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Efficacy of Chitosan Nanoparticles Against Bacterial Leaf Blight of Rice Caused by *Xanthomonas oryzae* Pv. *oryzae*

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Abstract

In this present study, we conducted a systematic examination of the influence of different concentrations (5–25mg/100ml) of chitosan nanoparticles (CNPs) on the germination and seedling growth of rice (RNR15048, IR64, MRM16 (Red rice) varieties. The antibacterial activity of CNPs at different concentrations (2-6mg/100ml) was tested against both human and plant bacterial pathogens. The CNPs showed significant antibacterial activity on *Bacillus subtilis, Clostridium sporogenes, Staphylococcus aureus, Salmonella abony, Pseudomonas aeruginosa, Escherichia coli* and *Xanthomonas oryzae*. A maximum zone of inhibition was observed against *X. oryzae* as compared to other pathogens, measuring 22.9±0.57 mm at 6 mg/100ml concentration. The synthesized CNPs were characterized by UV-Vis spectrophotometry, SEM (Scanning electron microscopy), FTIR (Fourier transform infrared spectroscopy) and XRD (X-ray diffraction) concerning the ideal CNPs absorption band, presence of nanoparticle (NP) bond, smooth surface of nanoparticle and diffraction peaks of NPs. The results indicate that CNPs have a maximum beneficial impact on seed germination and plant growth due to the effect of CNPs on seed coat at the concentration (25mg/100ml) under greenhouse conditions due to the antibacterial activity of CNPs against *Xanthomonas oryzae*.

Keywords : Chitosan nanoparticles, Rice, Characterization, Bacterial leaf blight, *Xanthomonas oryzae*

Agriculture is the primary source of income for almost 58 per cent of India's population (Manida and Nedumaran 2020). Rice (*Oryza sativa* L) is the most important source of income among several crops (Divya *et al.,* 2022). Rice is one of the world's most essential staple foods, feeding more than 3.5 billion people (Gadal *et al.,* 2019) and it is an excellent food source low in fat and high in starchy carbohydrates (Qudsia *et al.,* 2017). It is cultivated and consumed by 92 per cent of the world's population in Asia, which accounts for 55 per cent of the world's population (Yugander *et al.,* 2018). For millions of people around the world, rice contributes up to 20 per cent of their daily calorie intake. Rice output must be expanded to 852 million tonnes by 2035 in order to assure nutritional food security (Bazrkar-Khatibani *et al.,* 2019). It contains 80 per cent carbohydrates, 7-8 per cent protein, 3 per cent fat, and 3 per cent fiber which meets the energy needs of about 2 billion people in Asia. (Chaudhari *et al.,* 2018). Pests and diseases on rice plants cause farmers to lose output each year, as well as financial losses.

The main causes of rice diseases include bacteria, fungus, and viruses (Joshi *et al.,* 2016). There are numerous rice diseases, but the Bacterial leaf blight of rice is the one that has been examined in this paper. In both irrigated and rain-fed lowland habitats, bacterial leaf blight (BLB) is responsible for significant rice crop losses (Sana *et al.,* 2010). Diseases continue to be a major cause of output loss and poorer income on rice fields, despite advances in rice production technology and diseases lower output and quality while also raising production expenses (Wamishe *et al.,* 2013). The

gram-negative bacteria *X. oryzae* cause rice bacterial leaf blight (BLB) (Jiang *et al.,* 2020). The first case of bacterial blight disease (BLB) was discovered in India in 1951 (Jonit *et al.,* 2016). It grows best at a temperature of 25-30^{0}C and a pH of 6.5-7.5. (Saha *et al.,* 2015). Due to the formation of the pigment xanthomonadin which is unique to the genus. The colonies on solid glucose media are spherical, mucoid, convex, and yellow (Nino Liu *et al.,* 2006). This plant pathogen induces vascular disease by accumulating yellow-green patches on the upper leaves and edges which lead to grey to white lesions along the leaf veins, lowering rice quality considerably. (Shi *et al.,* 2016), The bacterium can be transmitted *via* irrigation, splashing, wind erosion, plant-to-plant contacts, cutting equipment used in transplantation and handling during transplanting (Saha *et al.,* 2015). Bacterial leaf blight disease can be controlled biologically, chemically or by providing genetic resistance to the disease through plant breeding, host resistance or changes in cultural practices (Mueen *et al.,* 2014). However, the chemicals are costly; their handling is hazardous; and they have long-term impacts that are harmful to humans and the environment (Islam *et al.,* 2016). Biological control has the ability to control the infection, however, the effectiveness of biological control agents (BCAs) must be improved (Kim *et al.,* 2007). Biodegradable and antibacterial composite reduce pollution and also improve plant defense and which can reduce bacterial blight by improving disease resistance in host plants. (Hassan and Abo-Elyousr, 2013). Chitosan is one of these novel particles. Chitosan has emerged as one of the most promising biopolymers for delivering agrochemicals and micro-nutrients in a cost-effective manner (Choudhary *et al.,* 2017).

Chitosan is a common natural polymer in the environment, and it is extracted from the shells of shrimp and other crustaceans (Negm *et al.,* 2020). Deacetylation of chitin yields chitosan, a deacetylated derivative of chitin (Priyadarshi *et al.,* 2020). Because of their biocompatibility, biodegradability and low toxicity, nanomaterials based on chitosan have emerged as attractive carriers of therapeutic agents for drug administration (Yang *et al.,*2014). Chitosan has a wide range of potential applications due to its chemical functional groups that can be changed to achieve specific purposes (Mohammed *et al.,* 2017).

Food, pharmaceutical, biotechnology, medicinal, textile, paper, agriculture and environmental uses have all been characterized using chitosan and derivatives (Bakshi *et al.,* 2020). Chitosan physical and chemical properties are influenced by its molecular weight and degree of deacetylation (Wang *et al.,* 2011). Generally, chitosan with lower molecular weights and lower degrees of deacetylation is more soluble and degrade faster than those with higher molecular weights (Bowman and Leong *et al.,* 2006).

The presence of chitosan in plants triggers a variety of defense mechanisms as is it usually used as a strong elicitor in plant disease control (Morin-crini *et al.,* 2019). Chitosan has antimicrobial properties that include bacteria, filamentous fungus, yeast and even virus (Divya *et al.,* 2017). Chitosan has grown in importance as a carrier-forming material because it may be used in a number of methods to make nanoparticles (Grenha *et al.,* 2012). Nanotechnology is new research that works with the nanoscale and nanoparticles are one of its building blocks (Agarwal *et al.,* 2018).

Solid colloidal particles having dimensions ranging from 1 to 1000 nm are known as nanoparticles (Peniche and Peniche 2011). Chitosan nanoparticles are smaller than chitosan, which could make them unique (Aliasghari *et al.,* 2016). The novel chitosan nanoparticles, which are made up of clusters of nanoparticles sizes vary from 10 - 80 nm which has shown promise in the field of nanomedicine, biomedical engineering, industrial applications and pharmaceuticals (Sharifi - Rad *et al.,* 2021). CNPs have been synthesized using a variety of techniques, there are now five techniques available. The reverse micellar technique, ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex (Divya *et al.,* 2017). Chitosan nanoparticles develop spontaneously when a polyanion such as a tripolyphosphate (TPP) is added to a chitosan solution and stirred continuously (Yien *et al.,* 2012). CNPs

are natural substances with outstanding physicochemical, antimicrobial and biological properties which make them environmentally friendly substances with non-harmful bioactivity. (Malmiri *et al.,* 2012). Although CNPs have a broad antibacterial range, their inhibitory efficacy against gram-negative (G) and gram-positive (G+) bacterial species differ (Chandrasekaran *et al.,* 2020). Because of its low solubility above pH 6.5, chitosan only has antibacterial activity in an acidic environment (Fei Liu *et al.,* 2001).

The current study focused on (i) Synthesis and characterization of chitosan nanoparticles, (ii) synthesized CNP screened against human and plant pathogens, (iii) CNPs effect on germination of seeds and seedling vigour of rice in a laboratory condition and (iv) effect of seed treatment with CNP against bacterial leaf blight with plant growth promotion in greenhouse conditions.

Material and Methods

Synthesis of Chitosan Nanoparticles

The Chitosan powder (50g) was purchased from Everest Biotech research institute in Basavanagudi Bangalore, Chitosan (5 mg) was dissolved in 50 ml of acetic acid (1%) prepared with sodium hydroxide solution while stirring at 120 rpm at RT for 30 min at pH 5.0. The CNPs were formed instantly upon adding 30 ml of tri-poly phosphate solution (TPP) (30 mg/ml) dropwise to 50 ml solution of chitosan under continuous magnetic stirring.

A milky-colored emulsion like the presence of CNPs was formed upon the ionic Interlink between the chitosan solution and TPP. The nanoparticles formed were centrifuged at 15,000 rpm at 4°C for 30 min. The pellet was suspended in water to remove residues of sodium hydroxide. The nanoparticles were formed from a reaction between TPP (negative charge) and positively charged amino groups of chitosan (Alireza Alishahi, 2014).

Characterization of Synthesized Chitosan Nanoparticles

The following techniques were used to characterize chitosan nanoparticles

Analysis by UV–Visible Spectroscopy

The sample is quantified for absorption spectra of the organic molecule (CNPs) by using ultraviolet and visible absorption spectroscopy (Double beam spectrometer 2203) at the wavelength range between 280-800 nm with 1 nm resolution (Uppal *et al.,* 2018).

Fourier Transform Infrared Spectroscopy

FTIR analysis was used to explore the functional groups linked to the surface of nanoparticles as well as the other surface chemical residue binding properties of chitosan nanoparticles. The liquid solution of CNPs was dried and the dried powder of CNPs was characterized using FTIR (IR Affinity-Shimadzu machine) analysis in the spectrum region of 4000-400 cm^{-1} with 4 cm^{-1} resolution.

Scanning Electron Microscopy (SEM)

The SEM (EVO LS 15) machine was used to examine the nanoparticle pictorial representation. The thin film of CNPs on the SEM grid was dried by placing the grids under a mercury lamp, after 5 min CNPs film exposed to the accelerated electrons which carry significant amount of kinetic energy to produce secondary electron which further produce SEM images

X-Ray Diffraction (XRD)

XRD machine (Rigaku Desktop Miniflex II) is utilized to identify and characterize the crystal structure of nanoparticles by irradiating the sample with an incident X-ray.To determine the crystalline phase, the maximum peak positions were compared to the standard files 2θ peak value (38.04, 44.3) (Bykkam *et al*., 2015)

Rice Varieties and Pathogens

Collection of Seeds of rice varieties such as IR64, RNR15048 and MRM16 (Red rice) that are highly susceptible to *Xanthomonas oryzae* pv. *oryzae* were purchased from Karnataka seed and agro suppliers in Hebbal Bangalore, Karnataka, India. The seeds were maintained at 4°C for future use. The human and plant pathogenic bacteria were collected from the Dept. of Microbiology and Biotechnology, Bangalore University, Karnataka, India, such as *E. coli, C. sporogenes,*

The Mysore Journal of Agricultural Sciences

S. aureus, B. subtilis, S. abony, P. aurginosa and plant pathogen *Xanthomonas oryzae*. The pathogens were maintained on nutrient broth and were kept at 37°C for future use.

Antibacterial Activity of Chitosan Nanoparticles

The disc diffusion method was used to determine the antibacterial activity of CNPs. The selected bacterial isolates were cultured for 24 h at 37°C in nutrient broth, then diluted to concentration of CFU (colony forming unit) 1. The inoculum was spread evenly on Muller Hinton agar (MHA) plates surface by using a sterile cotton swab. The CNPs were dissolved in sterile distilled water at various concentrations ranging from 2 to 6mg/1ml, uniform 5 mm-dia discs were soaked into 20ìl of varying concentrations CNPs and placed in each plate, and the plates were allowed to diffuse at room temperature for two to three h. The positive control was tetracycline (30g/ml) with distilled water and the negative control was distilled water. The plates were incubated for 24-28 h at 37°C. After incubation, the zone of inhibition around the discs was measured in mm. The experiment was carried out three times.

Seed Treatment With Chitosan Nanoparticles

Seeds of rice were surface-sterilized by using 4 per cent sodium hypochlorite for 3 minutes and then properly washed 5 times with sterile distilled water before being air-dried. To ensurehomogeneous coating, hundreds of surface-sterilized seeds were coated with 1 per cent carboxymethyl cellulose (CMC) as an adhesive and submerged in chitosan nanoparticle concentrations of 5, 10, 15, 20, 25mg/100ml (Fig. 1) and then kept in a rotary shaker for 6 h at 150rpm. The CNPs primed seeds were dried and preserved for further studies.

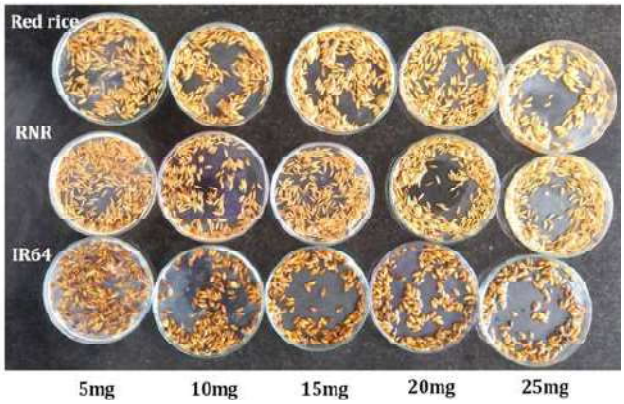

Fig. 1 : Different varieties of rice seeds soaked with different concentrations of CNPs

CNPs Influence on Germination of Seeds and Vigour under Laboratory Conditions

The CNPs treated IR64, RNR15048, and MRM16 (Red rice) Seeds were evenly distributed on a wet germination papers. The germination papers were then rolled and incubated for 14 days at 24±1°C. After incubation, the germination sheets were opened and root and shoot lengths were measured and the vigor index was calculated. The following formula was used to calculate the vigor index: Vigour Index (VI) = (Mean shoot length + Mean root length) x germination per cent. The experiment was conducted three times with four replicates of 100 seeds each.

Preparation of Bacterial Inoculum

Xanthomonas oryzae pv. *Oryzae (Xoo)* was cultured for 24 h in nutrient broth, after incubation, the bacterial cells were centrifuged and the inoculum was adjusted to 1×10^8 colony forming units (CFU). *Xoo* was grown by incubating it for 48 h at 28°C on a nutrient agar medium.

Effect of CNPs on Bacterial Leaf Blight and Growth Promotion in Greenhouse Conditions

Control seeds and CNPs treated seeds were allowed to germinate in the growth chamber separately (in photoperiod of light and shade for 24h at 25°C). After 25 days of treatment, the seedlings of rice were transplanted to clay pots (25 seedlings per pot, 20 cm diameter) filled with- autoclaved soil, farmyard manure (2:1:1) and sand then regularly watered and fertilized with NPK (0.2%).

The 100 seedlings were challenge inoculated by clip inoculation using sterile scissors dipped in 1x 10^8 CFU/ml of *Xoo* inoculum and grown under greenhouse conditions (Kauffman *et al.,* 1973). As a control, the seeds were treated with distilled water. The following treatments were utilized in a greenhouse setting: (1)

control (2) seeds treatment with CNPs alone at 25 mg/100 ml; (3) Treated with CNPs challenged with *Xoo* and (4) *Xoo* treated alone. After 14 days of pathogen inoculation, blight incidence, plant growth, and development indices. The percent of protection by CNPs against blight is calculated by using the formula: (Control-Treated)/Control ×100.

Results and Discussion

Synthesis and Characterization of Chitosan Nanoparticles

CNPs were produced at room temperature (25-28°C) by using an ionic gelation interaction between positively charged chitosan and negatively charged tripolyphosphate (TPP). The CNPs that were synthesized have a CNPs solution and these are insoluble in dilute acid, water and alkali solutions. For further confirmation, the produced CNPs were subjected to analytical studies such as UV-Vis spectroscopy, FTIR, XRD and SEM are the most common tests performed.

UV-Visible Spectroscopic Analysis of Chitosan Nanoparticles

The UV-Vis absorption spectrum is a potential procedure for the structural analysis of CNPs. The development of CNPs is suggested by the presence of an absorption band at about 221 nm which is a typical Plasmon band. The chitosan molecular weight, which acts as a controller of nucleation as well as stability had a small effect on the absorption bands (Fig. 2).

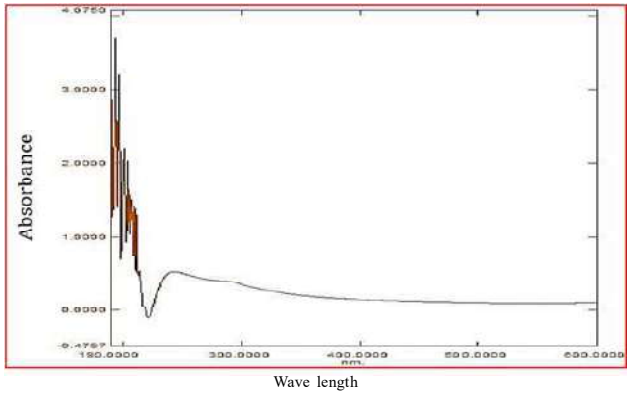

Fig. 2 UV - Visible spectra of synthesized CNPs

Scanning Electron Microscopic Analysis of CNPs

When TPP was added to chitosan solution, shapes of NPs with small spherical surfaces were generated according to SEM examination. Electrostatic interactions between polyanions (TPP) acting as crosslinkers and positively charged chitosan chains could shape the particles (Fig. 3).

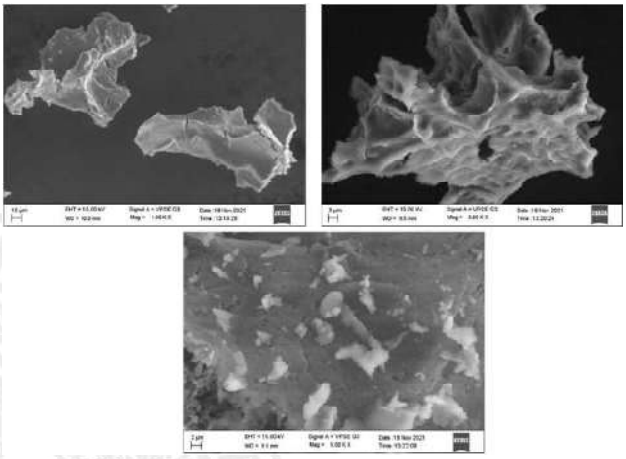

Fig. 3 : Scanning electron micro scopic analysis of CNPs

X-ray Diffraction Analysis of CNPs

The CNPs have diffraction peaks according to the XRD analysis, the XRD patterns of CNPs revealed large diffraction peaks at 2θ value, 20^0 indicating a high degree of semi-crystalline chitosan fingerprints. CNPs' XRD pattern revealed two unique diffraction peaks at 20.150 and 31.91^0 which are typical fingerprints of CNPs (Fig. 4). As a result, CNPs have an XRD pattern that is typical of an amorphous polymer.

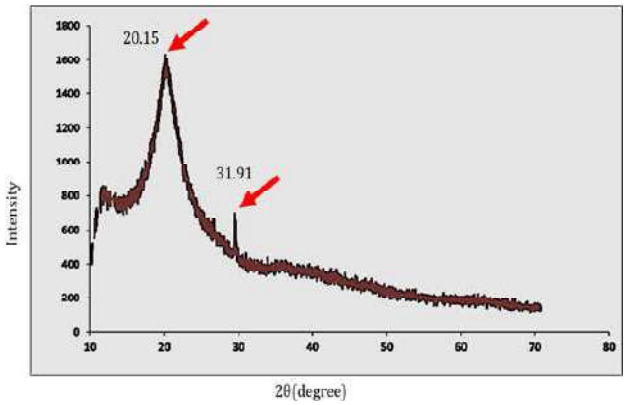

Fig. 4 : X-ray diffraction analysis of CNPs

Fourier Transform Infrared Spectroscopic Analysis of CNPs

FTIR spectra detects the functional groups of CNPs with peaks at 1250 cm^{-1} (alcohols), 3419.07 cm^{-1} (amines), 1450 cm^{-1}(aromatics), and 475 cm^{-1} (alkyl halides) (Fig. 5). It demonstrates that in the sodium polyphosphoric groups, polyphosphate interacts with chitosan's ammonium groups which enhances both intermolecular and intramolecular interactions in chitosan nanoparticles.

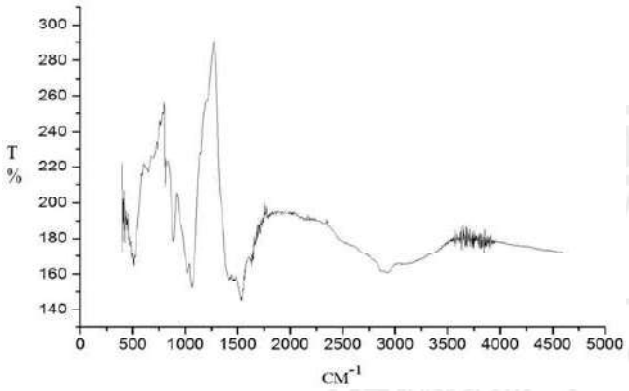

Fig. 5 : Fourier transform infrared spectrum of CNPs

Antibacterial Activity

The antibacterial activity of CNPs at different concentrations (2-6mg/100ml) was tested against both human and plant bacterial pathogens. The CNPs showed significant antibacterial activity on, *B. subtilis, C. sporogenes, S. aureus, S. abony, P. aeruginosa, E. coli,* and *X. oryzae*. Maximum zone of inhibition was observed against *X. oryzae* as compared to other pathogens, measuring 22.9±0.57 mm at 6 mg/100 ml concentration (Fig. 6 & Table 1). The results were compared with the standard (tetracycline).

Fig. 6 : Anti bacterial activity of synthesized CNPs against phytopathogenic strain *Xanthomonas oryzae* duplicates of different concentration after 24 h of incubation. 1-2mg/ml, 2-4mg/ml,3- 6mg/ml,4-8mg/ml and 5-positive control (PC) (Tetracycline), different concentration of CNPs used to examine the most effective CNPs concentration for antimicrobial activity

Effect of CNPs on Germination of Seeds and Seedling Vigour of Rice in a Laboratory Condition

The CNPs have significantly increased the mean root length, mean shoot length and vigour index of the germinated seedling as compared with untreated control (Table 2). The highest germination percentage

TABLE 1
Antibacterial activity of synthesized CNP's against different bacterial pathogens

Organisms	Concentration of CNP smg/mL)	Zone of inhibition (inmm)
Xanthomonas oryzae	PC	28.7 ± 0.33
	2	18.6 ± 0.54
	4	21.47 ± 0.66
	6	22.9 ± 0.57
	NC	0.0 ± 0.0
Escherichia coli	PC	32.2 ± 0.6
	2	13.33 ± 0.57
	4	17.78 ± 0.66
	6	18.65 ± 0.8
	NC	0.0 ± 0.0
Bacillus subtilis	PC	28.2 ± 0.6
	2	15.4 ± 0.57
	4	18.8 ± 0.57
	6	20.2 ± 0.66
	NC	0.0 ± 0.0
Clostridium sporogenes	PC	30.4 ± 0.33
	2	10.8 ± 0.6
	4	13.6 ± 0.57
	6	14.8 ± 0.33
	NC	0.0 ± 0.0
Staphylococcus aureus	PC	28.1 ± 0.36
	2	13.6 ± 0.57
	4	15.7 ± 0.66
	6	18.2 ± 0.89
	NC	0.0 ± 0.0
Salmonella abony	PC	29.1 ± 0.36
	2	0.0 ± 0.0
	4	0.0 ± 0.0
	6	0.0 ± 0.0
	NC	0.0 ± 0.0
Pseudomonas aurginosa	PC	28.1 ± 0.36
	2	14.6 ± 0.44
	4	16.4 ± 0.57
	6	19.7 ± 0.89
	NC	0.0 ± 0.0

Note : Mean values of three replicates ± standard errors.
NC - Negative control (Distilled water) ; PC - Positive control (Tetra cycline).

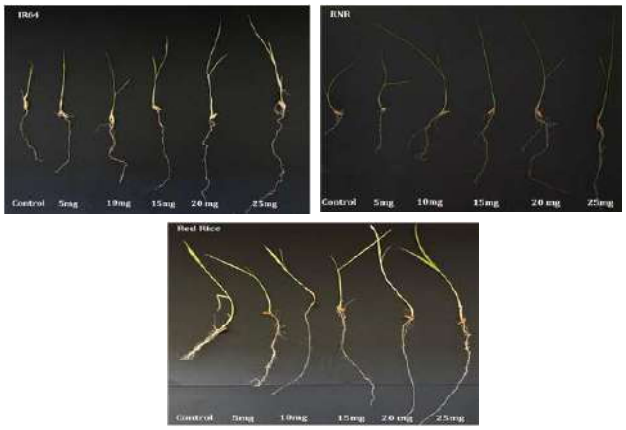

Fig. 7. Effect of CNP sonrice IR64, RNR, redrice variety seed germination at different concentrations (5mg-25mg/100ml), Untreated seedlings (control).

was recorded in IR64 variety at 25mg/100 ml with a germination percentage of 98.11%, followed by 20mg/100ml, 15mg/100, 10mg/100ml and 5mg/100ml, with 96.12, 95.0, 94.34 and 92.3 per cent, respectively as compared to control (88.56%) (Fig. 9). The seedling vigour of rice seeds was significantly increased by the CNPs different concentrations 5mg/100ml, 10mg/100ml, 15mg/100ml, 20mg/100ml, and 25mg/100ml by 1646.8, 2017.24, 2299, 2489 and 2741.06, respectively (Fig. 9).

The second highest germination percentage was recorded in RNR15048 variety (Fig. 9). The CNPs, 5mg/100ml, 10mg/100ml, 15mg/100ml, 20mg/100ml and 25mg/100ml significantly increased the seedling vigour of rice seeds by 1395, 1655.4, 1893.48, 2104.25 and 2341.44 (Fig. 9). respectively and increased percentage of seed germination by 90.2, 93.00, 93.66, 95.0 and 96.34 per cent when compared to control (87.71%).

The least germination percentage was recorded in MRM16(Red rice) variety (Fig. 9). The CNPs 5mg/100ml, 10mg/100ml, 15mg/100ml, 20mg/100ml and 25mg/100ml significantly increased the seedling vigour of rice seeds by 398.4, 578.7, 864.36, 1267.92, 1517.4,

TABLE 2

Influence of CNPs on rice seed germinationand seedling vigor

	Conc. (%) CNPs	Root length	Shoot length (g)	Germination (%)	VI
IR64	5	8.3 ± 0.57	9.6 ± 0.57	92.5 ± 2.3	1646.8 ± 9.3
	10	9.66 ± 0.89	11.8 ± 0.89	94.7 ± 3.4	2017.24 ± 8.6
	15	11.6 ± 0.57	12.6 ± 0.66	95.8 ± 3.6	2299 ± 12.3
	20	12.33 ± 0.89	13.6 ± 0.89	96.9 ± 2.4	2489.28 ± 13.6
	25	12.67 ± 0.66	15.3 ± 0.66	98.6 ± 3.6	2741.06 ± 11.3
	Control	7.8 ± 0.48	9.1 ± 0.57	88.4 ± 3.3	1487.2 ± 5.4
Red Rice	5	7.4 ± 0.66	9.2 ± 0.57	24.8 ± 2.8	398.4 ± 4.66
	10	8.79 ± 0.33	10.5 ± 0.66	30.7 ± 1.57	578.7 ± 4.45
	15	9.59 ± 0.66	10.99 ± 0.89	42.7 ± 2.6	864.36 ± 3.33
	20	11.18 ± 0.89	12.3 ± 0.66	54.9 ± 3.4	1267.92 ± 7.45
	25	11.89 ± 0.57	13.4 ± 0.89	60.7 ± 3.3	1517.4 ± 8.76
	Control	7.1 ± 0.33	7.3 ± 0.57	22.6 ± 1.89	316.8 ± 6.57
RNR	5	7.7 ± 0.54	7.8 ± 0.57	90.5 ± 2.3	1395 ± 5.33
	10	8.5 ± 0.66	9.3 ± 0.57	93.8 ± 3.67	1655.4 ± 7.23
	15	9.66 ± 0.33	10.7 ± 0.57	93.8 ± 3.21	1893.48 ± 9.89
	20	10.55 ± 0.57	11.6 ± 0.66	95.8 ± 3.33	2104.25 ± 10.34
	25	11.89 ± 0.48	12.5 ± 0.89	96.7 ± 2.89	2341.44 ± 14.21
	Control	7.1 ± 0.66	7.3 ± 0.34	87.5 ± 4.57	1252.8 ± 9.45

Note : Values are the means ± SE of three replicates. VI - Vigour Index

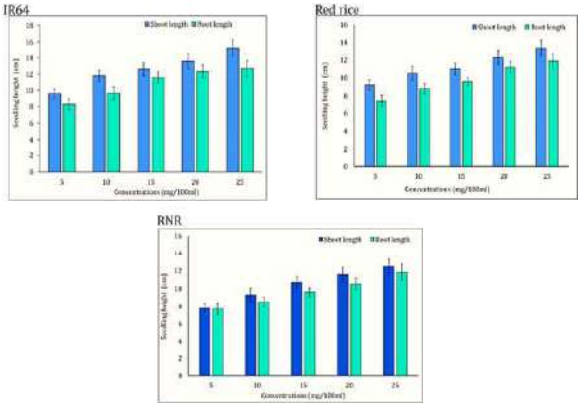

Fig. 8 Effect of chitos an nano particles on rice seed IR64, RNR15048, Redrice MRM16 varieties germination under laboratory conditions.

(Fig. 9), respectively and increased percentage of seed germination by 30.34, 42.0, 54.0 and 60.33 per cent as compared to control (22.45%).

In-vivo Studies under Greenhouse Conditions

The CNPs at 25 mg/100ml in IR64 were observed to be the optimal concentration for seed treatment for improvement of seed germination and vigour in *in-vitro* studies. It was further tested for its ability to elicit protection against bacterial leaf blight in greenhouse studies compared to control. The results showed that CNPs effectively reduced bacterial leaf blight when compared to control. The treatments significantly increased plant height, shoot length, root length. Control plants became completely diseased with the *X. oryzae*. In this study, disease incidence of

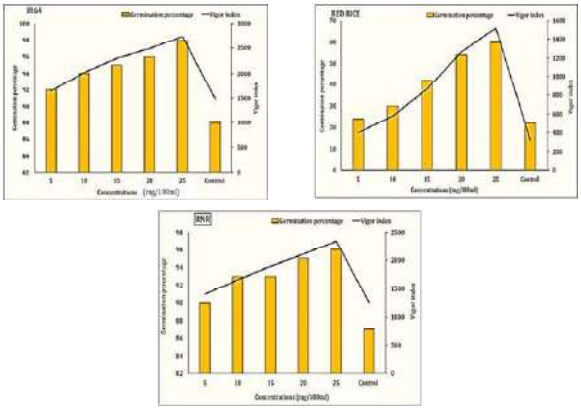

Fig. 9 Effect of rice IR64, RNR15048, Redrice MRM16 varieties seed treatment with CNPS at different concentrations (5-25mg/100ml) on seed germination and seedling vigour of rice under laboratory conditions.

bacterial leaf blight was significantly reduced by CNPs at 25 mg/100ml, and blight incidence was reported to be 71.06 per cent when compared with *X. oryzae* treated (94.16%) (Fig. 10).

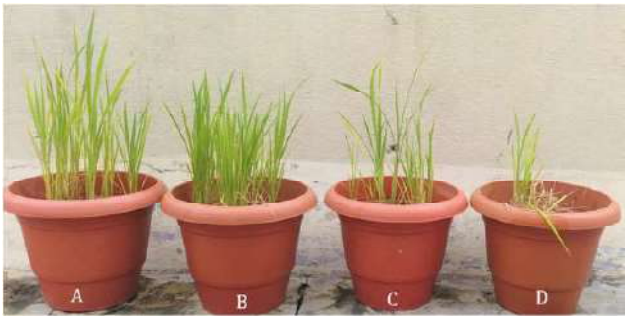

Fig. 10 Effect of CNP's in plant growth promotion on blight incidence under green house conditions. (A) CNP's treated alone (B) Control (C) Treated with CNP's challenged with *X. oryzae* (D) *X. oryzae* inoculated alone

The present investigation on Chitosan nanoparticles was analyzed for the efficacy of CNPs against Bacterial leaf blight caused by *Xanthomonas oryzae*. Chitosan nanoparticles have inherent antimicrobial and chelating properties, and the availability of modifiable functional groups (Bandara *et al.,* 2020). CNPs increase the efficiency of pesticides further reducing their concentration and decreasing its toxic effects and hazardous effects on the environment and crops (Xing *et al.,* 2016).

The CNPs presence stimulates the plant defense mechanisms which are used as a powerful elicitor in plant disease control. (Morin-crini *et al.,* 2019). In this study, the antibacterial activity of CNPs on human pathogens and plant pathogens was compared where CNPs showed good antibacterial activity on *Xanthomonas oryzae*.

Effect of CNPs on seed germination and seedling vigor of rice under laboratory conditions were examined results showed significantly increased mean shoot length and mean root length when compared with untreated control. *In-vivo* studies under greenhouse conditions were studied where results showed CNPs effectively reduced bacterial leaf blight when compared with control.

In this investigation disease incidence of blight was reduced by CNPs when compared to CNPs untreated

plants. CNPs was shown to be effective in reducing the infection caused by *Xanthomonas oryzae* pv. *Oryzae (Xoo)* in rice plant and potentially be used as an alternative to chemical management methods of Bacterial leaf blight in rice and as a promising future in agriculture for the management of a bacterial disease.

The current study identified the efficiency of the tested seed treatment with CNPs to induce defense against *X. oryzae* in rice plants. The outcomes indicate that the CNPs at different concentrations induced active defense reactions in rice against *X. oryzae*. The decreased blight incidence in rice by the treatment of CNPs may be an outcome of plant protection and plant growth promotion. Thus, we suggest that plant or seed treatment with CNPs could be an encouraging method to confirm increased yield development in present agriculture.

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