Summary and Conclusion

Shell waste produced by the seafood industry is one of the most important problems contributing significant environmental and health hazards. The most frequent method employed for its disposal is burning which becomes environmentally costly due to the low burning capacity of shells. In such a scenario, conversion of Shrimp shell waste to chitosan a commercially valuable product with a myriad of uses could serve as an effective mode of shell remediation.

Interest in chitosan, a linear polysaccharide obtained by deacetylation of naturally occurring chitin, arose in the 1930s owing to its non-toxicity and versatile applications. The chitosan nanoparticles (ChNP) prepared by ionic gelation of chitosan has greater activity owing to its small size and a large surface to volume ratio. Application of chitosan nanoparticle used to be limited to drug delivery and as an encapsulating compound. But recent years have seen the effective use of chitosan nanoparticles as an antimicrobial agent against both human, and plant pathogens. Though relatively new, the application of chitosan nanoparticles in agriculture, as a plant growth promoting agent and a plant protector is gaining more popularity as an alternative to use of harmful chemicals.

Crude chitin was collected from the shell of *Penaeus monodon* which was then processed to obtain chitosan. The chitosan yield was found to be 46%. Chitosan obtained had 5% moisture content, pH of 8 and 85% degree of deacetylation. Viscosity was 80cps. Residue on ignition was only 2% and was soluble in 1% acetic acid solution. The FT-IR, SEM and XRD data confirms the structure of chitosan. Chitosan forms nanoparticles spontaneously on the addition of polyanion tripolyphosphate which has greater antimicrobial activity
than parent chitosan. The chitosan nanoparticles (ChNP) were prepared by the ionic gelation method. The physiochemical characteristics of nanoparticles were analyzed using XRD, SEM, FTIR. The process was optimised for the concentration of chitosan, reaction time and the rotation speed of magnetic stirrer. 2mg/ml chitosan was found to be the optimum concentration yielding 21nm size nanoparticles. The reaction time of 60min and rotation speed of 700rpm was found to be the optimum conditions.

ChNP was found to have decreased cytotoxicity thus making it an ideal antifungal, antioxidant and coating agent. ChNP was shown to have decreased cytotoxicity with an LD50 value of 64.21mg/ml. The seed toxicity of ChNP was also analyzed to ensure the safety of ChNP application in plants. The toxicity study was done according to EPA guidelines and ChNP was found to be non-toxic. The soil toxicity of ChNP was studied prior to application and was found to be non-toxic. The microbial count of ChNP treated soil was 2.18 log CFU/ml at day 15 compared to 1.96 log CFU/ml on the same day. The soil dehydrogenase activity, ammonia and nitrate levels were also higher for ChNP treatment indicating soil nourishment by the ChNP application.

The presence of primary amine groups in repeating units of chitosan grants it several properties like antimicrobial activity, antioxidant activity and so on. ChNP compounds exhibited superior antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* in comparison with chitosan and chitin. ChNP had good antifungal activity against all *Rhizoctonia solani, Fusarium oxysporum, Collectotrichum acutatum, and Phytophthora infestans* compared to Amphotericin B. Significant antioxidant activity was obtained when compared to gallic acid
The tomato, chilly and brinjal coated with different concentrations of ChNP (1%, 2%, 3%, 4% and 5%) showed decreased weight loss compared to uncoated control. Cut flower varieties of carnation, rose and gladiolus dipped in 4% and 5% ChNP was found to have increased shelf life when compared to control.

Rice seeds were treated with different concentrations of ChNP at the different soaking time and kept for germination under controlled environmental conditions. Upon complete germination, the seedlings were sown in seed trays and growth was evaluated at 21 days after sowing (DAS). The germination percent, growth performance, seed vigour index and relative growth rates were analyzed. All treatments showed better results than the untreated control. The combination treatment of seed, soil and the foliar application was found to be most effective in enhancing the growth and yield of rice. The cellular uptake of ChNP was also studied to deduce the mechanism of action.

The biocontrol ability of chitosan nanoparticles (ChNP) against rice sheath blight pathogen (ShB) Rhizoctonia solani was studied in detail. The results of the study show that ChNP is a cost-effective alternative for the chemical fungicides with potential biocontrol efficacy. ChNP was found to suppress 90% disease in detached leaf assay and 75% under greenhouse conditions. The enzyme specific activity of all defence enzymes was found to be higher than the chemical control. The ChNP stored under room temperature was found to have elicited rice seed germination up to seven months.

**Conclusion**

Availability of bioactive compounds compatible with the environment is one of the main challenges of modern agriculture. ChNP is a promising alternative in this aspect, due to its biological activity and easy-to-obtain methods. The plant growth promoting the ability of
ChNP and also its plant defence induction capacity is a well-established fact. But its application in rice is an underexplored area. This work aimed to make a detailed study on the plant growth promotion ability and defence eliciting capacity of ChNP in rice crop. The toxicity of ChNP was studied in detail prior to plant application and was found to be non-toxic. ChNP treatment was found to be very effective in improving the growth and yield of rice. The application of ChNP had a positive effect in eliciting defence response against ShB thereby preventing crop loss. ChNP application can hence be recommended for rice crop as a cost-effective and safe alternative to the chemicals.

**Future Prospective**

The overuse of chemicals in agriculture sector is causing major environmental problems. Availability of eco-friendly solutions is the need of the hour. Chitosan nanoparticles is a one-word answer to almost all agricultural problems and can be used from seed germination to increase the shelf life of agricultural products.

The use of ChNP in agriculture is still in its initial phase and not many works are available in this respect. To the best of our knowledge, this is the first report on biocontrol ability of ChNP against rice sheath blight pathogen under greenhouse conditions. The result of the present work proves beyond doubt that ChNP is a potential elicitor of plant defence.

The gene expression studies of ChNP treated plants have to be done to confirm the exact mechanism of action. In the present work, plant growth promotion and biocontrol potential of ChNP were evaluated under greenhouse conditions. A further field study has to be done to standardise the formulations for commercial application.
Chitosan nanoparticles have been reported to have herbicide and pesticide properties also. A detailed study on the various properties and mechanism of action of ChNP can cause a revolutionary change in agriculture with ChNP emerging as a broad spectrum bioagent.