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SHORT COMMUNICATION

Synthesis of Nickel-Chitosan Nanoparticles for Controlling Blast Diseases in Asian Rice

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Abstract

Rice blast caused by *Pyricularia oryzae* is one of most devastating fungal diseases in rice, reducing the annual yield of rice worldwide. As an alternative to fungicide for curbing rice blast, synthesis of nickel-chitosan nanoparticles (Ni-Ch NPs) was performed with nickel chloride and assessed its efficacy in inflating plant growth and hindrance of *Pyricularia oryzae* (blast pathogen). Characterization of Ni-Ch NPs from SEM, TEM, and DLS analyses showed smooth- and spherical-shaped nanoparticles in the range of 20–70 nm. Colloidal stability of NPs was revealed from Zeta potential exhibiting polydispersity index of 0.22. EDX spectroscopy corroborated the presence of nickel (14.05%) in synthesized Ni-Ch NPs. A significant increase in germination and growth attributes in terms of shoot and root length and number of lateral roots over control was observed in paddy seeds on the treatment with Ni-Ch NPs. Furthermore, the application of NPs in paddy plants under glasshouse condition demonstrated a remarkable improvement in plant growth. Protein profiling of NP-treated plants revealed new polypeptides (Rubisco units) enlightening the enhanced photosynthetic rate. Also, Asian rice exhibited reduced blast symptoms on leaves treated with NPs under glasshouse condition while displaying 64% mycelia inhibition in Petri plates. All these results suggest that nickel-chitosan nanoparticles could be exploited as an effective plant growth promoter cohort in controlling rice blast disease.

Keywords Nickel-chitosan nanoparticles · Protein profile · Seed germination · *Oryza sativa* · *Pyricularia oryzae* · Rice blast

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Introduction

Rice blast disease as a result of *Pyricularia oryzae* is solicitude in tackling worldwide subsistence unpredictably accountable for roughly 30% food grain manufacturing drop globally—equivalent of nourishing 60 million populations. This forfeiture escalates the universal grain cost and diminishes customer well-being and food certainty [1]. Distinctive strategies had been developed to boom the productivity and conquer demanding situations of controlling rice blast diseases. Though rice blast may be curtailed through the use of fungicides and resistant cultivars, development of the latest pathogenic race hostility to commercially obtainable antifungal agent is an enduring problem [2]. To overcome this issue, nanomaterials are predominantly used in crop protection as an alternate to fungicides owing to their homogenous size, larger surface volume ratio, quirky optical properties, and ecologically secure [3]. Over the last few years, metal nanoparticles including Ag, Au, ZnO, nano-sized TiO_2, Al_2O_3, Fe_3O_4, CeO_2, and Cu NPs are reported to exert positive effects on plant growth [4–7]. These metallic nanoparticles had been shown to penetrate the biological tissue inducing physiological and biochemical modifications in plants [8, 9]. Conversely, different studies provided conflicting evidence about the positive nexuses of nanomaterials on grain sprouting and vegetative maturation in limiting environmental conditions [10–14].

Nickel (Ni), an essential plant micronutrient, was proclaimed to regulate seed germination in higher plants including cereal grains and legumes which is less toxic [15]. It activates enzymes such as urease and glycosidase-I required for nitrogen metabolism and assimilation [16]. It has been shown that nickel gets transported easily into plant system inducing physiological changes in plants [17, 18]. Gajewska and Sklodowska [19] described the use of nickel salt as fertilizer for promoting plant growth. Nowadays, biopolymers such as chitosan, cellulose, alginate, and pectin has fascinated researchers for synthesizing metal nanoparticles which acts as a good support preventing agglomeration and proven to be a better stabilizing agent as they allow better particle size control [20, 21].

Chitosan, a non-toxic, biocompatible, and biodegradable natural polymer, has the capability to chelate numerous metal ions such as Cu^{2+} and Zn^{2+} which can make an ideal composite based on the concept of nanotechnology. As well, chitosan has been shown to boost the yield and protein content emphatically in numerous plants likely maize, mulberry grains, broad bean, and corn [22]. In particular, metal-based chitosan nanoparticles (Cu-chitosan, ZnO-chitosan, Ag-chitosan) demonstrated an outstanding antimicrobial activity against phytopathogens accompanying plant growth promotion [23–26]. However, only few investigations have been concentrated on the antifungal activity of nickel nanoparticles [27]. In this context, we hereby describe, primarily, the synthesis of nickel-chitosan nanoparticles and assessed the effect of nanoparticles on growth improvement in Asian rice and control of *P. oryzae* causing rice blast disease.

Materials and Methods

Synthesis of Nickel-Chitosan Nanoparticles

In brief, 100 ml of 0.1% (w/v) chitosan solution was processed employing (0.1%, w/v) acetic acid. The mixture was precipitated with 10 N NaOH and centrifuged at 10,000 g for 30 min. The supernatant was discarded and immensely washed with distilled water to eliminate excess sodium hydroxide.

For the preparation of nanoparticles, 0.01% (w/v) of 25 ml nickel chloride solution was added dropwise to the prepared chitosan solution and stirred continuously for 12 h at 70 °C using a magnetic stirrer. To the resultant complex, 0.5 M NaOH was added dropwise to remove excess Cl_2 ions from the nanoparticles. The composite was finally centrifuged at 10,000 g for 10 min, concentrated under vacuum, and stored at 4 °C.

Physico-chemical Characterization of Ni-Ch NPs

The optical measurement of Ni-Ch NPs was carried out by UV–visible spectrophotometer (Varian Cary 100) ranging from 200 to 500 nm to authenticate the formation of nanoparticles. FTIR spectroscopic studies were documented in the span of 4000–400 cm^{-1} on Spectrum RX I to authenticate the functional groups and bonding of chitosan to nickel nanoparticles. The mean particle size, distribution, and polydispersity index (PDI) of Ni-Ch NPs were carried out by dynamic light scattering (DLS). The stability of the nanoparticle in suspension was determined by measuring the zeta potential of nanoparticles (Zeta sizer Nano ZS, Malvern). SEM analysis was being done to identify the dimension, shape, and surface arrangement of Ni-Ch NPs. EDX spectroscopy analysis was performed to determine the presence of elemental nickel in the nanoparticle. The size and morphology of nickel-chitosan nanoparticle was characterized using TEM (JEOL JEM-2100 F) by drop coating of the NPs onto a carbon-coated copper grid.

In Vitro Seedling Bioassay

The seeds of Asian rice (ADT-43) were obtained from Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu, India. Briefly, 50 paddy seeds were disinfected with 0.001% (w/v) sodium hypochlorite for 10 min and washed several times with distilled water. The disinfected seeds were placed in Petri plates holding a filter paper with 5 ml of 0.1% (w/v) Ni-Ch NPs (treated) and 5 ml of distilled water serves as control. Seed germination was recorded and the Petri dishes were kept in a growth chamber at 28 ± 2 °C for 10 days. Each experiment was repeated three times. The shoot length, root length, and number of lateral roots per plant were measured after 10 days of germination. Seedling vigor index was calculated using the formula as described by Abdul-Baki and Anderson [28]: Seed vigor index = (Germination %) × (Seedling length).

Inhibitory Effect of Ni-Ch NPs Against *Pyricularia oryzae*

The fungus *Pyricularia oryzae* was obtained from Tamil Nadu Rice Research Institute, Aduthurai, India. In vitro antifungal assay against *P. oryzae* was evaluated by the inhibition of mycelia growth (%). The *P. oryzae* mycelia bit (5.0 mm) was placed at the center of potato dextrose broth (PDA) plates containing 0.1% (w/v) Ni-Ch NPs as treated. The PDA plate without nanoparticles served as control. The plates were maintained at 28 °C for 7 days and monitored for mycelial growth. The percent inhibition of mycelial growth was measured. Each experiment was replicated thrice.

Effect of Ni-Ch NPs on Vegetative Growth and Rice Blast Control Under Glasshouse Environment

Ni-Ch NP-treated paddy seeds were placed in containers (27-cm width; 26-cm vertex) holding soil + manure, inoculated with spores of *Pyricularia oryzae* (1×10^5 spores ml^{-1}) under glasshouse condition. Plants grown in soil with spores of *P. oryzae* (1×10^5 spores ml^{-1}) alone served as control. Growth attributes with regard to shoot length, root length, and number of leaves were monitored regularly in treated and control plants. For all experiment, 50 plants were used and repeated thrice. Blast symptom including lesions was observed on leaves of the control and treated plants. Additionally, disease severity (DS) and percent efficacy of disease control (PEDC) were assessed as mentioned by Chester [29] and Wheeler [30].

$$\text{DS} = \text{Sum of all individual disease rating} \times 100/\text{Total number of plant assessed} \times \text{Maximum rating}$$

$$\text{PEDC} = \text{Disease severity in control} - \text{Disease severity in treatment}/\text{Disease severity in control} \times 100$$

Effect of Ni-Ch NPs on Protein Profiling

The control and treated (Ni-Ch NP + pathogen) leaves of paddy were collected on 25th day after treatment. Leaves (1 g/2 ml) were homogenized with sodium acetate buffer (0.02 M, pH 5.8) and centrifuged at 10,000 g for 10 min at 4 °C. From the supernatant obtained, the protein content was evaluated according to the Bradford method [31].

The samples (20 µg) were denatured for 3 min in a boiling water bath with sodium dodecyl sulfate (SDS) containing sample buffer and separated ahead with marker proteins following the procedure of Laemmeli [32]. Localization of polypeptides on PAGE was completed by staining with Coomassie Brilliant Blue.

Statistical Analysis

All the statistics were subjected to two-manner analysis of variance. The significance of variations among treatment approach was as compared by means of Duncan's multiple range tests at $P < 0.05$.

Results and Discussion

Metal and chitosan-based nanoparticles promoting plant growth and protection have been known for years finding its application in modern agriculture [33–36]. Naturally occurring polymers for managing rice blast disease are secure to environment and acts as an effective alternative to chemical fungicides. The utility of nano-composite which includes micronutrients complements the consumption and utility of minerals in cereal crops [37]. As well, chitosan acts as a carrier to obtain new polymeric material in order to prevent the undesirable nutrient loss to soil and can be modified easily without

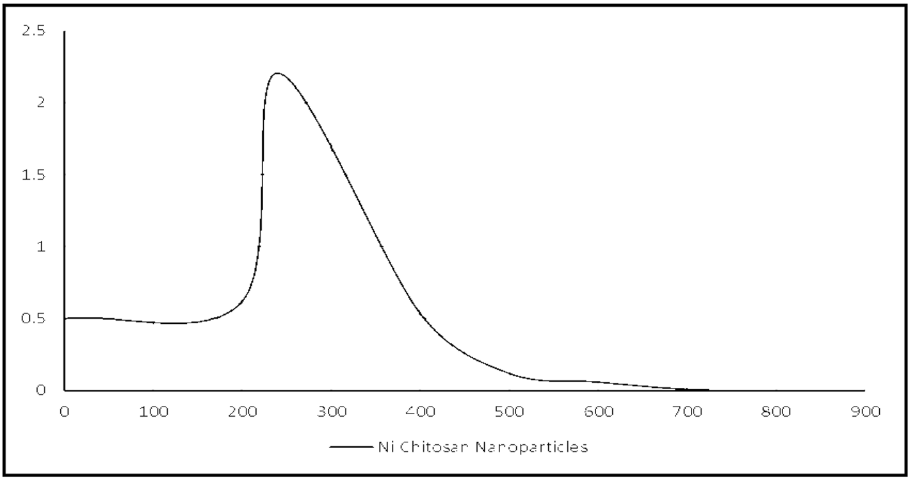

Fig. 1 UV–visible spectra of nickel-chitosan nanoparticles

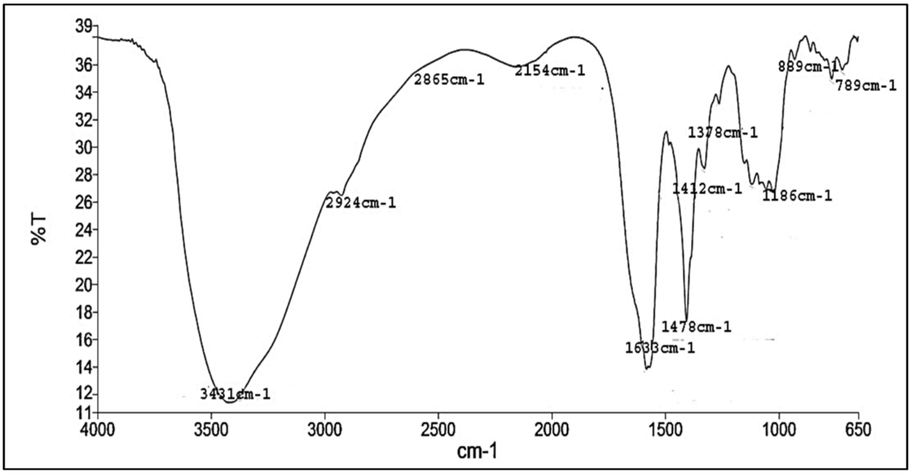

Fig. 2 FTIR spectra of nickel-chitosan nanoparticles

affecting its innate capabilities [38, 39]. The present study herein strongly demonstrated the control of rice blast by promoting the growth of paddy plants.

Characterization of Nickel-Chitosan NPs

The successful formation of nanoparticles was confirmed by a peak at 240 nm using UV–visible spectrophotometer (Fig. 1). FTIR analysis was done to track the interaction between nickel and chitosan nanoparticles. As depicted in Fig. 2, FTIR spectra exhibited the characteristic peaks at 3431 cm^{-1} (asymmetrical stretching frequency due to overlap of OH and -NH$_2$); 2924 cm^{-1} and 2865 cm^{-1} (-C-H stretching vibration); 1633 cm^{-1} (-C=O amide group); the band at 889 cm^{-1} stretching frequency shows -C–O–C bridge as well

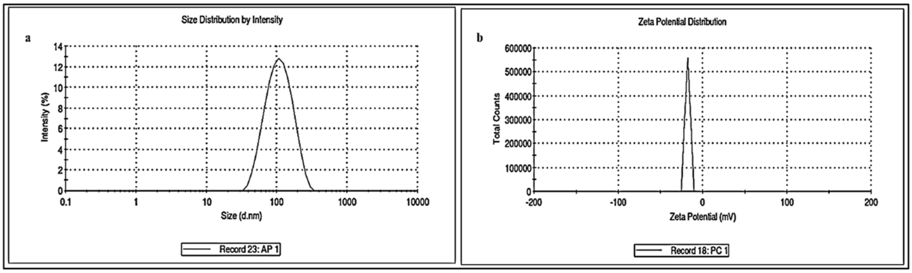

Fig. 3 Dynamic light scattering (**a**) and zeta potential distribution of nickel-chitosan nanoparticles (**b**)

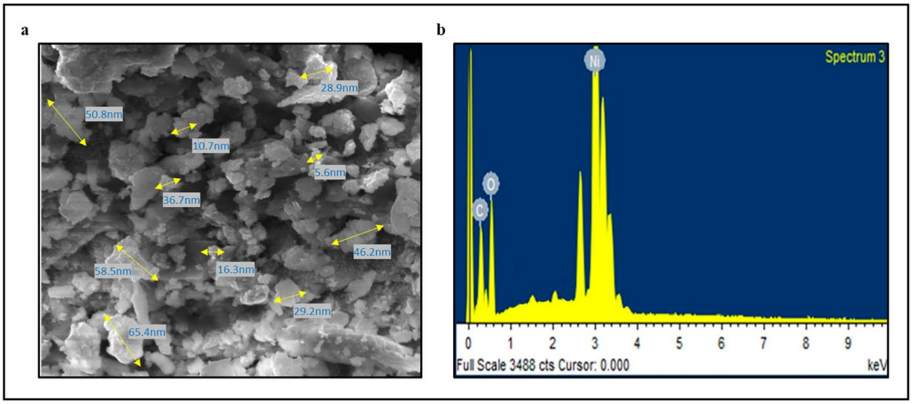

Fig. 4 Scanning electron microscopy image (**a**) and EDX analysis (**b**) of nickel-chitosan nanoparticles

as glycosidic linkage. The peak at 1420 cm^{-1} and 1072 cm^{-1} indicates the -CH$_2$ bending and -C–O–C stretching frequency which confirmed the glycosidic linkage formed between nickel and chitosan nanoparticle [40].

DLS analyses revealed the average size and narrow distribution of nanoparticle as 89.81 nm with a polydispersity index of 0.22 (Fig. 3a). As shown in Fig. 3b, the zeta potential of nickel-chitosan nanoparticle was found to possess negative surface charge (−17.8 mv) which implicated the colloidal stability of nanoparticles. Chitosan, a positively charged amino polysaccharide, in acidic condition can form strong chemical and physical bonds with metal ions insinuating the stability of nanoparticles [9, 23].

A SEM study revealed the well-dispersed and spherical shape of nanoparticles ranging from 30 to 50 nm (Fig. 4a). The EDX analysis inferred the elemental composition (14.05%) of the nanocomposite by exhibiting very strong signals at nickel-rich complex for nickel ions along with other weak signals displaying carbon and oxygen. The weak signals were attributed to the chitosan polymer surrounding nickel nanoparticle complex (Fig. 4b). The TEM micrograph disclosed the spherical shape and smooth surface of the discrete nanoparticles without any aggregation (Fig. 5). The mean size of the nickel-chitosan nanoparticle as observed by TEM was 30–40 nm. It was obvious that chitosan as a dispersing agent interconnects superficially to nickel-chitosan nanoparticles thereby modifying the morphology, structure, and magnetic properties [41]. This is in accordance with Hajipuor and Abolfathi [42] who prepared uniform size, spherical-shaped nickel nanoparticles supported

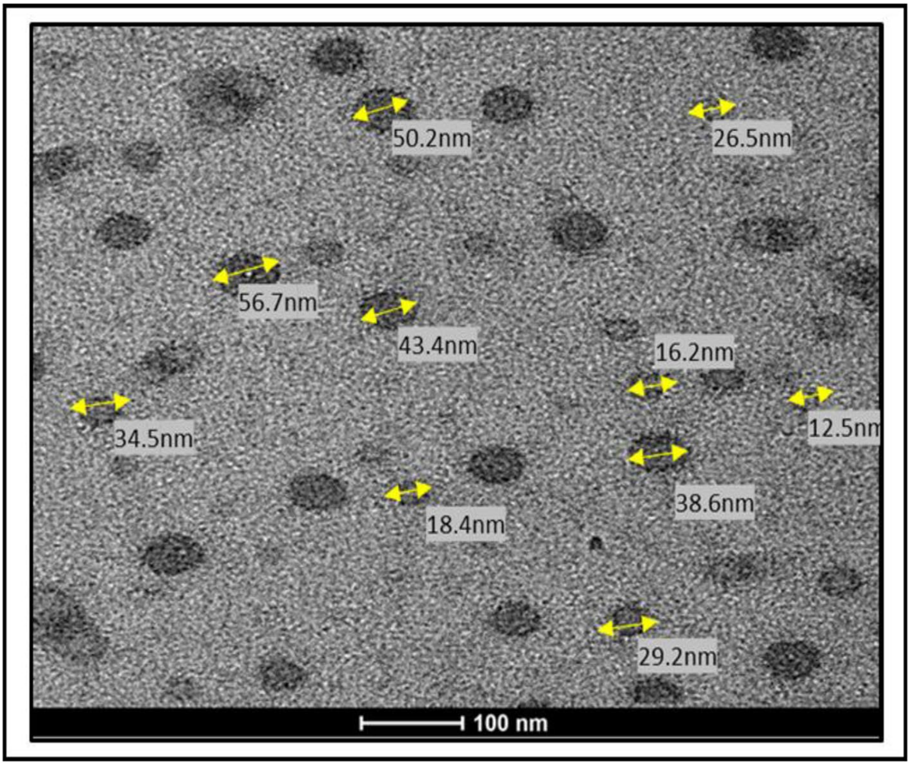

Fig. 5 Transmission electron microscopy image of nickel-chitosan nanoparticles

onto the chitosan surface. The dissimilarity in dimensions among TEM and DLS study was because of the techniques intricated in preparation of the samples [43].

Effect of Ni-Ch NPs on Seed Germination and Growth of Paddy

To check out the plant growth stimulating activity of Ni-Ch nanoparticles in paddy, the development criterion for instance shoot length, root length, number of lateral roots, and seedling vigor index was calculated. Germination studies of Ni-Ch NP on paddy seeds revealed 100% germination with increased seedling vigor index (2054) while control showed only 82% germination with less seedling vigor index (1305) comparatively (Fig. 6). Seed treatment of nanoparticles was reported to enhance the seed vigor and seedling development in many crops [44]. As depicted in Fig. 7a, a significant increase in shoot and root length was noticed in NP applied seedlings as compared to control. In general, plant growth promoting activity was found to be higher in Ni-Ch NP treatment. The Ni-Ch NP gets imbibed into the seeds and transported along with the photosynthates. Tan et al. [45] reported higher root number, root length, and plant fresh weight of the *Oryza sativa* treated with uniconazole encapsulated silica nanoparticles. Besides increased shoot length, the growth rate was higher in root tissue following exposure to NP exhibiting enhancement in lateral root formation compared to control. Root elongation is a more sensitive indicator to study the phytotoxicity of engineered nanoparticles as reported by Temsah and

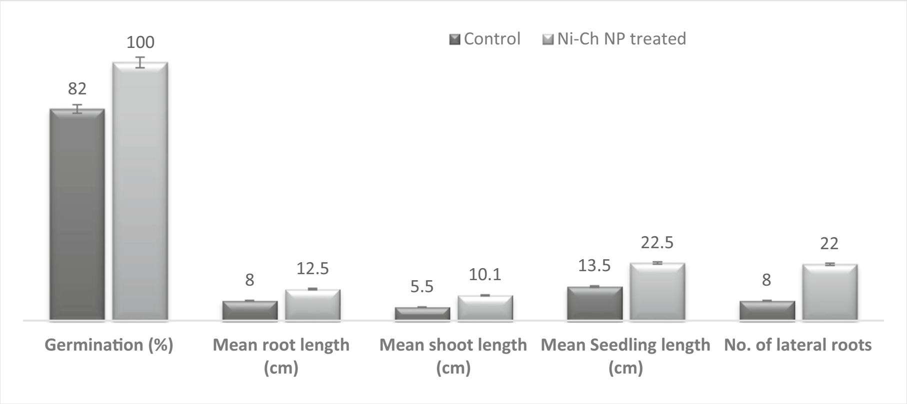

Fig. 6 Effect of Ni-chitosan nanoparticles on seed germination, shoot length, root length, and number of lateral roots in paddy

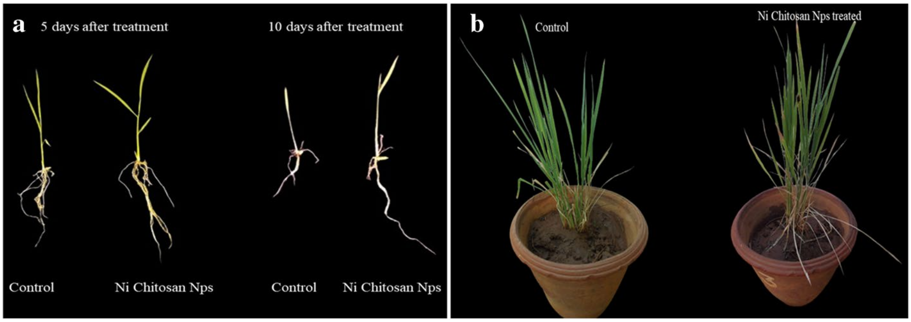

Fig. 7 Effect of nickel-chitosan nanoparticles on growth of paddy under "in vitro" (**a**) and glasshouse condition (**b**)

Joner [46]. At the root plane, rhizodermis lateral root junctions may anticipate easy access to NMs, largely near the root tip, although upper parts are impermeable since impregnation of suberin on the outer layer [47]. Furthermore, the negatively charged nanoparticles are translocated directly into root central cylinder and promote their migration ascending towards the aerial part of plant [48, 49].

It is noteworthy that NP treatment promoted the growth of paddy plants under pot condition showing no toxic effects (Fig. 7b). Some of the synthesized metal nanoparticles (iron oxide, ZnO, TiO_2, and copper) were straight away administered to soil as nano-fertilizers through irrigation or foliar spray in distinct plants, together with mung bean plant, cucumber, and rape [50–53]. Despite this fascinating evidence, soil amendment of nanoparticles may additionally create a risk to soil microorganisms dampening the use of metallic nanoparticles in agriculture [54, 55]. But metal chitosan nanocomposites are said to be much less toxic due to slow-release phenomenon and confirmed the ever-lasting effect in plants [52, 56, 57]. Chitosan nanoparticles had been used for controlled launch of nitrogen, phosphorus, and potassium in wheat by way of foliar uptake [4]. Because of excessive nitrogen content material and low *C*/*N* ratio, chitosan will be immediately used as a fertilizer to enhance crop growth. In a few different studies, chitosan nanoparticles loaded with three triazine herbicides have shown decreased environmental effect and less toxic effects on genotype of onion plants [58]. The nanoparticle treatment displaying positive impact may additionally be because of the dimension, form, strength, chemical framework, and effective electrical charge of nanoparticles [59].

Effect of Ni-Ch NPs on *Pyricularia oryzae*

The obliteration of mycelial growth was ascertained in Petri plates holding 0.1% (w/v) Ni-Ch NP after 7 days at 28 °C and the percentage of inhibition was measured. No inhibition was noticed in the control plates while the treated plates displayed (64%) higher growth inhibition (Fig. 8a). This observation is in agreement with Pandian et al. [60] demonstrating the antifungal activity of nickel nanoparticle against *Candida* and *Aspergillus* species. Antifungal activity of metal nanoparticles is dependent on concentration, exposure time, suspension preparation procedure, and the class of fungal strains [60, 61]. The inhibition of mycelial growth might be due to the electrostatic interplay between undoubtedly charged nickel ions and negatively charged microbial cellular membranes. The inhibitory effect also results from the chitosan interaction with phospholipid components of fungal membrane inflicting leakage of cell contents which ultimately results in cell death [62, 63]. Furthermore, chitosan readily binds with minor nutrients fabricating crucial elements inaccessible for normal fungal growth [64]. Therefore, the direct antifungal activity of NPs could have relevant implications in controlling blast pathogen.

To investigate the virtue of Ni-Ch NPs against blast pathogen, potting experiments were conducted by inoculating the spores of *P. oryzae* under glasshouse condition. After 25 days of pathogen infection, the disease was assessed for each leaf and the disease severity was calculated. Initial symptoms such as oval-shaped gray color lesions were observed in control. Later, it becomes dark and the entire leaves get affected while a significant control of blast disease was observed in NP-treated paddy (Fig. 8b). The disease severity was higher in control (63%) compared to the treated plants (21%). Percent efficacy of disease control was 23.4% in control while the treated plants showed 72% higher resistance to pathogen (Fig. 9). Cu-chitosan NPs have been outlined successful against early blight and *Fusarium* wilt of tomato [65]. In plants, chitosan is recognized by specific receptors available on the cellular plasma membrane together with pathogen-associated molecular pattern (PAMP)

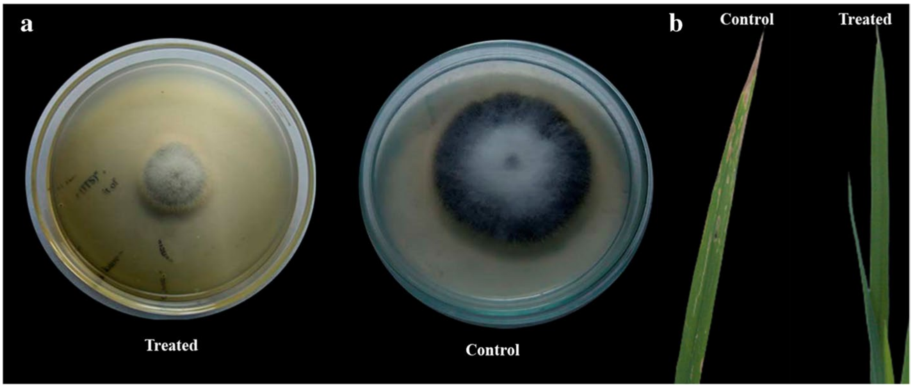

Fig. 8 Inhibitory effect of nickel-chitosan nanoparticles on *Pyricularia oryzae* under "in vitro" (**a**) and blast symptoms on the control and treated leaves under glasshouse condition (**b**)

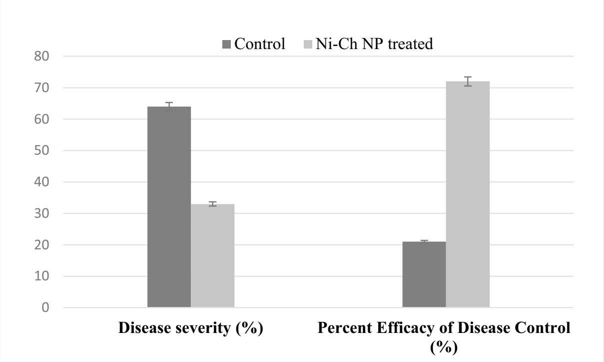

Fig. 9 Effect of nickel-chitosan nanoparticles in paddy plants after 25 days of pathogen infection under glasshouse condition

receptor. After this recognition, chitosan can spark off PAMP—brought on immunity, this is capable of setting off defense responses against potential fungal, bacterial, and viral pathogens [66].

Protein Profile

Protein content was found to be higher in leaves of treated plants compared to control (Fig. 10a). Metal NPs such as zinc oxide NPs increased the protein content by modulating the photosynthetic efficiency in tomato plants [67]. Also, Mehmood and Murtaza [68] reported that exposure of pea seeds to silver NPs significantly increased the carbohydrate and protein content. Multiwalled carbon nanotubes extended the number of

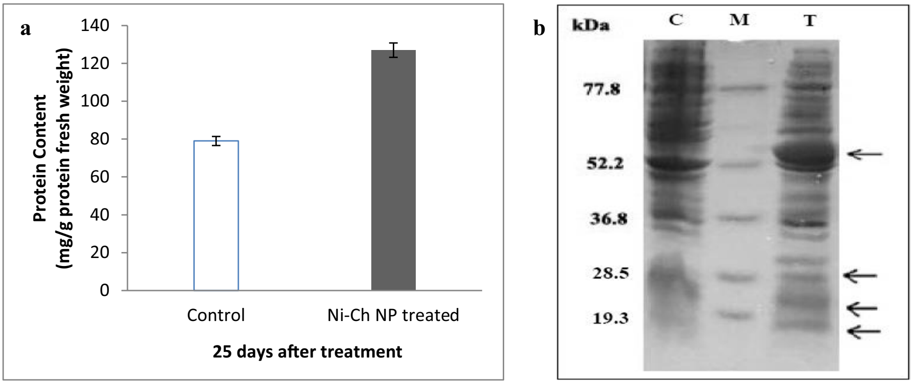

Fig. 10 Protein content in control and nickel-chitosan nanoparticle-treated leaves of paddy plants (**a**). Localization of polypeptides in leaves of paddy (**b**) on SDS-SPAGE (10% separating gel; 4% stacking gel); M, standard marker proteins of different molecular weights (Bio Rad Chem. Co., USA); C, control; T, nickel-chitosan nanoparticle treated

flowers and fruits in tomato by activating the proteins important for plant bloom and development [69].

Ribulose bisphosphate carboxylase/oxygenase (Rubisco), a vital photosynthetic enzyme, was quantified by electrophoresis [70]. SDS-PAGE separation of protein in leaves of paddy obtained on the 25th day treatment revealed polypeptides ranging from 25 to 110 kDa in control (pathogen alone) as well as the treated (Ni-Ch NPs + pathogen) plants (Fig. 10b). Apart from that, three new polypeptides were observed with molecular weights of 15, 18, and 28 kDa in leaves of treated paddy plants. As seen in Fig. 10b, a molecular mass of 52.2 kDa was predominantly expressed in treated seedlings compared to control. RuBP oxygenase-carboxylase (Rubisco), a key enzyme in photosynthesis, contains two large subunits (50–55 kDa) that are associated with two small subunits (12–18 kDa) comprising up to 50% of the total soluble protein and 26% of total nitrogen found in leaf material [71]. Perdomo et al. [72] highlighted the importance of Rubisco for enhancing the photosynthetic overall performance of wheat, rice, and maize at distinctive climatic conditions. Also, Rubisco and nitrogen courting rice were stated to promote leaf photosynthesis and plant growth [73].

In monocots, the degradation of Rubisco is constantly preponderant after complete growth of leaf blade leading to a prompt decrease in the Rubisco content. But in response to NPs, Rubisco tends to remain even after 25 days following exposure to NPs and pathogen which was confirmed by electrophoresis. This sincerely explains the position of NPs in enhancing the photosynthetic activity by means of mentioned expression of Rubisco eventually promoting plant growth.

Conclusion

From this study, it is clear that nickel-chitosan nanoparticles through seed and soil amendment can be used in modern-day agriculture for increasing productiveness and as a novel remedy for rice blast disease. Further studies on biochemical and molecular aspects underneath field situations are compulsory to validate the exact mechanisms.

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Author Contribution Ramalingam Parthasarathy: conceptualization, methodology, writing—original draft. Sathiyanarayanan Anusuya: writing—review and editing—original draft. Appu Manikandan: review and editing. Chelliah Jayabaskaran: supervision, project administration.

Data Availability Not applicable

Declarations

Ethical Approval Not applicable

Consent to Participate Not applicable

Consent for Publication Not applicable

Competing Interests The authors declare no competing interests.

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