot, number of grains/spike, 100 grain weight and grain yield. Results of this study reveal that Cu-NPs have the potential to enhance growth and yield of wheat but their effect is concentration dependent. Further experimentation is required to explore the most effective concentration of Cu-NPs and mode of application for yield maximization of wheat.

Keywords: Copper nano-particles, Wheat, Chlorophyll, Growth, Yield.

Introduction

Agricultural production needs to maximize on sustainable basis to meet the continuously increasing food demand of mushrooming population. Global warming may lead to increased probability of drought and temperature stresses. Continuously increasing use of agrochemicals is threatening human health and environment. Producing more and quality food from diminishing land and water while sustaining agricultural resource base in an environment friendly way is a formidable challenge of this century. To increase productivities in a resource efficient way agriculture needs to be reinforced and revitalized with innovating science-based technologies. Nanotechnology seems to have a promising potential to make more sustainable future of agriculture.

Wheat is one of the most important staple food crops in the world. Yield of the crop is a complex trait controlled by genetic, environmental and management factors. Target oriented, cheaper and efficient technologies have to be employed for sustainable increase in production of wheat crop [1]. Nanotechnology might offer tremendous potential to boost agricultural production to fulfill food, feed and fiber requirements of humans [2].

Nanotechnology deals with atomic or molecular aggregates of 1 to 100 nm in size [3]. A vast application in almost every discipline of biosciences is due to profoundly modified physio-chemical properties of nanoparticles associated with smaller size and large surface area [4]. Nanoparticles of gold (Au), silver (Ag), copper (Cu), zinc (Zn), aluminum (Al), silica (Si), zinc oxide (ZnO), cesium oxide (Ce_2O_3), titanium dioxide (TiO_2) and magnetized iron (Fe) have found applications in agriculture [5]. Possible applications of nanoparticles in agriculture include crop production, protection and improvement, fertilizer and irrigation management. Use of nanoparticles in crop sciences is consistently increasing. Several advantageous effects of nanoparticles have been observed on crop plants [6]. Soaking of cotton seeds in silver nanoparticles produced favorable effects and reduced the amount of fertilizers applied by half [7]. Effect of different nanoparticles on germination [8], growth, physiological activities [9], water and fertilizers use efficiency [10], root growth, branching, biomass and photosynthetic pigments [11]has also been reported. Wheat seeds treated with metal nanoparticles exhibited enhanced nutrient use efficiency, photosynthetic activity, grain quality and increased yield [12]. Metal nanoparticles also enhanced photosynthetic activity and nitrogen metabolism in many crop plants including wheat [13]. Improvement in leaf area, pod dry weight and grain yield of soybean by exposure to nano-iron oxide was also postulated [14]. Application of nano SiO_2 and TiO_2 enhanced germination, improved growth and nitrate reductase activity in soybean [10]. Enhanced Hill reaction and chloroplast activity resulting in increased evolution of oxygen in spinach was recorded with application of nano TiO_2 [15]. Nano TiO_2 were also found to increase several times the activity of rubisco carboxylase [16].

Nanoparticles blended with fertilizers may provide a more efficient means to distribute pesticides and fertilizers reducing the quantities of these chemicals introduced into the environment

[17]. Use of nanoparticles as fertilizer has a great potential to enhance crop yield and minimize environmental hazards [7]. Metal nanoparticles when applied as foliar spray enhanced crop production [18].

In fact, nanotechnology is a rapidly developing discipline substantially influencing every field of science and biology. Nanotechnology certainly holds the potential to rejuvenate agriculture [19] and is expected to become a dynamic economic force in the near future. Convincingly nanotech-based reorientation of agriculture can boost production of quality food in a resource and environment friendly manner. Exploring comprehensive application profile of nano-particles may revolutionize research in crop science and transform agriculture into industry. Copper nanoparticles show positive effects on germination [8] but are phytotoxic at seedling growth [20]. However, fewer studies have been reported on exploring the potential of copper nanoparticles in crop growth. Visualizing enormous beneficial aspects of metal nanoparticles present study was conducted to find out the possible role copper nanoparticles can play in enhancing growth and increasing yield of wheat crop.

Material and Methods

Copper nano-particles (Cu-NPs) were synthesized by reduction method using organic extract. A solution of $CuSO_4.5H_2O$ was prepared in distill water and reduced by stepwise addition of 250ml of onion extract with continuous stirring (3000- 4000 rpm) by magnetic stirrer at 100°C in water bath. Translucent yellowish green color was indication for conversion of Cu^+ into Cu-NPs. Molar mass of $CuSO_4.5H_2O$ depending upon purity was so adjusted to finally prepare stock solution of 100ppm Cu-NPs. Size of Cu-NPs was determined by Zeta Particle Analyzer at Nuclear Institute of Biology and Genetic Engineering, (NIBGE) Faisalabad. Maximum number of Cu-NPs in solution ranged between 12 to 20nm (Figure 1).

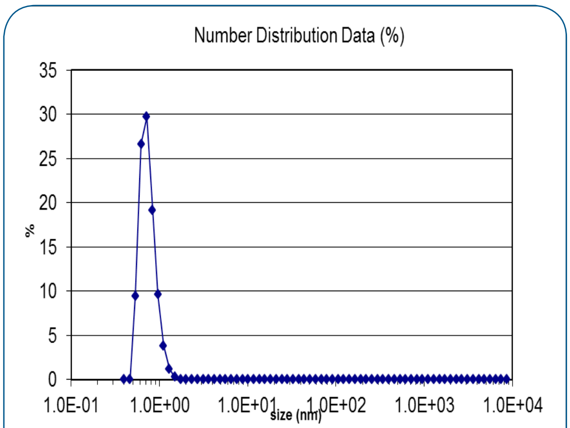

Figure 1: Zeeta analyzer peak for copper Nano-particles

Pakistani wheat cultivar Millat-2011 was used to conclude the role of Cu-NPs on germination, growth and yield. To study the effect of Cu-NPs on seed germination three layers of sterilized filter papers were fitted in petri plates and soaked with different concentrations of Cu-NPs. Distill water was used as control. Fifty sterilized seeds were placed in each petri-plate. Preliminary experiments were conducted by using 2, 4, 6, 8 and 10ppm Cu-NPs. Results showed highly toxic effects of Cu-NPs on germination. In the next experiment little lower concentrations of Cu-NPs (0.2, 0.4, 0.6, 0.8 and 1.0 ppm) were used to observe their effect on germination. Completely Randomized Design with three replications was employed for experimental layout. Germination percentage and index were calculated by following formula:

$$\text{Germination percentage} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds}}$$

Germination index was calculated by following formula [21]: Germination Index (GI) = Σ Gi/Di

where Gi is the difference in number of seeds germinated between the count i and the preceding count, and Di is the day of each count.

Second experiment was conducted to document the effect of Cu-NPs on seedling growth of wheat. Seeds were sown in petri-plate having filter papers soaked with distill water for seed germination. Plastic pots of 500ml capacity were filled with MS medium blended initially with 0, 2, 4, 6, 8 and 10ppm Cu-NPs. One week old seedlings were transferred to the solution supported by holes in thermopole sheets fitted in pots. It was observed (results not presented) that all these concentrations of Cu-NPs adversely affected growth of wheat seedlings (Figure 2).

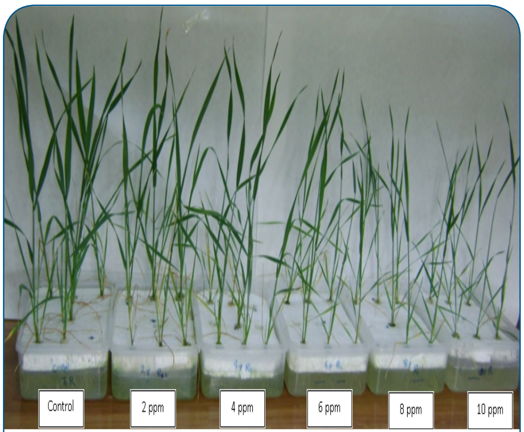

Figure 2: Effects of higher concentration of copper nanoparticles on growth

Subsequently 0.2, 0.4, 0.6, 0.8 and 1.0 ppm were used. MS without Cu-NPs served as control. The experiment was arranged in Completely Randomized Design with three replications. After 4 weeks of seedling growth in solution culture data on leaf area, chlorophyll content (measured with Chlorophyll Meter SPAD-502), fresh weight, dry weight and root dry weight was recorded.

Third experiment was application of nanoparticles to soil filled in pots to investigate the effect of Cu-NPs on yield of wheat. Soil from field was taken, completely homogenized after mixing recommended doses of fertilizers NPK and filled in pots. Wheat seeds were grown in petri-plates. One week old seedlings were transplanted into pots (ten/pot). After one week thinning was done leaving five plants of almost equal size in each pot. Completely Randomized Design with three replications was employed for experimental layout. Solutions equivalent to field capacity water containing 0, 2, 4, 6, 8 and 10ppm Cu-NPs were applied as different treatments. Nonetheless, Cu-NPs did not produce significant effect as compared to control (Figure 3).

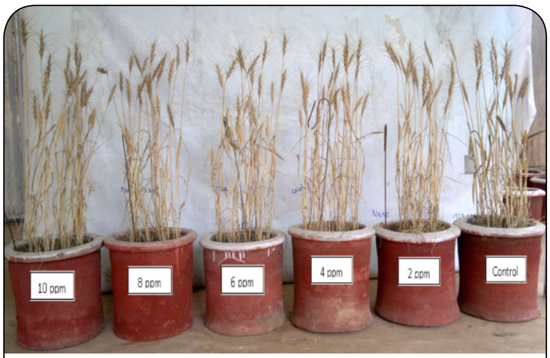

Figure 3: Response of wheat plants to lower concentration of copper nanoparticles

Consequently, Cu-NPs concentrations were modified till 10, 20, 30, 40 and 50ppm of Cu-NPs produced significant effects. Simple water was applied as control. Water was applied according to the requirement of plants. Data was recorded for leaf area, chlorophyll content, number of spikes/pot, number of grains/spike, 100-grain weight and grain yield/pot. Data collected was subjected to statistical analysis using software Statistix 8.1 for interpretation of results and inferences.

Results

Data pertaining to effect of copper nanoparticles on germination response of wheat cultivar Millat 2011 is presented in Table 1.

Analysis of data indicated that lower concentrations of Cu-NPs (0.2 to 0.8 ppm) did not significantly influence percent

Figure 4: Effect of Copper Nanoparticles on root growth of wheat

Consequently, MS medium blended with 0.4ppm Cu-NPs produced healthier and vigorous plants with higher chlorophyll content and stronger root system.

Impact of soil applied Cu-NPs to wheat plants in pots are presented in Table 3.

Progressive increase in chlorophyll content and leaf area was observed with application of 10, 20 and 30ppm Cu-NPs.

Increasing the level of Cu-NPs to 40 and 50ppm was accompanied by a significant reduction in chlorophyll and leaf area. In general, addition of 10 to 40ppm Cu-NPs in pots produced significantly higher leaf area and chlorophyll than those of control plants.

Table 1: Germination response of wheat seed to copper nanoparticles

Treatments	Concentration of copper nanoparticles					
	0 ppm	**0.2 ppm**	**0.4 ppm**	**0.6 ppm**	**0.8 ppm**	**1 ppm**	**LSD**
Germination %	94.67 a	94.67 a	96.0 a	96.67 a	96.67 a	82.67 b	**8.3020**
Germination Index	13.77 a	14.48 a	13.96 a	14.43 a	14.49 a	12.32 b	**1.0308**

*Means sharing similar letters do not differ significantly at 5% level of probability

germination and germination index of wheat seed in comparison to control. However, 1.0ppm level of Cu-NPs adversely affected both germination percentage and germination index. It was observed that lower concentration of Cu-NPs (up to 0.8ppm) did not affect on germination. Further increase in concentration of Cu-NPs became toxic lowering germination percentage and index.

Response of wheat seedlings grown in MS medium supplemented with different levels of Cu-NPs varied significantly (Table 2).

Addition of Cu-NPs up to 0.4 ppm significantly increased leaf area, chlorophyll content, plant fresh weight, dry weight and root dry weight over control. Further increase in level of Cu-NPs caused significant drop in values of the growth parameters except plant fresh weight that started decreasing at 0.8 ppm. Chlorophyll content and root dry weight matched with those of control plants at 1.0ppm. Declining trend in growth parameters was observed at concentration higher than 0.4 ppm. In general, significantly higher values than those of control plants for growth parameters were recorded at all levels of Cu-NPs. The plants treated with 0.4ppm Cu-NPs were visibly compact, vigorous and greener in color with stronger root system (Figure 4).

Similar trend was observed for number of spikes/pot, number of grains/spike, 100 grain weight and grain yield per pot. Nonetheless, the best results were achieved with application of 30ppm Cu-NPs to wheat in pots. Therefore, 30ppm Cu-NPs applied in soil may be considered the best for inducing good growth and maximum yield (Figure 5)

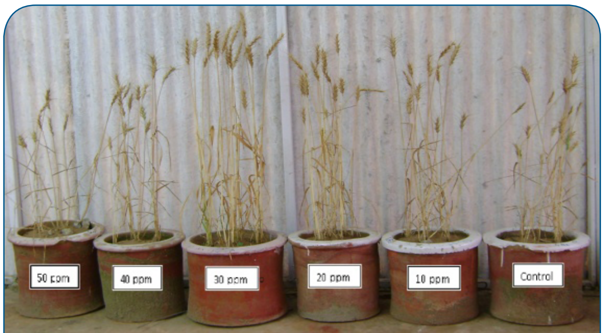

Figure 5: Effect of copper nanoparticles on plant growth and yield

according to the results of this study. Microscopic analysis indicated (Figure 6) absorption of nanoparticles by plant roots.

Table 2: Effect of Copper Nanoparticles on Growth Parameters of Wheat

Treatments	Concentration of Copper nanoparticles						
	0 ppm	0.2 ppm	0.4 ppm	0.6 ppm	0.8 ppm	1 ppm	LSD
Leaf Area (cm^2/plant)	6.81 e	8.87 c	12.83 a	10.53 b	10.10 b	7.90 d	**0.5974**
Chlorophyll Contents (SPAD units)	38.28 c	40.43 c	51.23 a	46.87 b	48.50 ab	37.97 c	**4.0357**
FW (g/plant)	1.0467 c	1.9167 b	2.4900 a	2.2967 a	1.8800 b	1.8600 b	**0.2291**
DW (g/plant)	0.0633 c	0.0967 b	0.1167 a	0.1133 b	0.0933 b	0.0867 b	**0.0126**
Root DW/plant (mg)	0.0223 e	0.0493 b	0.0580 a	0.0330 c	0.0320 cd	0.0253 de	**0.0072**

*Means having similar letters do not differ significantly at 5% level of probability

Table 3: Effect of Copper Nanoparticles on Yield Parameters of Wheat

Treatments	Concentration of copper nanoparticles						
	0 ppm	10 ppm	20 ppm	30 ppm	40 ppm	50 ppm	LSD
Leaf Area (cm^2/plant)	6.847 e	8.980 c	10.783 b	12.793 a	10.290 b	8.263 d	**0.5473**
Chlorophyll Contents (SPAD units)	38.433 c	41.667 c	46.480 b	51.367 a	50.400 ab	37.833 c	**4.8054**
Grains per Spike	23.333 cd	25.333 b	27.667 b	30.667 a	20.667 de	19.333 e	**2.7175**
Spikes per Pot	13.00 c	13.33 c	16.33 b	19.33 a	11.67 c	9.00 d	**2.5506**
100 Grain Weight (g)	4.0800 d	5.1033 c	5.7567 b	6.4500 a	3.8067 d	3.1800 e	**0.2805**
Grain yield per Pot (g)	6.4200 d	8.5700 c	10.873 b	13.513 a	5.1000 e	4.0867 f	**0.335**

*Means having similar letters do not differ significantly at 5% level of probability

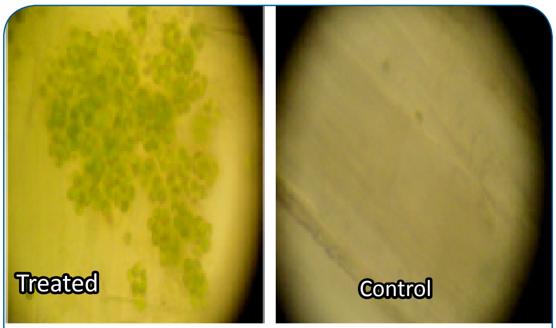

Figure 6: Micrograph of root indicating absorption of nanoparticles

Discussion

Different nanoparticles have been applied to several plant species with variable and contrasting effects. Metal nanoparticles are considered to modify physiological and biochemical processes in plants thereby affecting their germination and growth favorably or otherwise. Several studies conducted to investigate the role of nanoparticles on germination of seeds have reported variable and concentration dependent effects of nanoparticles. Our study revealed that Cu-NPs either do not affect seed germination at lower concentration (up to 0.8ppm) or impair germination at 1 ppm and above. Silver nanoparticles were reported to adversely affect germination of lettuce and cucumber seeds [22](Barrena *et al.*, 2009) and enhanced germination of wetland plant [23]. Relatively fewer studies have been reported on application of Cu-NPs. Shah and Belozerova [8] observed favorable effect of Cu-NPs on germination of lettuce seeds in contradiction with our results. However, Adhikari *et al.* [24] reported that germination of soybean and chickpea was not checked up to 2,000 ppm concentration of CuO nanoparticles. Further experimentation is needed to precisely explore impact of Cu-NPs on germination of wheat seed for conclusive results.

Application of 0.4ppm Cu-NPs in MS medium seems better for good seedling growth. However, higher concentration proved phytotoxic for wheat plants. This might be due to more bioavailability, absorption and accumulation of nanoparticles leading to toxic effects. Concentration of Cu-NPs higher than 0.4 ppm in MS medium produced declining trend in growth parameters. Application of 2 – 10ppm Cu-NPs applied to pots in soil did not produce significant effects as compared to control indicating low bioavailability and absorption of soil applied nanoparticles. Nonetheless, application of 30ppm Cu-NPs to soil in pots proved best for growth and yield of wheat. Therefore, 30ppm Cu NPs applied to soil seems equivalent to 0.4ppm applied in MS medium. Declining trend in growth and yield at concentrations higher than 30 ppm might be due to more absorption of nanoparticles leading to phytotoxic effects. Several studies have reported phytotoxic effects of Cu-NPs on growth in contradiction with our results. Adverse effects of Cu-NPs on root [24,25], seedling growth [8] and shoot growth [26] on different plants including wheat have been reported. However, it was noted that phytotoxic effects were concentration dependent. Concentration of Cu-NPs used in all the studies reporting toxic effects were higher than 200ppm [8,27,28]. Exposure of plants to elevated levels of Cu-NPs increases bioavailability. Consequently massive accumulation of nanoparticles in roots and shoots occurs [24] leading to phytotoxicity. DNA damage induced by Cu-NPs [29] may be responsible for toxic effects. Lower concentration of Cu-NPs (not more than 1.0 ppm) in solution culture and less than 50 ppm in pots used in our experiments did not prove toxic for wheat plants. Maximum growth and yield was recorded with 30 ppm in pots. Nanoparticles induced increased activity of chloroplast [15], rubisco [16], antioxidant enzyme system [30] and nitrate reductase [10] might be the possible underlying mechanism responsible for enhanced growth and yield. So far, no study has reported effects of Cu-NPs on yield of wheat. Other nanoparticles have been found to increase wheat yield by 20-25% [12]. Nano-iron oxide particles on soybean increased grain yield by 48% in comparison with control [14]. Effect of Cu-NPs seems concentration dependent and higher concentration may be harmful to plants.

Conclusion

Cu NPs certainly have potential to enhance growth and yield of wheat. Soil application of 30ppm Cu-NPs may increase yield of wheat crop significantly to match the food demand of growing population. However, comprehensive experimentation is needed to determine the best concentration, mode and time of application in addition to exploring underlying physiological mechanism responsible for enhanced growth and yield.

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