



Cu-Chitosan Nanoparticles Stimulated Defence Responses against Bacterial Pustule Disease in Soybean Crop

Swati ^{a,b*} and Arunabh Joshi ^a

^a Maharana Pratap University of Agriculture and Technology, Udaipur-313001, India.
^b Rajasthan Agriculture Research Institute, Durgapura, Jaipur-302018, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Assessment of Cu-chitosan nanoparticles on controlling Bacterial disease by enhancing the activity of different antioxidant and defence enzymes in soybean.

Study Design: Randomized block Method

Place and Duration of Study: Department of Molecular biology and Biotechnology (MBBT), RCA, Maharana Pratap University of Agriculture and Technology, Udaipur Sub humid southern plain region of India between 2017-2019.

Methodology: We synthesized, characterized and tested the efficacy of Copper-chitosan Nanoparticles against Bacterial Pustule disease in Soybean. Activity of defence related enzymes [polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL)], and antioxidant [peroxidase (POD) and superoxide dismutase (SOD)] were estimated after treating the plants with various concentrations of Cu-chitosan nanoparticles.

Results: Three treatments of Cu-chitosan treatments (0.02, 0.06 and 0.10%) were found most efficient in disease control in both laboratory and net house condition) experiments. Cu-chitosan Nanoparticles application to soybean plants significantly enhanced the activity of defence enzymes (PAL and PPO) and antioxidant enzymes (SOD and POD) from 1.1 to 2.29 folds.

Conclusion: Cu-chitosan NPs developed in this study were effective in controlling disease by enhancing the activity of different defence related enzymes [polyphenol oxidase (PPO) and

*Corresponding author: E-mail: swatijhajhria90@gmail.com;

phenylalanine ammonia-lyase (PAL)], and antioxidant [peroxidase (POD) and superoxide dismutase (SOD)]. This could be an important nonmaterial research in the field of agriculture due to better compatibility of biodegradable Cu-chitosan nanoparticles with biological control. In this study, the effect of Cu-chitosan nanoparticles to boost defence responses against *Xanthomonas axonopodis* pv. *Glycine* were evaluated.

Keywords: Chitosan; antioxidants; defence enzymes and copper chitosan nanoparticles.

1. INTRODUCTION

“The continuous use of agrochemicals causes several adverse effects including, deterioration of soil health and increased resistance in plant pathogenic microbes and also affect the non-target organisms” [1] and [2]. “To deal with sustainable production and increasing challenges of food security, significant technological advancements and innovations have been made in these recent years in agriculture sector” [3,4,5]. “To meet the increasing food demand of exploding global population through the uses of natural and synthetic resources such continuous agricultural innovations are crucial. In particular, nanotechnology has potential to provide effective solutions to the multiple agriculture-related problems. To bridge the gap between bulk materials and atomic or molecular structures, nanoparticles provide a great scientific interest. Over the last two decades, a significant amount of research has been carried out on nanotechnology emphasizing its plentiful applications in agriculture sectors” [6,7].

Due to versatile, biodegradable, non-toxic and biocompatible nature chitosan is being exploited in agriculture [8,9]. It is well recognized as an antimicrobial [10,11] immuno modulatory [12,13,14,15] and plant growth promotory agent in concentration of 0.04 to 0.16% [16]. “As compared to bulk chitosan, chitosan based nanoparticles have higher physiological and biochemical responses due to its high volume ratio and surface to surface charge” [17,18,19]. “Hence, chitosan based Nanoparticles have been used for various applications in agriculture” [20,21,22,23]. Recently, chitosan based NPs have been evaluated as potent inducer of defence related enzymes [polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL)], and antioxidant [peroxidase (POD) and superoxide dismutase (SOD)]. Transcript analysis of chitosan NPs treated plants showed that increased level of defence responses was due to high defence related genes expression [24,25,26]. These finding supported the enhanced innate immunity of plants by chitosan

component of Nanoparticles [27,28,29]. In our previous studies, we have reported Cu-chitosan NPs as an effective antibacterial and plant growth promotory agent.

In this study, we reported for the first time the efficacy of Cu-chitosan NPs to induce the Defence Related Enzyme in Soybean under *in vitro* and net house conditions. Our results persuasively established Cu-chitosan Nanoparticles as a persuasive inducer of defence related enzyme for efficient control of Bacterial pustule disease of soybean.

2. METHODS AND MATERIALS

2.1 Preparation and Characterization of Cu-Chitosan Nanoparticles

Ionic gelation method was followed to prepare Cu-chitosan nanoparticles. This method was developed in our laboratory. Characteristics of these synthesized NPs were same as we reported in our earlier publications [30,31,18].

The Soybean cultivars JS-335 which is susceptible towards bacterial pustules disease was being used during the course of research. The Seed lot were selected carefully by visual assessment to ensure the healthy and disease free vigour seeds. Highly infectious bacterial pustule disease causing agent *Xanthomonas axonopodis* pv. *glycines* were procured.

Seeds were treated for 4h in different concentrations of cu-chitosan nanoparticles viz. 0.02, 0.04, 0.06,0.08, 0.10 and 0.12 % w/v along with controls [bulk chitosan (0.01%), CuSo₄ (0.01%) and Untreated] in aqueous suspension. These 4 hr treated seeds were sown:

2.1.1 In plastic pots

In plastic pots filled with commercial coco soil at laboratory conditions under 16/8h light and dark condition. Foliar application of various concentrations of Cu-chitosan NPs as mention above was used after emergence of first trifoliolate

stage. Enzyme assay were conducted in leaves after 12, 48 and 72h of foliar application.

2.1.2 In earthen pots

"In earthen pots filled with standard potting soil obtained from field and kept in net house in natural environment. Foliar spray of Cu-chitosan NPs (until run-off) was applied after emergence of first trifoliate stage. Artificial inoculation of *X. axonopodis* pv. *glycine* was carried out after 35 days of sowing as describe earlier" (Kim *et al.*, 2011). "Second spray of Cu-chitosan NPs (0.02, 0.04, 0.06, 0.08, 0.10 and 0.12% w/v) along with controls [untreated, bulk chitosan (0.01%) and CuSO₄ (0.01%)] in aqueous suspension was applied after disease occurrence. After 48h of second foliar spray various enzymes assay were conducted in inoculated plants as well as in control" [32,33].

2.2 Measurements of Enzyme Activity

"Activity of antioxidant [superoxide dismutase (SOD) and peroxidase (POD)] and defence enzymes [phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO)] were estimated in 3rd leaf after 24 h of foliar spray of various treatments. For enzymes extraction, 0.2 g samples were homogenized in 5 ml of extraction Buffer (phosphate Buffer for SOD and PPO at pH 7.4 and 6.8, respectively; tris-HCl Buffer at pH 7.5 for POD and borate Buffer at pH 8.8 for PAL).

The homogenates were centrifuged at 10,000xg for 20 min at 4 °C and supernatants were taken for enzymes assay. SOD (EC 1.15.1.1) activity was determined at 560nm, as reduction of nitro-blue tetrazolium (NBT) as an indicator of superoxide anion production. POD (EC 1.11.1.7) activity was measured spectrophotometrically as described by Chance and Maehly by oxidation of guaiacol in the presence of hydrogen peroxide. Increase in absorbance at 470nm was recorded due to formation of tetra guaiacol. PPO (EC 1.10.3.1) was assayed according to Taneja and Sachar and activity was expressed as change in absorbance at 490nm. PAL (EC 4.3.1.5) was estimated as described by Moerschbacher where the deamination of L-phenylalanine to trans-cinnamic acid and ammonia was measured at 290nm. Activities of all the enzymes were expressed in µmol/min/g tissue" [34].

2.3 Disease Assessment

Disease severity and disease incidence was assessed in plants by using an earlier described scale by Bull and Koike [35]. By calculating the proportion of diseased plant in each treatment, disease incidence was determined. Disease severity was evaluated by rating the most severely damaged are on the plant on a scale of 0 to 5 (Table 1). Further, the formula given by Chester (1959) and Wheeler (1969) was used to calculate the percentage efficacy of disease control (PEDC) and disease severity (DS).

$$\text{Disease severity} = \frac{\text{Sum of all individual disease rating}}{\text{Total number of leaf assessed} \times \text{Maximum rating}} \times 100$$

$$\text{PEDC} = \frac{\text{Disease severity in control} - \text{Disease severity in treatment}}{\text{Infection index in control}} \times 100$$

Chart 1. Experimental details

Variety	JS-335
Number of treatments	9
T ₁	Untreated control
T ₂	Bulk chitosan
T ₃	CuSO ₄ (0.01%)
Cu-chitosan nanoparticles	
T ₄	Cu-chitosan NPs – 0.02%
T ₅	Cu-chitosan NPs – 0.04%
T ₆	Cu-chitosan NPs – 0.06%
T ₇	Cu-chitosan NPs – 0.08%
T ₈	Cu-chitosan NPs – 0.10%
T ₉	Cu-chitosan NPs – 0.12%
Replications	03
Mode of experiment	<i>In vitro</i> and Net house conditions

Table 1. Rating scale for bacterial disease

Description	Disease rating
Plant with no visible symptoms	0
A few individual lesions	1
Many individual lesions	2
Small patches of coalesced lesions	3
Medium sized patched of coalesced lesions	4
Large sized patched of coalesced lesions	5

2.4 Statistical Analysis

Statistical analysis of the data was performed with JMP software version 12. The significant difference among treatment groups were determined using the Turkey Kramer HSD at $p=0.05$. All experiments were performed in three replications (triplicates) and each replication consisted of minimum three (for pot experiments) and ten samples (for field experiments) from randomly selected plants.

3. RESULTS AND DISCUSSION

“Previously, in maize, finger millet and tea plants, chitosan nanoparticles have been reported as immune modulator through induction of defence/antioxidant enzyme activity” [15,20]. *In vitro* antibacterial test was conducted to illustrate the degree of growth inhibition of Cu-chitosan NPs against *Xanthomonas axonopodis*, before conducting the pot and Net house experiments for disease responses. A considerable % control was noticed in the experiment. Same batch of Cu-chitosan NPs were found effective in our previous studies in inhibiting *Pseudomonas syringae* [20] and mycelia growth of *Alternaria solani* and *Fusarium oxysporum* in *in vitro* experiments [18].

3.1 Defence Related Enzyme Activity

SOD activity was found higher in Cu-chitosan NPs treatments at 12 hr. In addition, maximum activity was recorded in 0.06% of Cu-chitosan NPs which was 1.40 fold higher than control. At 48 hr Cu-chitosan Nanoparticles treatments showed higher activity compared to control. Similarly, at 72 hr 0.02 to 0.06 and 0.10% of Cu-chitosan nanoparticles enhanced activity was recorded as compared to bulk chitosan treated and control plants. Maximum activity was recorded in 0.04% of Cu-chitosan NPs which was 1.32 folds higher than control (Fig. 1A)

At 12 hr and 48 hr POD activity was recorded higher in control but at 72 hr 0.02 and 0.06%

concentration of Cuchitosan NPs showed higher activity compared to control, bulk chitosan and CuSO_4 treated plants. In addition, maximum activity was recorded in 0.02% of Cu-chitosan NPs which was 1.38 fold higher than control (Fig. 1B).

PAL activity was significantly higher in 0.04% to 0.10% of Cu-chitosan NPs at 12 hr. At 48 hr 0.06% of Cu-chitosan NPs recorded enhanced activity compared to Control, Bulk chitosan and CuSO_4 treated plants which was 2.29 fold higher than control. Similarly, at 72 hr 1.1-2 folds higher activity was found in 0.02 to 0.10% NPs treated plant leaves as compared to control and bulk chitosan treated plants (Fig. 1C).

PPO activity was found higher in 0.04 and 0.06% of Cu-chitosan NPs treatments at 12 hr. In addition, maximum activity was recorded in 0.06% of Cu-chitosan NPs which was 1.6 folds higher than control. At 48 hr 0.04 to 0.08% of Cu-chitosan NPs enhanced activity was recorded as compared to control, CuSO_4 and bulk chitosan treated plants. In addition, Maximum activity was found in 0.04 and 0.06% of Cu-chitosan NPs which was 1.30 fold higher than control. At 72 hr 0.02 to 0.08% of Cu-chitosan NPs treatments showed higher activity compared to control, CuSO_4 and bulk chitosan treated plants (Fig. 1D).

3.2 Defence Related Enzyme Activity under Net House Condition

SOD activity was significantly higher in 0.02, 0.06 and 0.10% of NPs (Fig. 2A). Similarly, 1.5-2 folds higher POD activity was recorded in 0.02 to 0.06% NPs treated plant leaves as compared to control and bulk chitosan treated plants (Fig. 2B). The activity of PAL was also enhanced by NPs treatments (0.06-0.10%) as compared to control, bulk chitosan and CuSO_4 treatments (Fig. 2C). Likewise, Cu-chitosan NPs treated plants leaves showed 1-2 folds increased PPO activity as compared to control and bulk chitosan treatment (Fig. 2D).

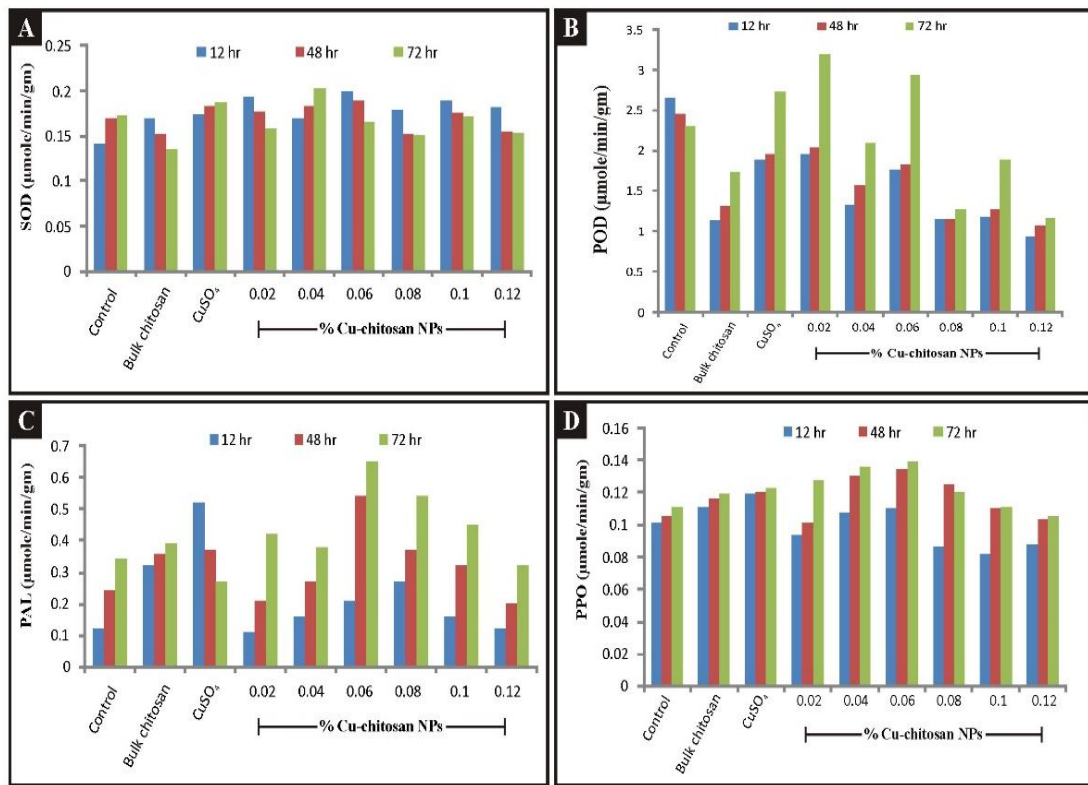


Fig. 1. Effect of Cu-chitosan NPs on (A)SOD (B) POD (C)PAL and (D) PPO enzymes activity in soybean plant leaves after 12, 48 and 72 hr of foliar spray in pots (under laboratory condition). Control with water. Bulk chitosan, (0.01%) dissolved in 0.1% acetic acid. CuSO₄ (0.01%)

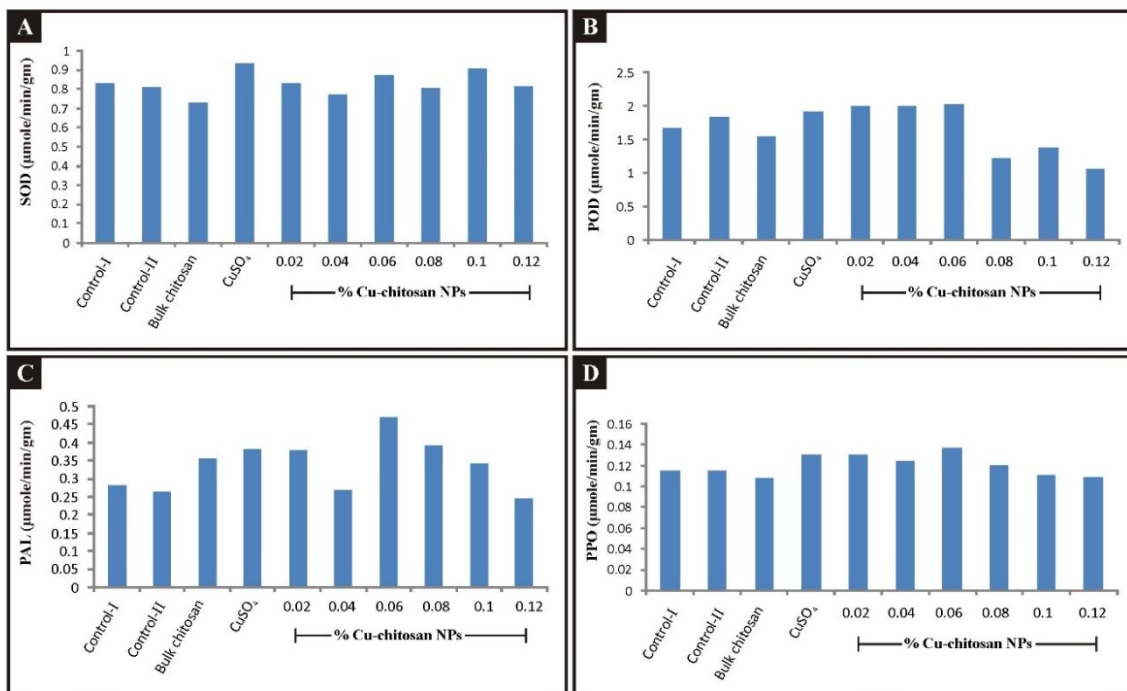


Fig. 2. Effect of Cu-chitosan NPs on (A)SOD (B) POD (C)PAL and (D) PPO enzymes activity in soybean Plant leaves after 48 hr of foliar spray in pots (under laboratory condition). Control with water. Bulk chitosan, (0.01%) dissolved in 0.1% acetic acid. CuSO₄ (0.01%)

Table 2. Effect of Cu-chitosan NPs on bacterial pustule disease in pots (under net house condition)

Treatment (%)	Disease Severity (%) ^A	PEDC (%) ^A
Control 1	66.3 ± 0.88 ^a	0.00 ± 0.00 ^f
Control II	64.3 ± 1.33 ^a	3.67 ± 1.33 ^f
Bulk chitosan (0.01)	40.0 ± 1.15 ^c	39.2 ± 1.94 ^d
CuSO ₄ (0.01)	26.3 ± 0.88 ^e	60.1 ± 1.25 ^a
Cu-chitosan NPs		
0.02	49.0 ± 1.15 ^b	26.1 ± 1.74 ^e
0.04	50.0 ± 0.00 ^b	24.6 ± 0.00 ^e
0.06	33.3 ± 1.20 ^d	49.7 ± 1.81 ^b
0.08	34.3 ± 0.66 ^d	48.2 ± 1.00 ^{bc}
0.10	37.6 ± 0.88 ^{cd}	43.2 ± 1.32 ^{cd}
0.12	39.3 ± 0.33 ^c	40.6 ± 0.50 ^d

3.3 Disease Occurrence

After 4 hr of seed treatment followed by two foliar sprays, control plants showed average disease severity 64.30%. All plants treated with Cu-chitosan NPs (0.02 to 0.12%) showed significant antibacterial activity and express lower disease severity 50.0% to 33.3%. CuSO₄ showed 26.3% and 40.0% disease severity was found in bulk chitosan. PEDC was found maximum (49.7%) at 0.06% of Cu-chitosan NPs. Significantly higher PEDC value was recorded in 0.06% of Cu-chitosan NPs from all other treatments at statistical level (Table 2).

Three treatments of Cu-chitosan treatments (0.02, 0.06 and 0.10%) were found most effective in disease control in pot (both laboratory and net house condition) experiments. Cu-chitosan NPs application to soybean plants significantly enhanced the activity of antioxidant enzymes and defence enzymes from 1.1 to 2.29 folds.

4. CONCLUSION

Cu-chitosan NPs developed in present investigation were found effective in controlling the disease by enhancing the activity of the antioxidant (superoxide dismutase and peroxidase) and defence enzyme (phenylalanine ammonia-lyase and polyphenol oxidase). In this study, the potential of Cu-chitosan NPs anticipated that these nanoparticles could be further exploited in large scale experiments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kashyap PL, Xiang X, Heiden P. Chitosan nanoparticle-based delivery systems for sustainable agriculture. *Int. J. Biol. Macromol.* 2015;77:36–51.
2. Zhan J, Thrall PH, Papaix J, Xie L, Burdon JJ. Playing on a pathogen's weakness: using evolution to guide sustainable plant disease control strategies. *Ann. Rev. Phytopathol.* 2015;53:19–43
3. Dwivedi S, Saquib Q, Al-Khedhairi AA, Musarrat J. Understanding the role of nanomaterials in agriculture. In microbial inoculants in sustainable agricultural productivity. Singh DP, Singh HB, Prabha R, Eds. Springer: New Delhi, India. 2016;271–288.
4. Kou TJ, Yu WW, Lam SK, Chen DL, Hou YP, Li ZY. Differential root responses in two cultivars of winter wheat (*Triticum aestivum* L.) to elevated ozone concentration under fully open-air field conditions. *J. Agron. Crop Sci.* 2018;204:325–332.
5. Xiao M, Song F, Jiao J, Wang X, Xu H, Li H. Identification of the gene Pm47 on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi. *Theor. Appl. Genet.* 2013;126:1397–1403.

6. Lv M, Liu Y, Geng JH, Kou XH, Xin ZH, Yang DY. Engineering nanomaterials-based biosensors for food safety detection. *Biosens Bioelectron.* 2018;106:122–128.
7. Prasad R, Bhattacharyya A, Nguyen QD. Nanotechnology in sustainable agriculture: Recent developments, challenges, and perspectives. *Front. Microbiol.* 2017;8:1014.
8. Katiyar D, Hemantaranjan A, Singh B. Chitosan as a promising natural compound to enhance potential physiological responses in plant: A review. *Indian J. Plant Physiol.* 2015;20:1–9.
9. Xing K, Zhu X, Peng X, Qin S. Chitosan antimicrobial and eliciting properties for pest control in agriculture: A review. *Agronomy Sustain. Develop.* 2015;35:569–588.
10. Goy RC, Morais ST, Assis OB. Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth. *Revista Brasileira de Farmacognosia.* 2016;26:122–127.
11. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. *Int. J. Food Microbiol.* 2010;144:51–63.
12. Amborabé BE, Bonmort J, Fleurat-Lessard P, Roblin G. Early events induced by chitosan on plant cells. *J. Exper. Bot.* 2008;59:2317–2324.
13. Popova E, Domnina N, Kovalenko N, Sokornova S, Tyuterev S. Effect of chitosan and vanillin-modified chitosan on wheat resistance to spot blotch. *Appl. Biochem. Microbiol.* 2016;52:537–540
14. Sathiyabama M, Manikandan A. Chitosan nanoparticle induced defence responses in finger millet plants against blast disease caused by *Pyricularia grisea* (Cke.) Sacc. *Carbo. Poly.* 2016;154:241–246.
15. Sathiyabama M, Parthasarathy R. Biological preparation of chitosan nanoparticles and its *in vitro* antifungal efficacy against some phytopathogenic fungi. *Carbo. Polymers.* 2016;151:321–325.
16. Kananont N, Pichyangkura R, Chanprame S, Chadchawan S, Limpanavech P. Chitosan specificity for the *in vitro* seed germination of two *Dendrobium* orchids (Asparagales: Orchidaceae). *Scientia Horticul.* 2010;124:239–247.
17. Saharan V, Pal A. Chitosan Based Nanomaterials in Plant Growth and Protection. *Springer Briefs in Plant Sci.*; 2016.
18. Saharan V. Synthesis and *in vitro* antifungal efficacy of Cu–chitosan nanoparticles against pathogenic fungi of tomato. *Int. J. Biol. Macromol.* 2015; 75:346–353.
19. Van SN, Minh HD, Anh DN. Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. *Biocat. Agric. Biotechnol.* 2013;2:289–294.
20. Chandra S, Chakarborty N, Dasgupt A, Sarkar J, Panda K, Acharya. Chitosan nanoparticle: A positive modulator of innate immune responses in plants. *Sci. Rep.* 2015;5:1-13.
21. Saharan V. In International Conference on Advances in Biotechnology (BioTech). *Proceedings.* 2014;23.
22. Swati, Joshi A, Saharan V. Cu- chitosan nanoparticles: As an antibacterial agent against bacterial pustule disease of Soybean [*Glycine max* (L.)]. *International Journal of Chemical Studies.* 2018;6:994-999.
23. Swati, Joshi K, Joshi A. Cu-chitosan nanoparticle induced plant growth and antibacterial activity against bacterial pustule disease in soybean [*Glycine max* (L.)]. *Journal of Pharmacognosy and Phytochemistry.* 2020;9:450-455.
24. Chester KS. How sick is the plant? In: *Plant pathology an advances traits* (Eds. Horsfall JG, Diamond AE.). Academic Press, New York. 1959;1:199-242.
25. Kim KH, Park JH, Kim MY, Heu S, Lee SH. Genetic mapping of novel symptom in response to soybean bacterial leaf pustule in PI 96188. *Journal of Crop Science and Biotechnology.* 2011;14(2):119-123.
26. Moerschbacher BM, Noll UM, Flott BE, Reisener HJ. Lignin biosynthetic enzymes in stem rust infected, resistant and susceptible near-isogenic wheat lines. *Physiological and Molecular Plant Pathology.* 1988;33:33-46.
27. Taneja SR, Sachar RC. Induction of polyphenol oxidase in germinating wheat seeds. *Phytochemistry.* 1974;13:2695-2702.
28. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural Sustainability and Intensive Production Practices. *Nature.* 2002;418:671–677.

29. Wheeler BEJ. An Introduction to plant disease. John Wiley and Sons Limited, London. 1969;301.
30. Sathiyabama M, Bernstein N, Anusuya S. Chitosan elicitation for increased curcumin production and stimulation of defence response in turmeric (*Curcuma longa* L.). *Ind. Crops Prod.* 2016;89:87–94.
31. Swati, Choudhary MK, Joshi A, Saharan V. Assessment of Cu- chitosan nanoparticles for its antibacterial activity against *Pseudomonas syringaepv. glycinea*. *International Journal of Current Microbiology and Applied Sciences.* 2017;6:1335-1350.
32. Jung WJ, Mabood F, Souleimanov A, Smith DL. Induction of defence-related enzymes in soybean leaves by class IId bacteriocins (thuricin 17 and bacthuricin F4) purified from *Bacillus* strains. *Microbiological Research.* 2011;167:14-19.
33. Khan W, Prithviraj B, Smith DL. Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves. *Journal of Plant Physiology.* 2003;160:859-863.
34. Choudhary RC, Kumaraswami RV, Kumari S, Sharma SS, Pal A, Ralia R, Biswas P, Saharan V. Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). *Scientific Reports.* 2017;7. Article number: 9754.
35. Odubanwo BO, Popoola AR, Ojo DK, Ganiyu SA. Yield performance of soybean accessions on field naturally infected with bacterial pustules (*Xanthomonas axonopodis* pv. *glycines*). *African Crop Science Society.* 2013;11:309-315.

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