

Research Article

Effect of Cu²⁺/Chitosan Nanoparticles on the Growth of Tomato (*Solanum Lycopersicum Mill*) Seedling

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Cu-chitosan nanoparticles were synthesized and evaluated for seedling growth in tomato (*Solanum lycopersicum Mill*). Effectiveness of Cu-chitosan in promoting tomato plant growth were studied. Ionic gelation method was used for synthesis of nanoparticles. Particle size of Cu-chitosan nanoparticles was found 295.4 ± 2.8 nm analyzed by DLS i.e. dynamic light scattering. These synthesized nanoparticles were used for seed treatment for 6hrs. Different concentrations were used for the present investigation and various growth parameters viz Fresh weight, shoot length, root length and no. of leaves were recorded. Cu-chitosan nanoparticles at 50ppm has significant effect on almost all the parameters whereas concentration 800 ppm shown inhibitory effect. This is because Cu-chitosan Nanoparticles may commendably supply copper as a micronutrient for the plants, while the uncontrolled uptake of copper ions from the commercial fungicide inhibits the healthy development of plants.

Keywords: Cu-chitosan Nanoparticles, Growth, Ionic gelation method

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Introduction

Tomato (*Lycopersicon esculentum L.*) is one of the common vegetable crops, available throughout year and an economical source of minerals and antioxidants. Nanotechnology is a potential and emerging field with multiple applications in different areas of study. The beneficial effects of the use of nanoparticles in agriculture have already been proven. Chitosan is a natural polymer, which has been used in agriculture to stimulate crop growth. Chitosan is produced from chitin which is abundantly found in cell wall of fungi and forms exoskeleton of insects and crustaceans. Chitosan is poly (1,4)-2-amino-2-deoxy- β -D glucose, which is deacetylated product of chitin. Being highly basic in nature, chitosan has some unique properties like metal ion chelation, reactive to polyanions, non-antigenic. In case of agricultural point of view, it has got some useful properties like act as fungicide, as elicitor, soil modifier, ability to form films etc. Further they are safe and non-toxic to environment as well as biodegradable. Chitosan solubilizes only at acidic pH and it precipitates out when added to the cultures at physiological pH so there is a need to develop a dispersion system to check the antifungal activities of it. Thus, chitosan nanomaterial mainly used for plant defence and yield increase as a natural biocontrol as well as elicitor [1-5]. Ionic gelation method was used for preparation of Cu-chitosan NCPs [6,7]. This is because Cu-NPs effectively deliver copper as a micronutrient for the plants, while the uncontrolled uptake of copper ions from the commercial fungicide (copper hydroxide) inhibits the healthy development of plants. Importantly, despite the principal function of Cu-NPs as a fungicide, Cu-NPs may be applied as fertilizer for tomato plants. Thus, the application of Cu-NPs effectively promotes the growth of tomato plants. Therefore, these results suggest that copper nanomaterials may be used as both a source of micronutrients in cases of soil copper deficiency and as a fungicide. In terms of sustainability, this multifunctionality has the potential to positively impact the environment.

Material and Methods*Materials*

Low molecular weight Chitosan which is 80% N-deacetylated, Sodium tri-polyphosphate anhydrous (TPP), CuSO₄ and acetic acid were procured from Department of Molecular Biology and Biotechnology, RCA, MPUAT, Udaipur, Rajasthan, India.

Preparation of Cu²⁺ chitosan nanoparticles by using ionic gelation technique which is based on the ionic interactions between the positively charged primary amino groups of chitosan and the negatively charged groups of polyanion, such as sodium tripolyphosphate (TPP). Cu²⁺ added in 0.01 concentration 6,7.

Characterization of Cu-chitosan nanoparticles

Seed treatment and growing condition: To test the effect of Cu²⁺ chitosan nanoparticles on tomato seedling growth seeds were treated by soaking in different concentrations of Cu²⁺ chitosan nano-formulations (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) for 6 hrs and sown in trays having media (vermiculite + perlite + coco powder). Seedlings were watered and maintained under greenhouse conditions. When the plants were 30 days old various growth parameters viz Fresh weight, Shoot length, Root length and No. of leaves.

Results and Discussion

Results of experiments revealed that the seedling growth of tomato under different treatments showed different growth rate in terms of the fresh weight, shoot length (cm), root length (cm) and no. of leaves (Table 1). These experiments were conducted as per the recommendation of International Seed Testing Association (1976). Synthesized nanoparticles were characterized by DLS and SEM analysis. Particle size of Cu-chitosan nanoparticles was found 295.4 ± 2.8 nm analyzed by DLS i.e. dynamic light scattering (Figure 1). SEM (Scanning Electron Micrograph) showed sphere shaped nanoparticle as shown in Figure 2. In 6 hrs treated seeds fresh weight was recorded maximum in 50 ppm concentration of Cu-chitosan nanoparticles and 800 ppm showed inhibitory effect (Figure 3). Shoot length was recorded maximum in 400 ppm and minimum in control and 800 ppm (Figure 4). Root length was found maximum in 50 ppm concentration and 800 ppm also showed inhibitory effect (Figure 5). No. of leaves were found maximum in 50 ppm (Figure 6). Even though various methods have been developed in the recent years for the synthesis of nanoparticles, still there is a need for development of new shape-selective synthesis strategies, because nanoparticles express more biological interaction in living system^{9,10,11,12}. The findings of the present study reveal that copper nanoparticles have potential to enhance the growth of tomato seedlings.

Table 1 Effect of Cu-chitosan Nanoparticles concentrations (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) in different growth parameters. Values represent the mean (\pm) standard error of three independent experiments. Each value is mean of triplicate. The results were significant at $p < 0.05$.

Treatment	Fresh Weight	Shoot length (cm)	Root length (cm)	No. of leaves
Control	0.79 \pm 0.1 ^B	8.64 \pm 0.15 ^C	5.2 \pm 0.22 ^D	6.33 \pm 0.2 ^B
20 ppm	1.31 \pm 0.03 ^A	10.7 \pm 0.12 ^{AB}	6.9 \pm 0.19 ^{AB} ^C	7.26 \pm 0.13 ^B
50 ppm	1.42 \pm 0.09 ^A	11.2 \pm 0.17 ^A	8.31 \pm 0.47 ^A	12.25 \pm 0.19 ^A
400 ppm	1.29 \pm 0.1 ^A	11.5 \pm 0.34 ^A	7.17 \pm 0.49 ^A ^B	12.17 \pm 0.79 ^A
600 ppm	1.33 \pm 0.08 ^A	11.43 \pm 0.4 ^A	6.61 \pm 0.15 ^{BCD}	12.04 \pm 0.34 ^A
800 ppm	1.19 \pm 0.04 ^A ^B	9.91 \pm 0.25 ^B	5.35 \pm 0.28 ^{CD}	7.51 \pm 0.28 ^B

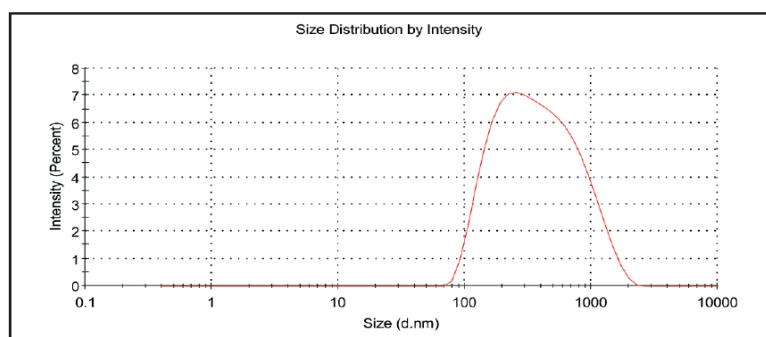


Figure 1. DLS analysis of Cu-chitosan Nanoparticles.

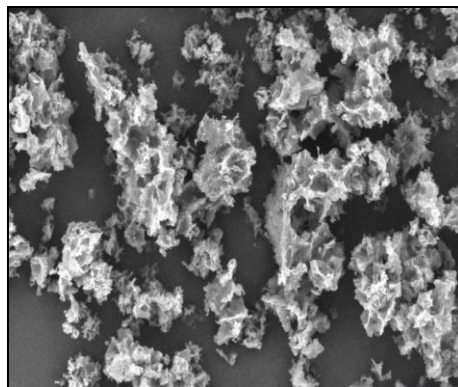


Figure 2. SEM micrographs of Cu-chitosan Nanoparticles at 9.2mm \times 1.00K.

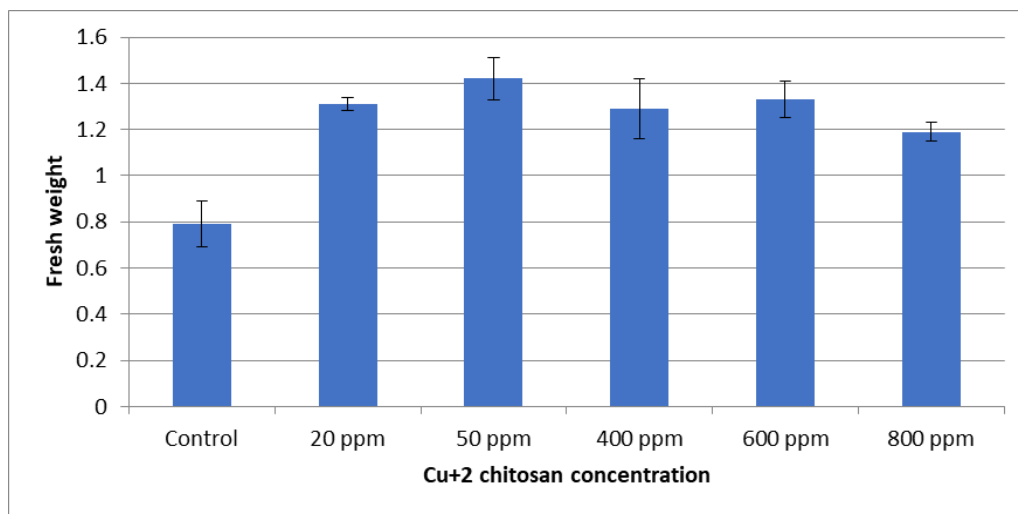


Figure 3. Graphical representation of effect of different concentrations of Cu²⁺ chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on fresh weight.

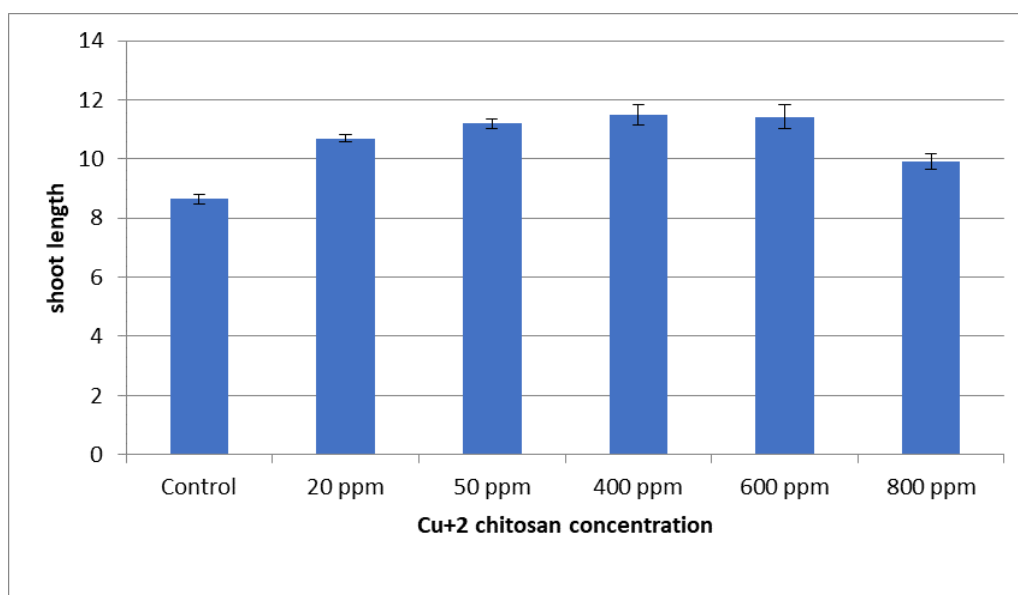


Figure 4. Graphical representation of effect of different concentrations of Cu+2 chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on shoot length.

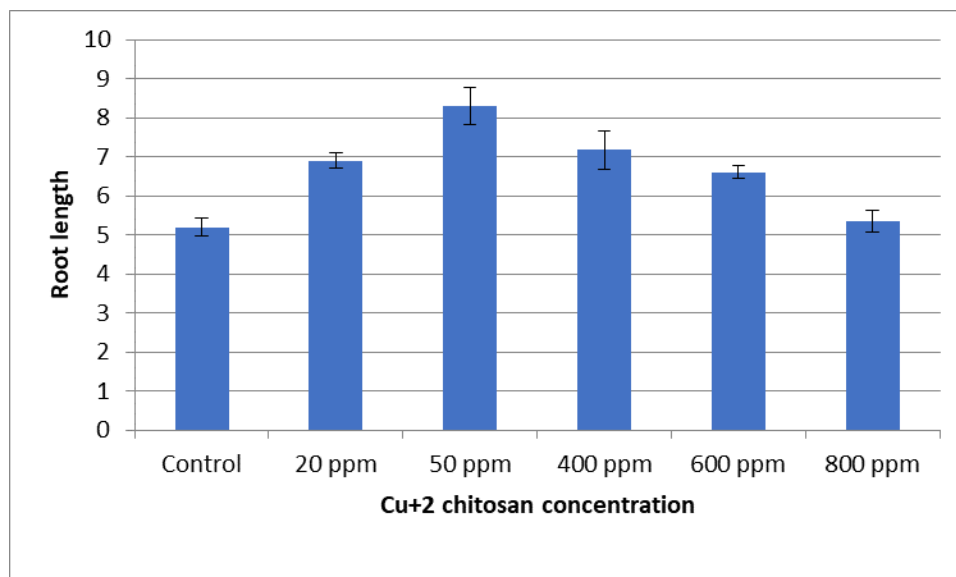


Figure 5. Graphical representation of effect of different concentrations of Cu+2 chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on root length.

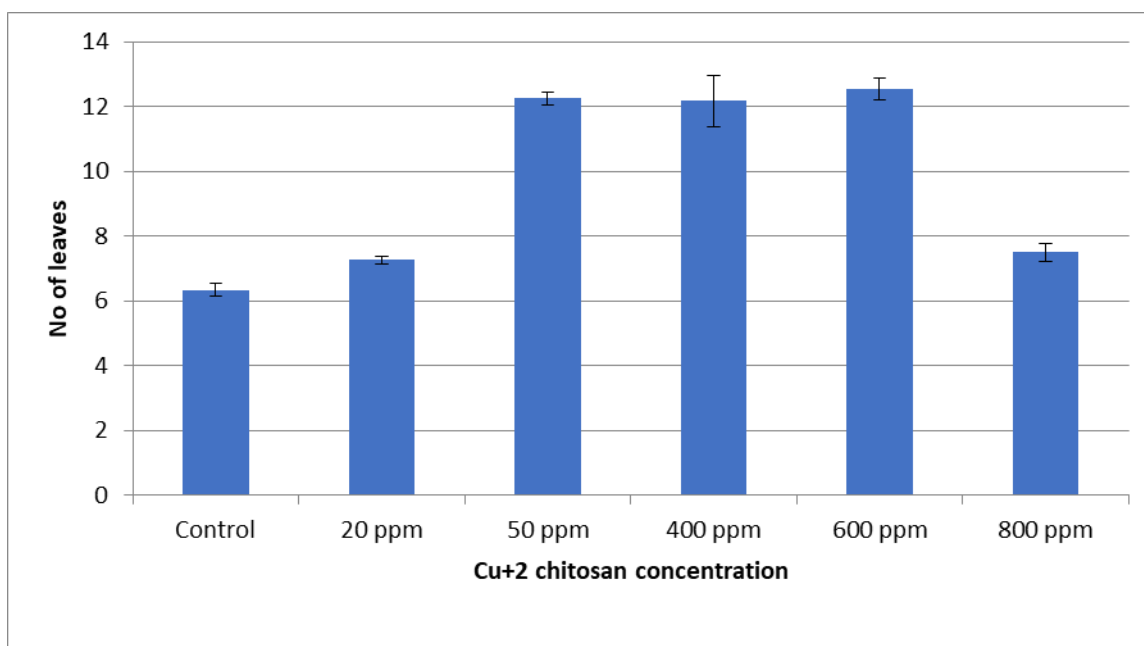


Figure 6: Graphical representation of effect of different concentrations of Cu+2 chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on number of leaves.

Statistical Analysis

The statistical analysis for the data was performed with JMP software version 12 using Turkey Kramer HSD test for determining significant differences among treatment at $p = 0.05$ level 14. Each experiment was repeated twice with minimum three replications (SAS, 2010).

Conclusion

The findings of the present study reveal that copper nanoparticles have potential to enhance the growth of tomato seedlings. Among the various concentrations (50 ppm and 400 ppm) of Cu chitosan nanoparticles considered as the optimum level for the growth of tomato (*Solanum lycopersicum* Mill). However higher concentration (800 ppm) of Cu chitosan nanoparticles affects the plant growth. The outcome of the present

study will be useful in finding the potential of nanoparticles in crop improvement and other agricultural applications.

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