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Effect of green synthesized copper nanoparticles on seed germination and seedling growth in wheat

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Abstract

The green synthesis and application of metallic nanoparticles has become a significantly important branch of nanotechnology. In present study, we report the green synthesis of Copper nanoparticles using leaf extract of *Moringa oliefera* as reducing agent and tested their effects on seed germination as well as seedling growth. The exposure of copper to leaf extract resulted in rapid reduction of copper ions leading to the formation of copper NPs in the solution. The UV visible spectroscopy and X-ray Diffraction analysis confirmed the synthesis of Cu NPs. Biologically synthesized Cu NPs were applied to evaluate the effect on seed germination and seedling growth of *Triticum aestivum,* an economically important food crop. Seeds were treated with different concentrations of Cu NPs (25ppm, 50ppm, 75ppm and 100ppm) and tested against control. Higher percentage of seed germination was observed in seeds treated with low concentrations of Cu NPs. Also, a significant increase in root fresh weight, root dry weight, root length, and root elongation was recorded at lower concentrations of Cu NPs as compared to the control. However, the treatment with Cu NPs above 75ppm showed delayed germination and overall inhibitory effect on seedling growth. The results of this experiment revealed that the application of Cu NPs enhanced the germination in wheat.

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Introduction

Nanotechnology is an innovative and promising field of interdisciplinary research. It founds a wide range of applications in various diverse fields like electronics, medicine, pharmaceutical, food processing, textile and agriculture.

Nanotechnology has enormous potential uses and benefits. Nowadays tremendous research has been carried out to explore the positive impacts of nanoparticles on the plant growth and development and few studies also reported their negative effect as well. Nanoparticles are the atomic or molecular aggregates having size dimension between 1 to 100nm. They have diverse and unique physic-chemical properties as compared to other bulk materials (Net *et al.*, 2006).

Seed germination is crucial stage in plant growth and it is also an important phenomenon in agriculture because it is regarded as thread of life of plants that ensure its survival (Zaka *et al.,* 2016).

The recent advances in nanotechnology and its applications and utilization in the field of agriculture is tremendously increasing; so it is quite persuading to study or explore the role of Cu NPs in the germination of seeds.

Wheat *Triticum aestivum* L. commonly called as wheat belongs to family Poaceae is a staple food crop providing nutrition to global population and also a very adaptable food crop (Mladenov *et al.*, 2012).

Unfortunately yield of wheat crop in Pakistan has been decreasing from many years due changing climatic conditions to biotic and abiotic stresses. So, it is the need of the hour to increase the yield of major staple food of the country for the accomplishment of food requirements of the people by application of modern technologies (Asseng *et al.*, 2011).

Green synthesis is a cost effective, eco-friendly method to prepare nanoparticles which does not require high temperatures or pressures and importantly toxic chemicals.

It helps to have better progression than chemical and physical methods. Synthesizing nanoparticles using plants is more beneficent than any other biological ways as it would be difficult to maintain the cell cultures. Many other green methods were reported in the synthesis of nanoparticles using plant extracts (Mittal *et al.*, 2013).

Among all metallic nanoparticles Cu NPs are well known due to their unique properties and diverse applications. Copper nanoparticles are certainly the most widely used nanomaterials and they have been used in textile industries, water treatment, sunscreen lotions, as antimicrobial agents etc.

Copper is necessary element required in many physiological responses of plant. Plants require Copper for normal growth and development. Copper is structural element in protein and participates in photosynthetic electrons transport, mitochondrial respiration, oxidative cell response, cell wall metabolism and hormones signaling (Sharma *et al.*, 2012).

However, role and interaction of Cu at nanosize range still need to be explored. Cu NPs are of immense curiosity due to its easiness of preparation and noteworthy promising physical and chemical properties (Manceau *et al.*, 2008). Copper NPs coated vetch seeds showed an increase in ascorbic acid and carotene content approximately three and four times respectively (Churilov *et al.*, 2000).

The effects of Cu NPs on the seedling growth of mung bean and wheat were studied using plant agar culture media for easy dispersion of nanoparticles without any precipitation (Lee *et al.*, 2008).

There is need to explore the most appropriate concentration of Cu NPs for stimulatory effects on wheat cultivars. Effect of Cu NPs on plants are concentration dependent as low concentration of Cu

NPs improved shoot/root ratio in lettuce (Shah and Belozerova, 2009) while high concentration reduced seedling growth in mungbean (Lee *et al.*, 2008), wheat (Lee *et al.*, 2008) and zucchini (Stampoulis *et al.*, 2009).

Keeping in view the available literature, present study was planned to evaluate the effects of green synthesized Cu NPs on various aspects of wheat seed germination.

Materials and methods

The study was conducted following a complete randomized design with three replicates in the lab, Department of Botany Pir Mehr Ali Shah Arig Agriculture University Rawalpindi.

Green synthesis of copper nanoparticles

Green synthesis of Copper nanoparticles was carried out by using leaf extract of *Moringa oliefera* Plant.

Preparation of plant extract

For preparation of plant extract healthy and fresh green leaves of medicinal plant were collected and washed first with tap water followed by distilled water to eliminate all the dust and visible dirt particles. After washing leaves were cut into tiny pieces and dried at room temperature. 10-20g of finely incised leaves was taken and transferred into 250 ml flask containing 100 ml distilled water and boiled for 15 minutes.

The extract was then filtered thrice by using What man No. 1 filter to remove all solid particles. The obtained clear solution was refrigerated at 4°C in 250 mL flask for further experiment. Sterility conditions were maintained at each and every step of the experiment to avoid contamination. (Banerjee *et al.*, 2014).

Method of preparing nanoparticles

Copper nano particles were synthesized by reducing Copper sulphate pentahydrate ($CuSO_4.5H_2O$) with plant extract by modifying the method of (Banerjee et *al.*, 2014).

A solution was prepared using by dissolving 589.8mg of Copper sulphate pentahydrate ($CuSO_4.5H_2O$) per liter of distilled water.

The solution was boiled and reduced step by step by addition of 10-20 ml of plant extract along with continuous boiling until color of solution changes to bluish green. Through this method stock solution of Cu NPs 150 ppm concentration was obtained (Fig.1). This stock solution was diluted for further treatments. UV visible spectroscopy and X-ray Diffraction analysis confirmed the synthesis of copper nanoparticles (Fig.2).

Seed collection and experimentation

Seeds of Wheat variety Galaxy-13 were collected from Institute of Crop Sciences, National Agriculture Research Centre (NARC), Islamabad. Healthy seeds were surface sterilized in 10% Sodium Hypochlorite solution for 15 min and washed thoroughly with distilled water. Copper nanoparticles in different concentration (0, 25, 50, 75 and 100ppm) were prepared directly in distilled water and then dispersed by ultrasonic vibration for approximate one hour.

All the seeds were stored in dark at room temperature. Viability of seeds was checked by suspending them in distilled water.

The settled seeds at the bottom were selected for further experiment. The sterilization of seeds was carried out by suspending seeds in 5% sodium hypochlorite solution for 10 minutes and then rinsed thoroughly with distilled water.

After surface sterilization, the rinsed seeds stirred for about 2 hours in Cu NPs suspension by using magnetic stirrer.

Three layers of filter paper were placed into each Petri plate and 5ml of the respective particle suspensions were added using a Pasteur pipette. Twenty five seeds were then transferred into each Petri dish at controlled temperature of 25±1°C.

Approximately 15ml of each concentration (0, 25, 50, 75 and 100ppm) of Cu NPs was supplied to every test plantlets for 14 days. Distilled water was served as control. After 14 days of growth, the shoot and root lengths were long enough to be measured by using a scale.

A set of seeds was used without providing any treatment as a control. Germination rate of the seeds was recorded daily for fourteen days and Germination percentage was calculated till the last day.

The data for all the other growth related parameters which appeared early such as shoot length; root fresh weight and root biomass was recorded.

Germination percentage (gp)

Seeds were taken as germinated when radicle has emerged from the seed coat. Percentage germination frequency Percentage germination was recorded by using following formula. (Iqbal *et al.* 2016).

Germination Percentage = Number of seeds germinated / Total number of seeds X100.

Germination Speed Index (GSI)

The germination test was performed with daily calculation of that number of seeds that showed protrusion of primary root with length of ≥2mm always at the same time during entire trial.

The germination speed index was calculated by sum of seeds germinated each day, divided by number of days passed between the seedling and germination. According to Maguire formula

$GSI= G1/n1+G2/n2+.......+Gi/ni$

Where:

G= Number of seeds germinated each day

N= Number of days elapsed from seedling until the last count

Seedling Vigor Index (SVI)

Seedling vigor Index was calculated by using method of Abdul-Baki and Anderson (1973) and expressed by Ushahra and Malik (2013).

Seedling Vigor Index = (Root length + Shoot length) X Germination Percentage.

Fresh and Dry Weight of plants (g)

The fresh weight was estimated through weighing plant material in precision scale, and dry weight was calculated through weighing in precision scale after drying the material in oven at temperature of 70°C till constant weight. At the end of experiment, plumule and radical length as well as fresh weight was recorded. So dry weights were calculated by placing the plants in oven at 70°C for 48 hours and then weighed with sensitive scale.

Statistical analysis of data

Statistical analysis of each treatment was carried out with three replicates and all the results were presented as mean ± SD (Standard deviation). Analysis of results was performed by one way ANOVA with Statistix 8.1.

Results and discussion

In present study, different concentrations 0, 25, 50, 75 and 100 ppm) were prepared in distilled water and further used for the treatment in wheat seeds to explore their effects on seed germination and early seedling growth. Significant positive results on shoot and root elongation were observed for all seeds as compared untreated control germination.

Seed germination results revealed that Cu NPs at lower concentrations enhanced seed germination as well as promoted seedling growth in wheat, however at higher concentration of Cu NPs slightly adverse effects.

The results revealed that the effect of Cu NPs was significant on germination percentage in $P \leq 0.05$. Highest germination percentage (80.1a) was recorded at 75ppm Cu NPs.

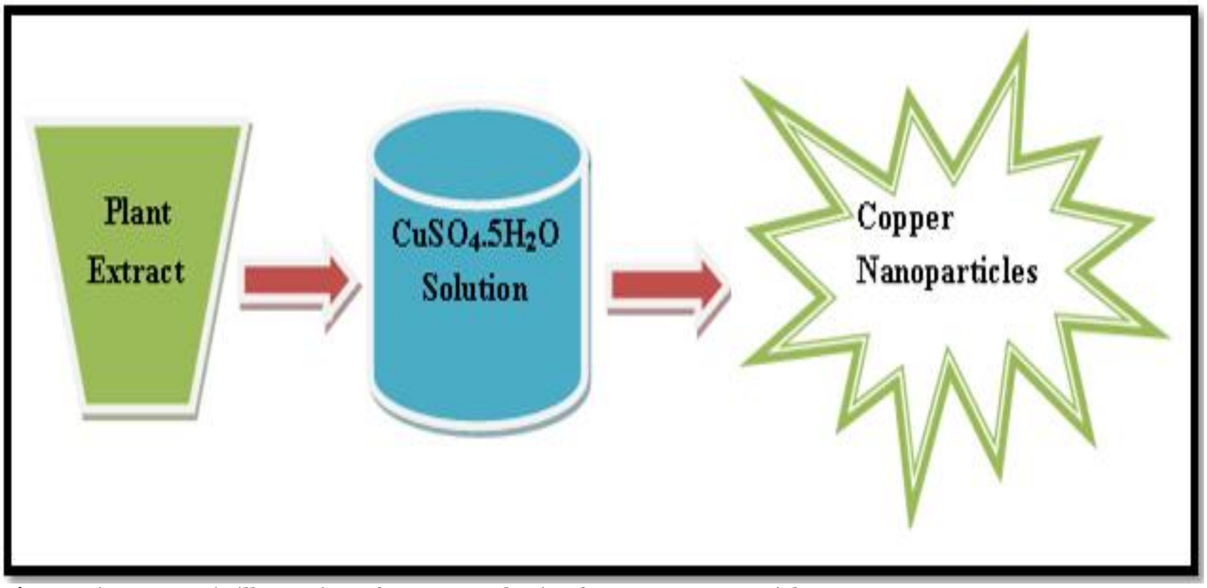

Fig. 1. Diagrammatic illustration of Green Synthesis of Copper Nanoparticles.

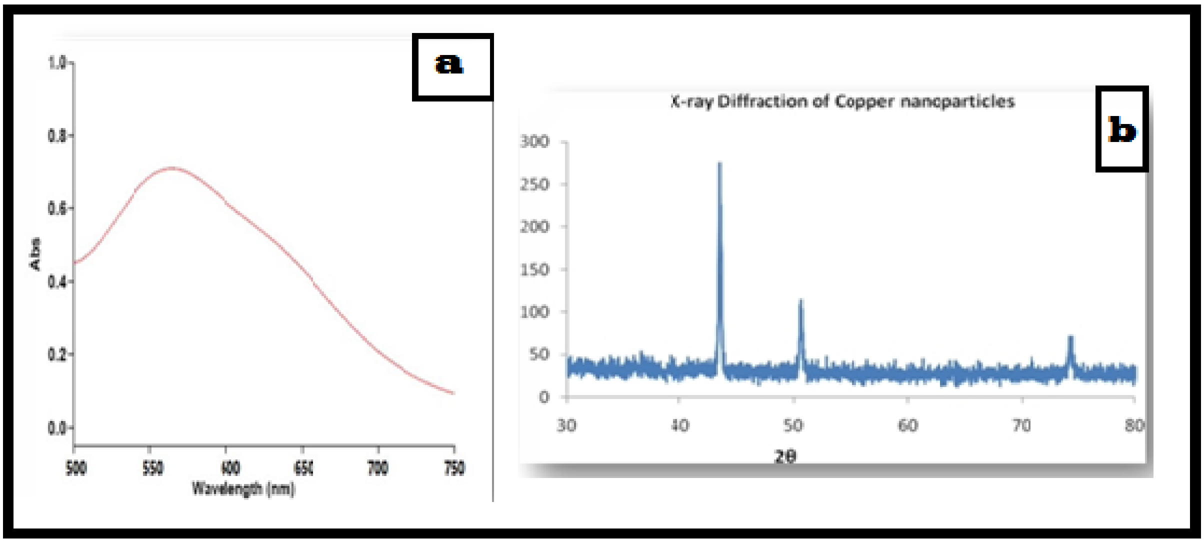

Fig. 2. UV Visible Spectrum and X-ray Diffraction Pattern of Green Synthesized Copper Nanoparticles.

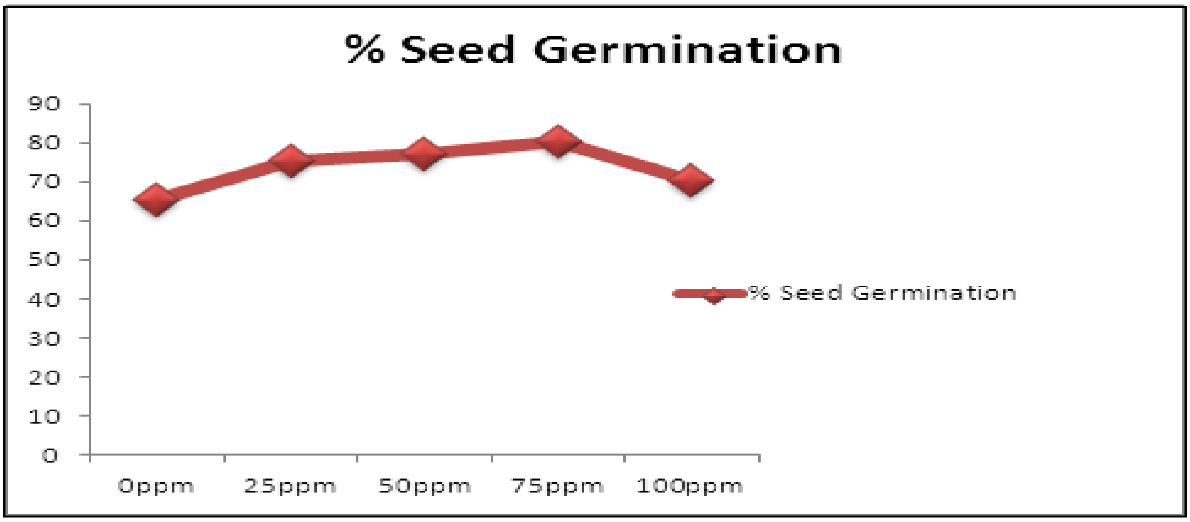

Fig. 3. Effect of Cu NPs on germination percentage in wheat.

Overall the results of this study showed that application of Cu NPs can increase the germination in wheat.

Among all the treatments 75ppm Cu NPs proved best because highest values for germination percentage (80.1a), germination time (4.9a), seedling vigor index (373a) as well as seed germination index (2.98a) was recorded at this concentration (Fig. 3, 4, 5). Root system was also well recognized and developed in less time in treated experiment as compared control.

Highest values for root fresh weight (2.98a) and root biomass (1.48a) were also recorded at 75ppm Cu NPs (Fig. 6, 7).

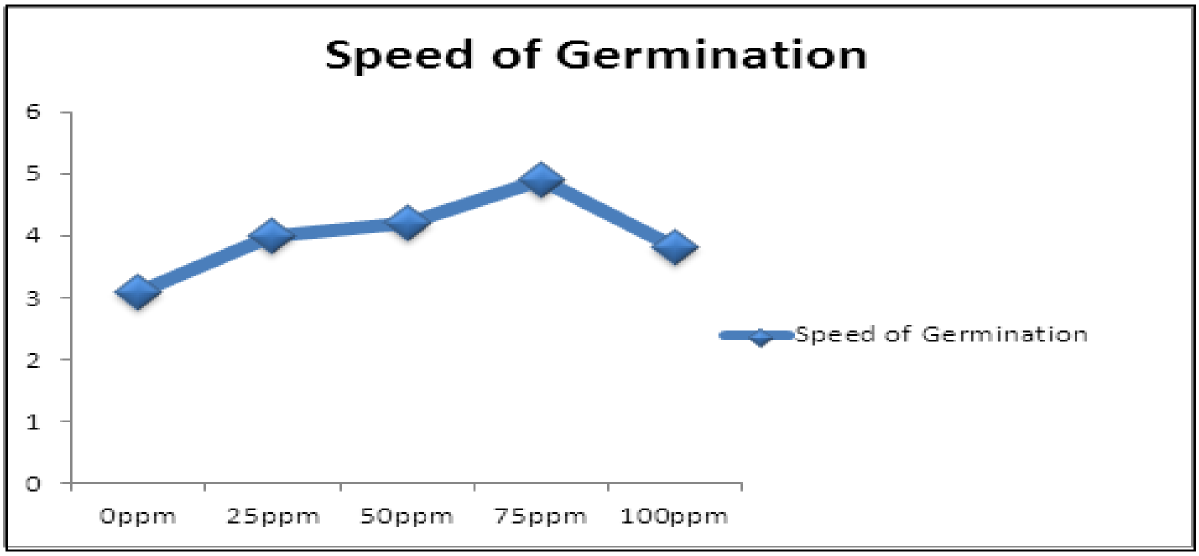

Fig. 4. Effect of Cu NPs on germination speed in wheat.

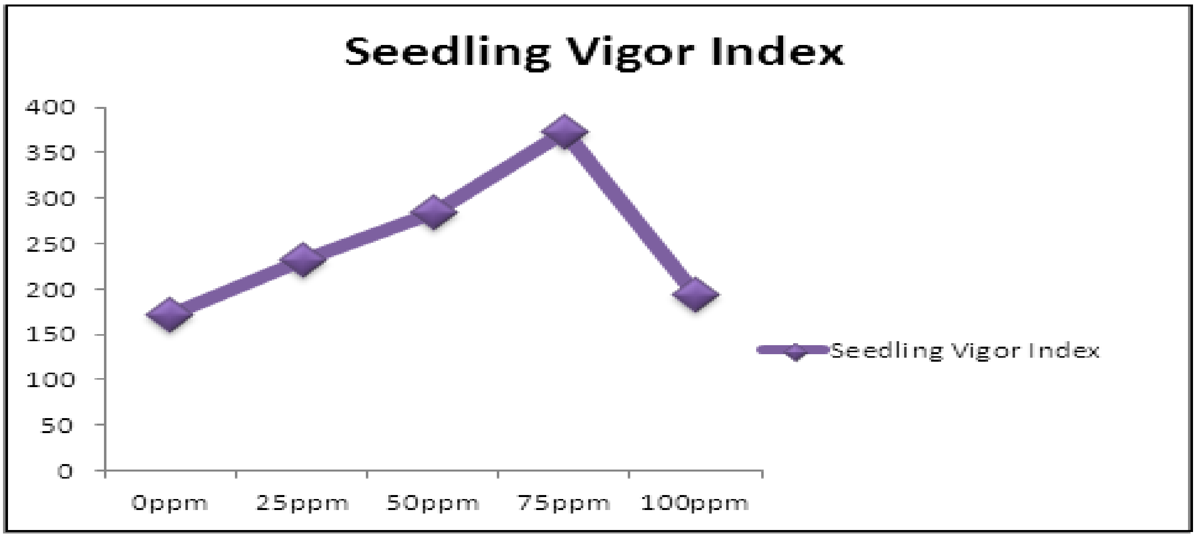

Fig. 5. Effect of Cu NPs on seedling vigor index in wheat.

So overall the results of present study showed that the level of seed germination and subsequent seedling growth was reduced with increasing concentration of Cu NPs.

Exposure of wheat seeds to Cu NPs revealed a clear dose dependent inhibitory effect Exposure of the Wheat seeds to the showed a clear and dose-dependent inhibitory effect on their consequent germination success and on the growth of that seedlings that did germinate.

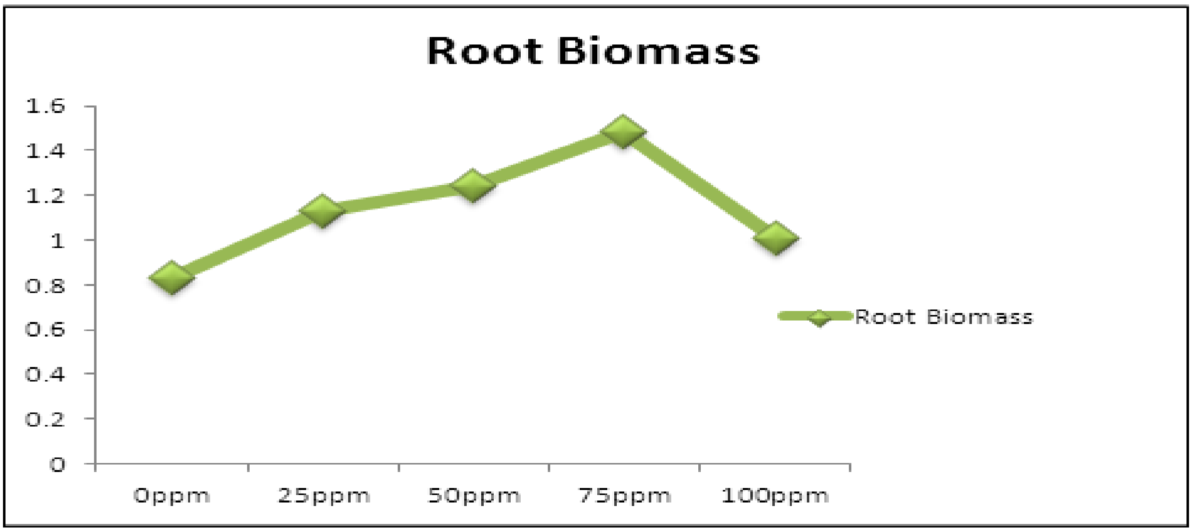

Fig. 6. Effect of Cu NPs on root biomass in wheat.

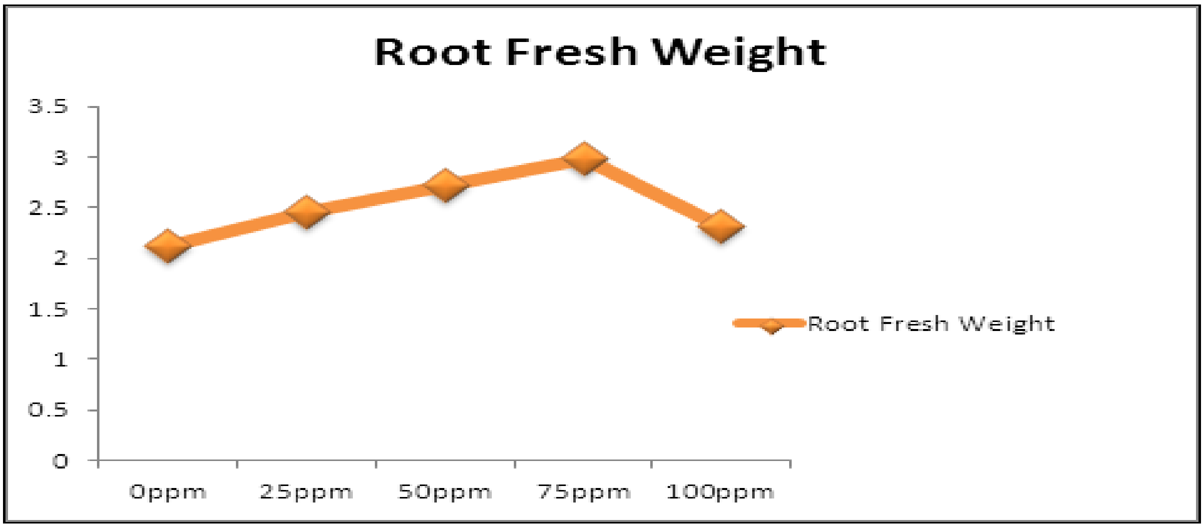

Fig. 7. Effect of Cu NPs on root fresh weight in wheat.

Conclusion

Nanomaterials are intended to be the materials for the new millennium. Nanoparticles of size below 100nm fall in the transition zone between individual molecules and the corresponding bulk materials, which can generate both positive plus negative biological effects in living cell. There is increasing amount of research on the biological effects of nanoparticles on higher plants. Only few studies have been reported on the promotory effects of nanoparticles on plants in low concentrations.

Wheat seeds responded to Cu NPs with higher seeds germination percentage compared to control (Fig. 6). Graphical abstracts. The increased growth rate of the seedlings might be due to the enhanced uptake of water and nutrient by the treated seeds. These results suggest that release of Cu NPs into the environment could have only positive effects on plant communities. Enhanced seed germination as well as early plant growth is vital to achieve crop productivity, especially for crops that otherwise show poor germination rates. The profound effect on the early stages of plant growth may be followed by similar enhancements at later stages as well, and by applying nanoparticles we may be able to improve plant productivity too.

In conclusion, the results of present study showed that the application of Cu NPs significantly promoted seed germination potential in wheat. Application of Cu NPs enhanced seed germination percentage, mean germination time, germination index, seedling vigor index as well as seedling fresh and dry weight. It was observed that the accumulation and uptake on nanoparticles was dependent on the exposure concentration.

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