

## Chitosan Based Nano-shield to Combat Biotic Stress in Plants

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Global warming, population blast and dwindling arable lands are amongst the major issues requiring a sustainable solution to feed the nine billion world population by 2050. In the efforts to increase crop yields and food production, pesticides and agrochemicals have become an important component of world-wide agriculture systems. But, chemical pesticides, in use, are rapidly losing their potency against plant pathogens. Furthermore, their uncontrolled and rampant use has raised serious concerns of developing resistance in pathogens by mutations [1]. The extensive and unrestrained applications of these agrochemicals also lead to another exigent issue of environmental pollution. The perpetual use of pesticides causes negative impact on non-target organisms. Therefore, potential attention needs to be paid to develop biomaterial based biodegradable agrochemicals for safe and sound application in crops.

Nanotechnology can play an important role in modern agriculture to address the severity of plant diseases. From past 5 to 8 years, nanomaterials have also been utilized in agriculture fields mostly in crop protection [2]. Plant pathogenic fungi cause plant and seed diseases to the most economically important crops and result in quantitative and qualitative losses in agriculture. Chitosan, a versatile non-toxic, biocompatible and biodegradable biomaterial, can be exploited in agriculture via nanotechnology approach. It is well recognized as an antimicrobial, immuno-modulatory and plant growth promoting agent. Chitosan based nanoparticles provoke higher physiological and biochemical responses in plants as compared to their bulk form because of their higher surface to volume ratio [3]. Our research group has successfully prepared various chitosan based nanoparticles like chitosan-saponin and Cu-chitosan using ionic gelation method. Their particle size, polydispersity index (PDI), zeta-potential and structures were determined using diverse techniques involving DLS, FTIR, TEM and SEM. These studies highlighted the stability and porous nature of Cu-chitosan nanoparticles. Different nanoparticles were investigated, in *in-vitro* model, against plant pathogenic fungi namely *Alternaria alternata*, *Macrophomina phaseolina* and *Rhizoctonia solani* at concentrations ranging from 0.001 to 0.1%. We found that amongst the various nanoformulations, Cu-chitosan nanoparticles were most effective at 0.1% concentration and showed 89.5, 63.0 and 60.1% growth inhibition of *A. alternata*, *M. phaseolina* and *R. solani*, respectively. At this concentration, these nanoparticles also showed maximum (87.4%) inhibition rate of spore germination of *A. alternata* [4].

We further evaluated the laboratory synthesized Cu-chitosan nanoparticles and found substantial growth promoting effect on tomato seed germination, seedling length, fresh and dry weight at 0.08, 0.10 and 0.12% level. Studied in *in-vitro* model, these nanoparticles at 0.12% concentration caused 70.5 and 73.5% inhibition of mycelia growth and 61.5 and 83.0% inhibition of spore germination in *A. solani* and *F. oxysporum*, respectively. When evaluated on tomato plants in pot experiments, Cu-chitosan nanoparticles (0.12% concentration) exhibited 87.7 and 61.1 per cent efficacy of disease control (PEDC) in early blight and Fusarium wilt, respectively [5].

In another set of experiments, our group has investigated the impact of Cu-chitosan nanoparticles on physiological and biochemical changes during maize seedling growth. We recorded higher values of per cent germination, shoot and root length, root number, seedling length, fresh and dry weight, and seed vigor index at 0.04 - 0.12% concentrations of Cu-chitosan NPs as compared to water, CuSO<sub>4</sub>, and bulk chitosan treatments. Cu-chitosan nanoparticles at the same concentrations induced the activities of  $\alpha$ -amylase and protease enzymes and also increased total protein content in germinating seeds. We further observed that increased activities of  $\alpha$ -amylase and protease enzymes corroborated with decreased content of starch and protein, respectively, in the germinating seeds. Our studies decipher that Cu-chitosan nanoparticles enhance the seedling growth of maize by mobilizing the reserved food, primarily starch, through the higher activity of  $\alpha$ -amylase. We found that our laboratory synthesized nanoparticles were able to release Cu<sup>+2</sup> slowly into maize seeds that might have reduced the chances of copper intoxication. It is also possible, by employing similar strategy, to deliver other useful micronutrients in a slow and steady fashion [6].

In continuation, we investigated the efficacy of Cu-chitosan nanoparticles to boost the defense responses against Curvularia leaf spot disease (CLSD) of maize and plant growth promoting activity. Foliarly applied nanoparticles activated the defense response of treated plants as evidenced by higher activities of superoxide dismutase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase. CLSD of maize was substantially checked by Cu-chitosan nanoparticles both in pot and field conditions. Plant height, stem diameter, root length, root number and chlorophyll content also improved by nanoparticles in pot experiments. Encouraging results were subsequently recorded in field experiment. Increased

plant height, ear length, ear weight/plot, grain yield/plot and 100 grain weight were recorded after treatment with nanoparticles. To summarize, these nanoparticles can find definite place in modern agriculture because these are better compatible, have biological control and imitate the natural elicitation of plant defense and antioxidant system. The synthesized Cu-chitosan nanoparticles can be a good choice for disease protection and sustainable growth [7].

Employment of chitosan-based nanoparticles in the agriculture sector is still in a burgeoning phase. Significant potentials have been reported in *in-vitro* and a few *in-vivo* studies in plant growth and protection by chitosan-based nanomaterials. Use of nano-chitosan for agrochemicals (pesticides, micronutrients, fertilizers and plant growth hormones) delivery would be the most promising field in coming times for nanotechnology application in agriculture [8,9]. It can be envisaged that a slow and steady release of agrochemicals (or other encapsulated materials) will defiantly exerts a profound effect in crop plants without increasing the agrochemicals doses. Additionally, nano-encapsulated agrochemicals with the phenomenon of slow release can reduce the environmental hazard. Further, a smart delivery system could be developed by chitosan nanomaterials for crops where agrochemicals encapsulated in nano-chitosan are released through external stimulation. Further research should be focused to generate information of molecular interactions of chitosan in plants to fully exploit this molecule in the field of agriculture.

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