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Effect of Cu^{+2} Chitosan Nanoparticles on Chilli (*Capsicum annum L.*) Seedling Development

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ABSTRACT: Synthesized Cu–chitosan nanoparticles were assessed for seedling development in chilli (*Capsicum annum* L.). Ionic gelation method was used for synthesis of Cu- chitosan nanoparticles. These synthesized nanoparticles were used for seed treatment for 6hrs. Different concentrations were used for the present investigation and various growth attributes like fresh weight (in gms), shoot length (in cm), root length (in cm) 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 Cu as a micronutrient for the plants, while the abandoned application of Cu ions from the commercial fungicide constrains the vigorous growth of plants. In agricultural field, utilization of chitosan based nanomaterials especially for growth, development and protection is rare as compared to bulk chitosan. So, Chitosan based nanoparticles may clutch enormous promises regarding their application in plant growth and development.

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

INTRODUCTION

Chili (*Capsicum annuum* L.) is a spice and a fruit vegetable commonly cultivated, is an tremendous source of health allied phytochemical compounds, such as vitamin C, provitamin A, vitamin E, flavonoids, and capsaicinoids that are very essential in human health (Thuesombat *et al.,* 2014). Nanotechnology is a prospective and evolving field with various uses in diverse areas of study. Chitosan which is a natural polymer has been used in agriculture to stimulate crop development. Chitosan is produced from chitin which is abundantly found in cell wall of fungi and forms exoskeleton of insects and crustceans. Chitosan which is a deacetylated form of chitin. Being highly basic in nature, chitosan has some unique properties like metal ion chelations, 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 defense and yield increase as a natural biocontrol as well as elicitor (Darvill, 1992: Agarwal *et al*., 2002; Badawy and Rabea, 2011; Chen *et al*., 2014 and Saharan *et al*., 2015). Ionic gelation method was used for preparation of Cu-chitosan NCPs (Du *et al*., 2009 and Ezra *et al*., 2004). Due to metal nanoparticles variability and random environmental harmfulness has eminent serious griefs in crops (Lin and Xing 2008; Kumari *et al.*, 2009; Dimkpa *et al.*, 2012 and Siddiqui *et al*., 2015). The current situations stimulate the environmentally free compounds such as chitosan as a safe substitute to synthetic chemical compounds. Biodegradable and non-toxic nature of chitosan biopolymer as a nano form have a great importance in agricultural nanotechnology. So Cu-NPs efficiently supply Cu as a micronutrient for the plants, while the abandoned application of Cu ions from the commercial fungicide inhibits the healthy growth of plant life. Significantly, despite the principal function of Cu-NPs as a fungicide, Cu-NPs may be useful as fertilizer for chilli plants.

MATERIAL AND METHODS

Materials. Low molecular weight Chitosan which is 80% *N*-deacetylated, Sodium tri-polyphosphate anhydrous (TPP), $CuSO_4$ and acetic acid were procured from Department of Molecular Biology and Biotechnology, RCA, MPUAT, Udaipur, Rajasthan, India.

Preparation of Cu^{+2} chitosan nanoparticles by using ionic gelation method which is a ionic interactions among the positively charged primary chitosan amino groups and the negatively charged polyanions, such as sodium tri-polyphosphate (TPP) (Saharan *et al*., 2013 and Saharan *et al*., 2015). Cu^{+2} added in 0.01 concentration (Fig. 1A and B).

(A)

(B)

Fig. 1: (A) Synthesis of Cu-chitosan nanoparticles using ionic gelation method. (B) Cu-chitosan nanoparticle formulation

Seed treatment and growing condition: To test the Cu^{+2} chitosan nanoparticles efficacy on chilli seedling development, seeds were treated by soaking in different concentrations of Cu^{+2} 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 attributes like fresh weight, shoot length, root length and no. of leaves.

RESULTS AND DISCUSSION

Experimental results have shown that the seedling development of chilli under different treatments showed different growth rate in terms of the fresh weight, shoot length (cm), root length (cm) and number of leaves (Table 1). These experiments were accompanied as per the approval of International Seed Testing Association (1976). In 6 hrs treated seeds fresh weight was recorded maximum in 50 ppm concentration of Cu-chitosan nanoparticles and 800 ppm showed inhibitory effect (Fig. 2). Shoot length was recorded maximum in 50 ppm and minimum in 800 ppm (Fig. 3). Root length was found maximum in 50 ppm concentration and 800 ppm also showed inhibitory effect (Fig. 4). Number of leaves were found maximum in 400 ppm and shown inhibitory effect on 800ppm (Fig. 5).

The findings of the present study reveal that copper nanoparticles have potential to enhance the growth of chilli seedlings. Among the various concentrations (50 ppm and 400 ppm) of Cu chitosan nanoparticles considered as the optimum level for the growth of chilli (*Capsicum annum* L.). However higher concentration (800 ppm) of Cu chitosan nanoparticles affect the plant growth.

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 (±) standard error of three independent experiments. Each value is mean of triplicate. The results were significant at $p < 0.05$.

Treatment	Fresh Weight (gm)	Shoot length (cm)	Root length (cm)	No. of leaves
Control	0.45 ± 0.04^{A}	8.38 ± 0.21^{ABC}	4.77 ± 0.22^{B}	5.72 ± 0.15^{A}
20 ppm	0.44 ± 0.06^{AB}	8.71 ± 0.08^{AB}	5.33 ± 0.33^{B}	5.83 ± 0.12^{A}
50 ppm	0.51 ± 0.02^{A}	9.25 ± 0.12^{A}	6.61 ± 0.1^{A}	6 ± 0.09^{A}
400 ppm	0.43 ± 0.04^{AB}	8.25 ± 0.34^{BC}	4.94 ± 0.38^{B}	6.02 ± 0.11^{A}
600 ppm	0.38 ± 0.01^{B}	7.75 ± 0.04^{C}	4.62 ± 0.28^{B}	5.33 ± 0.22^{A}
800 ppm	0.22 ± 0^{AB}	5.19 ± 0.18^{D}	2.96 ± 0.13^{C}	4.12 ± 0.19^{B}

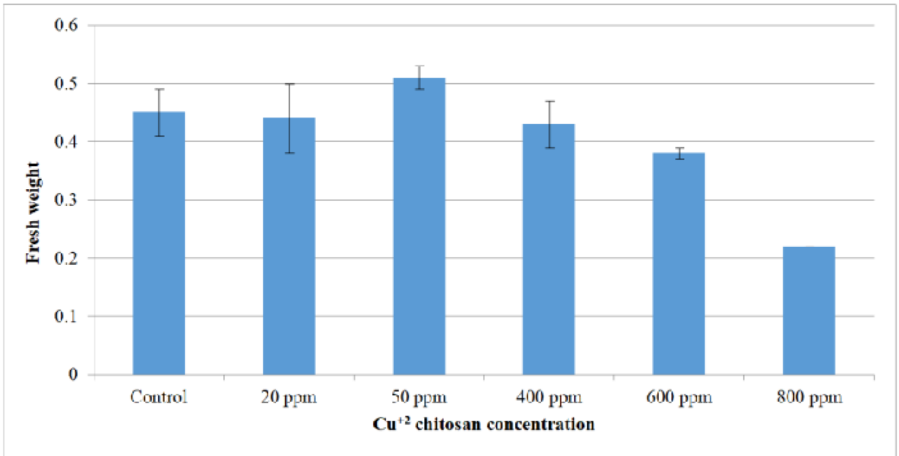

Fig. 2. Graphical representation of effect of different concentrations of Cu^{+2} chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on fresh weight.

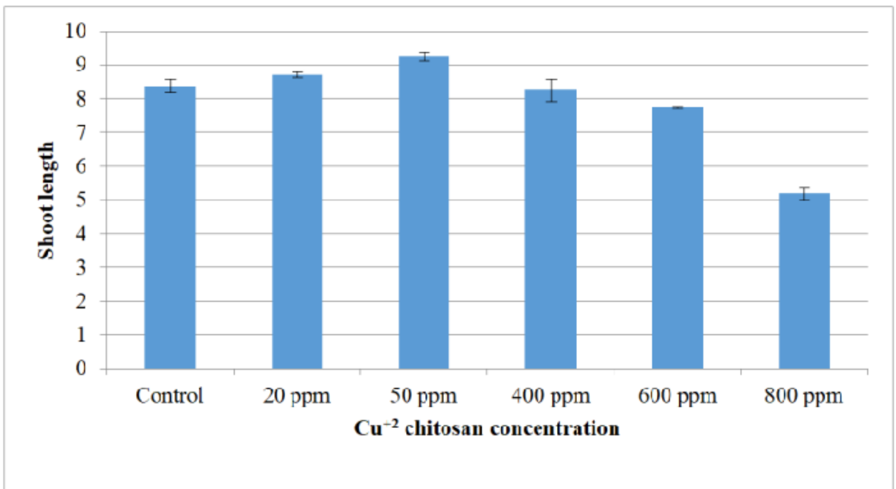

Fig. 3. Graphical representation of effect of different concentrations of Cu^{+2} chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on shoot length.

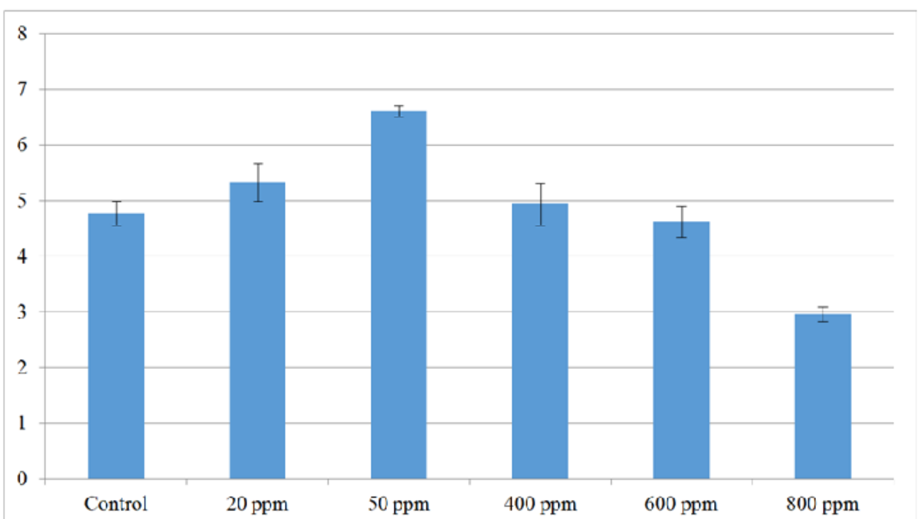

Fig. 4. Graphical representation of effect of different concentrations of Cu^{+2} chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on root length.

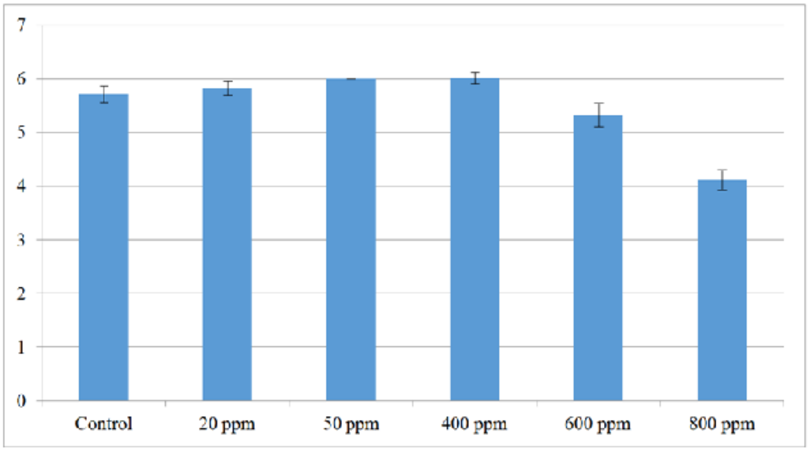

Fig. 5. Graphical representation of effect of different concentrations of Cu^{+2} chitosan nanoparticles (Control, 20 ppm, 50 ppm, 400 ppm, 600 ppm, 800 ppm) on number of leaves.

Therefore, Cu-chitosan nanoparticles efficiently stimulate the growth of chilli plants. Therefore, the present investigation recommend Cu chitosan nanoparticles may be used as a micronutrient source in cases of soil Cu scarcity as well as a fungicide also. In terms of sustainability, this y has the potential to positively influence the surroundings.

On the basis of published reports, it has been revealed that Nanotechnology unwraps a large scope of innovative application in the field of agriculture, because nanoparticles have unique physicochemical properties, i.e., high surface area, high reactivity, tunable pore size, and particle morphology. Nanoparticles employ their influence on plant growth and development (Ali *et al*.,2021; Boutchuen, *et al*., 2019; Shang *et al*., 2019; Ratna *et al*., 2018; Siddiqui *et al*., 2015 and Thuesombat *et al*., 2014). Therefore, the present research article highlights the key role of Cu-chitosan nanoparticles in growth and development of chilli seedlings. The applicable elucidation of physiological, biochemical, and molecular mechanism of nanoparticles in plant leads to superior plant growth and development.

Statistical Analysis. JMP software version 12 was used for statistical data analysis for determining significant differences among treatment at p = 0.05 level 14. Experiments were repeated two times with minimum three replications (SAS, 2010).

CONCLUSION

The findings of the present study reveal that Cu-chitosan nanoparticles have potential to enhance the growth of chilli seedlings. Among the various concentrations (50 ppm) of Cu chitosan nanoparticles considered as the optimum level for the growth of Chilli (*Capsicum annum* L.). However higher concentration (800 ppm) of Cu chitosan nanoparticles affect 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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