RESEARCH ARTICLE

Copper Nanoparticles using Onion *(Allium cepa)* Extract and their Application in Plant Growth

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ABSTRACT

Rapidly expanding use of nanoparticles in every section of life necessitates finding their potential to increase food production to match with increasing population. This study was conducted in NTRC (Nano Technology Research Centre) lab to determine the potential of copper nanoparticles (Cu-NPs) for enhancing growth of wheat. Cu-NPs were prepared from onion extract. Their characterization carried out. (UV-VIS Spectroscopy, Atomic Force Microscopy, Scanning Electron Microscopy, Energy Dispersive x-ray Spectroscopy). In pots Cu-NPs of (15, 25, 35, 45and 55 ppm) concentration significantly increased growth of wheat as compared with control. However, 35ppm Cu-NPs treated pot produced significantly higher root length; shoot length, germination percentage, chlorophyll content, fresh weight, dry root weight. Results of this study reveal that Cu-NPs have the potential to enhance growth of wheat but their desired effect is depending on its concentration.

Keywords: Copper nanoparticles, Onion Extract, Plant Growth, SEM – EDS, UV-Visible spectroscopy.

INTRODUCTION

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. 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. Target oriented, cheaper and efficient technologies have to be employed for sustainable increase in production of wheat crop (Abdul *et al.,*2015)). Nanotechnology deals with atomic or molecular aggregates of 1 to 100 nm in size. Nanoparticles of gold (Au), silver (Ag), copper (Cu), zinc (Zn), aluminium (Al), silica (Si), zinc oxide (ZnO), caesium oxide (Ce_2O_3), titanium dioxide (TiO_2) and magnetized iron (Fe) have found applications in agricul-

ture (Josef and Katarína, 2015). There are 17 elements that are known to be essential for plant growth and development. To increase food production it is necessary to use the different technologies in agriculture. Fertilizers produced with nanotechnology have special importance. Fertilizers derived from nanotechnology have started to attract attention in agriculture now a days. This study was undertaken to determine the effect of nanotechnology liquid fertilizers on plant growth and yield (Melek *et al.,* 2014).

A nanofertilizer refers to a product that delivers nutrients to crops in one of three ways. The nutrient can be encapsulated inside nanomaterials such as nanotubes or nanoporous materials, coated with a thin protective polymer film, or delivered as particles or emulsions of nanoscale dimensions. Zinc–aluminium layered double-hydroxide nanocomposites have been used for the controlled release of chemical compounds that regulate plant growth. Nanosize titanium dioxide has been incorporated into fertilizers as a bactericidal additive. The seed germination in the presence of CuNPs showed an increase in shoot to root ratio compared to control plant] (Maria *et al*.,2010) Treatment of ZnO NPs with the mean particle size of 25 nm at 1000 ppm concentration was found to promote both peanut seed germination and seedling vigour and in turn showed early establishment in soil manifested by early flowering and higher leaf chlorophyll content. The treatment of *Catharanthus roseus* plant for 70 days with Fe_3O_4 NPs resulted in significant increase of the leaf growth parameters and carbohydrate contents in comparison with control plants . (Naderi and Shahraki ,2013), Some other NPs, e.g. TiO_2 NPs, Al_2O_3 NPs or single walled or multiwall carbon nanotubes were found to have beneficial effect on plant growth.

In this research paper we discuss the green synthesis of *Cu-NPs* using onion extract and their application in wheat plant growth. The results show positive effects in wheat 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 of wheat.

MATERIAL AND METHODS

2.1 Preparation of PE

The onion (*Allium cepa L.*) is a vegetable and is the most widely cultivated species of the genus Allium. The onion was first washed; air dried and was finely chopped. Then the chopped onion was used for making its aqueous extract.

2.2 Synthesis of Cu-NPs

A solution of $CuSO_4.5H_2O$ (100 ppm) was taken in conical flasks and Copper ions were reduced by stepwise addition of 250 ml of onion extract carried out by ultra sonicated at 100°C in water bath. Translucent yellowish green colour was indication for conversion of Copper ion into Cu-NPs. The reaction mixture was kept in refrigerator for 24 hours incubation and was further used to carry out its characterization and its use in study of wheat plant growth activity.

2.3 Characterisations of Cu-NPs

The reduction of copper ions was monitored using UV – VIS Spectrum of the synthesized aqueous nanoparticle solution using onion extract. Analysis of UV – VIS Spectrum (Systronic 2203) of the aqueous mixture was done in the range of 200 – 600 nm.
SEM Analysis (HITACHI) was done to analyze the particle size and morphology of synthesized copper nanoparticles. The SEM Analysis was carried out at IIT – Bombay. EDS Analysis (HITACHI) was done to analyze the elemental composition of the copper nanoparticle solution. AFM was also done to view surface background of Cu-NPs and their size. The AFM analysis (NANOSURF) was done at Nano Technology Research Lab, Birla College, Kalyan.

2.4 Cu-NPs for Wheat plant growth

From stock solutions (100 PPM) (15 ppm, 25 ppm, 35 ppm, 45 ppm and 55 ppm) concentrations of Cu-NPs were prepared. In plastic pot soil was homogenized after mixing recommended doses of NPK fertilizer (Maxi Grow powdered plant food). Wheat seeds washed with sterile distilled water. Washed wheat seeds were sowed into soil. In each pot 40 seeds were sowed with specific distance. On the sowed seeds thin layer of 1-2 cm soil was spread. One of the pots labelled as control pot while other pots labelled as concentrations of CuNPs. In the remaining pots we added 15 ppm - 55 ppm concentrations of nanoparticles along with sowed seed and NPK. Water was applied according to the requirement of plant in control. Cu-NPs as fertilizer were sprinkled in remaining pots. After two week thinning was done leaving ten plants of almost equal size in each pot. Cu-NPs were applied as thrice in week, because wheat

was grown in winter season needed less water and less nanofertilizers. After four weeks growth data were recorded for root length, shoot length, chlorophyll content, dry root weight, fresh shoot weight and germination percentage. The germination percentage was calculated by using following formula.

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

RESULTS AND DISCUSSION

3.1 Synthesis of CuNPs

The CuNPs were prepared using onion extract by ultra sonication at various pH. Translucent yellowish green colour with no precipitation obtained in pH 4 onion extract hence pH 4 is suitable for synthesis of CuNPs. Optimum temperature for synthesis of CuNPs is at 100°C. The CuNPs prepared using above method was stable up to 5-6 weeks at room temperature (Fig.1-A and B).

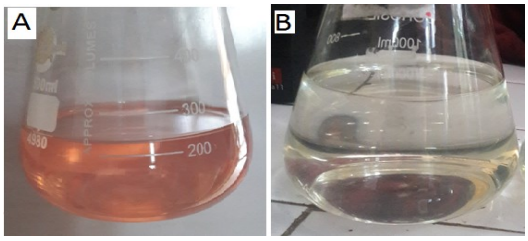

Fig.1 A) onion extracts at pH4 B) synthesised CuNPs using onion extract

3.2 UV-Visible spectrum of CuNPs

The UV-Visible spectrum of CuNPs obtained using pH 4 onion extract was shown in Fig.2.

The maximum absorption peak at 260 nm shows formation of CuNPs.

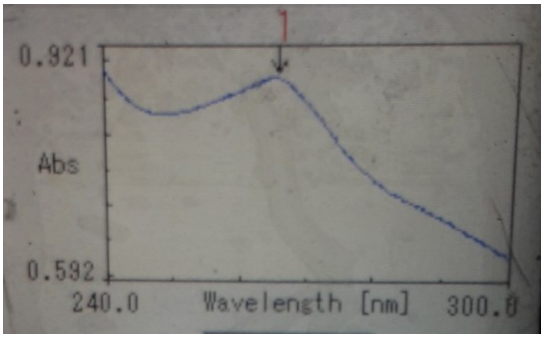

Fig.2 UV-Visible absorption spectrum of synthesised CuNPs using onion extract

3.3 SEM Analysis of CuNPs

The SEM image of zero valent copper nanoparticles is shown in Fig 3. The CuNPs appear round in shape and the average particle size found ranges from 21nm – 23nm.

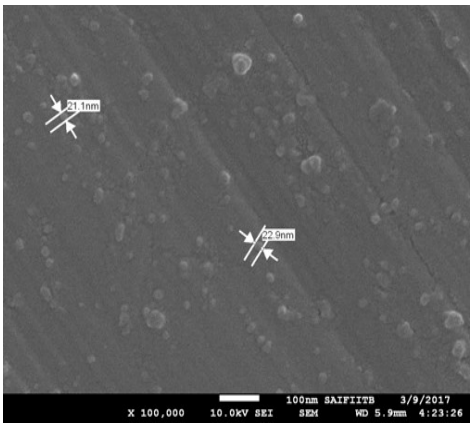

Fig.3 SEM image of synthesised CuNPs using onion extract

3.4 Elemental Analysis

The elemental analysis & chemical characterization of CuNPs shows that the solution contains copper, iron, aluminium, carbon, oxygen, chlorine elements in it.

The high peak of aluminium is the result of the aluminium-coated grid on which the sample was placed. However, from the spectral range it is evident that aluminium is also an integral element in the solution. The presence of other metals might be due to onion plant extract used for synthesis. The higher percentage of carbon and oxygen is observed because of the plant material used. Elemental analysis shown in fig.4 confirms the weight and atomic percentage of CuNPs is 17.83 and 33.51% respectively. It also contains low percentage of Carbon, Oxygen, Iron, Sulphur and Chlorine.

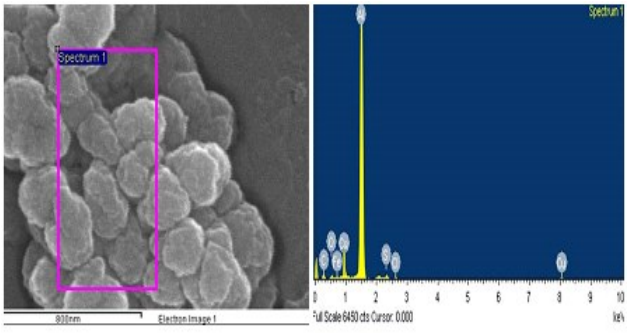

Fig.4 EDS image of synthesised CuNPs using onion extract

3.5 AFM Analysis

The external morphology of CuNPs was recorded using AFM shown in fig. 5. The maximum particle size of CuNPs was found to be 35 nm having spherical shape.

3.6 Cu-NPs for Wheat plant growth

The growth of root and shoot of wheat plant was analysed after four weeks. The photographic images results shows that the control pot and CuNPs treated pot of concentration 15-55 ppm shown in fig.6. The 35 ppm concentration treated CuNPs pot comparatively showed better results in term of rootv length, shoot length, germination percentage, fresh shoot weight, root dry weight and chlorophyll content shown in Table 1.

Table 1, shows as compared to control, different concentrations CuNPs treated wheat crop gave the better results. 35ppm CuNPs treated wheat crop shows higher chlorophyll content, shoot length, root length, germination percentage, root dry weight, fresh shoot weight as compared to control. Wheat crop treated above 35ppm concentration of CuNPs affect the crop growth and also affects the other parameters. Increasing the level of CuNPs to 45 and 55ppm was accompanied by a significant reduction in chlorophyll.

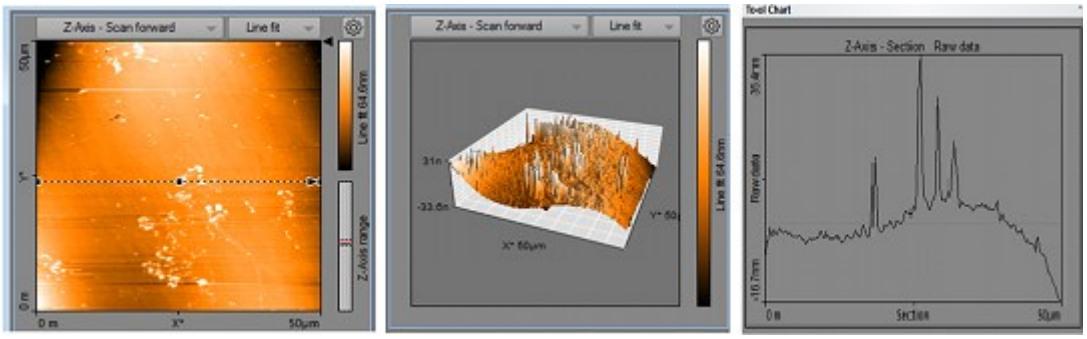

Fig.5 : AFM image of synthesised CuNPs using onion extract

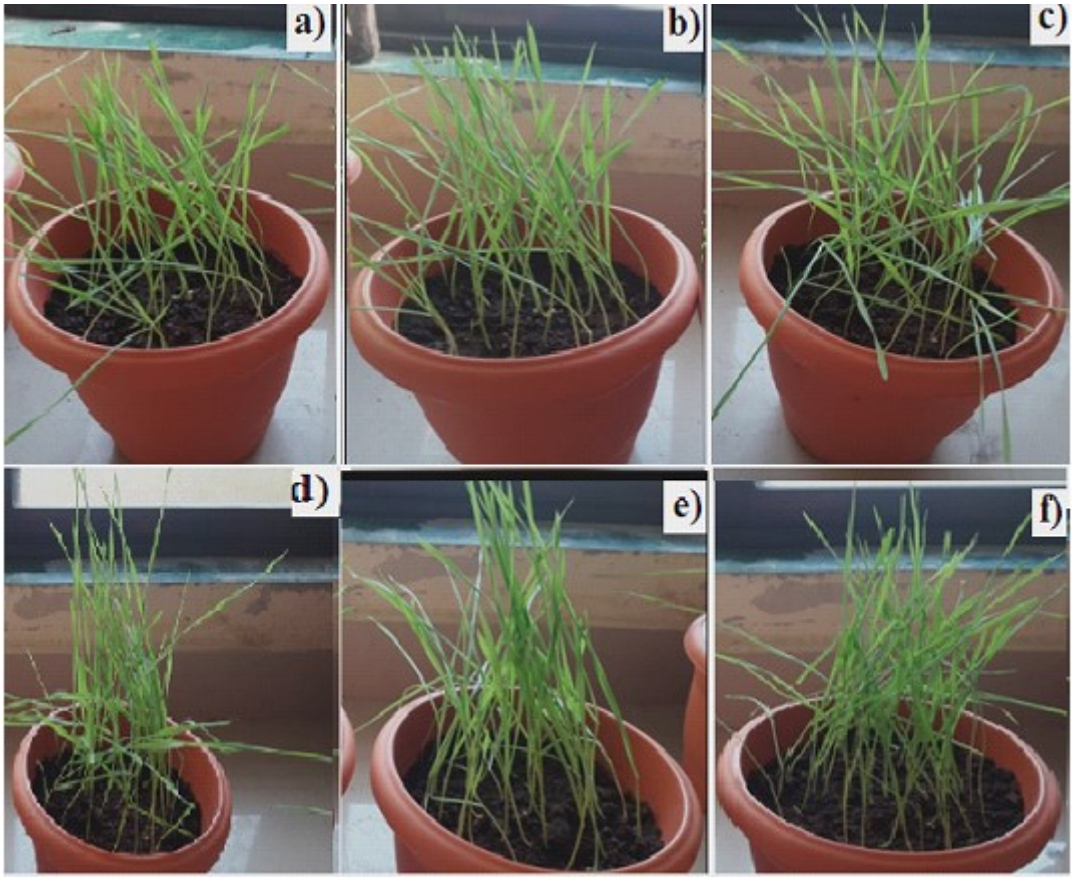

Fig. 6: Result for wheat growth a) control pot CuNPS treated pot of concentration b) 15 ppm c) 25 ppm d)35 ppm e) 45 ppm f) 55 ppm

Table 1. Results of Control and Different Concentrations CuNPs Treated Wheat Crop

Measured parameters	Control	CuNPs Concentration in ppm				
		15	**25**	**35**	**45**	**55**
Root length (cm)	5	14	15	19	16	14
Shoot length (cm)	20	27	32	37	31	29
Germination percentage (%)	0.575	0.600	0.675	0.900	0.750	0.600
Root dry weight (mg)	150.9	173.7	192.7	210	125	82
Fresh shoot weight (mg)	211.2	734	976	1123.4	787	406
Chlorophyll content (mg/g)	4.0284	5.3694	6.0270	11.5523	8.1235	6.5275

CONCLUSION

From the present study we can successfully conclude that large scale eco-friendly production of zero valent CuNPs is possible by using aqueous extract of *Allium cepa.* The CuNPs are synthesized at 4 pH not only formed uniform size particles but also increase stability up to 4-5 weeks at room temperature. The colour change of the plant extract to translucent green is due to the phenomenon of surface Plasmon resonance of CuNPs.

The UV absorption peak at 260 nm shows formation of CuNPs. The SEM image shows particle size of CuNPs is 21nm-23nm. The elemental analysis shows the elemental percentage composition of copper, iron, aluminium, carbon, oxygen, chlorine present in solution. AFM Image of CuNPs shows the maximum particle size is 35 nm.

The fertilizer activity of the synthesized Cu-NPs was evaluated. It was observed that wheat crop treated with various concentrations (15 to 55ppm), 35ppm concentration gives better result in terms of parameters like chlorophyll content, shoot length, root length, germination percentage, dry root weight and fresh shoot weight as compared to control. After the 35ppm concentration, it is observed that wheat growth declined. CuNPs certainly have potential to enhance growth of wheat crop. 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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