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Short communication

Preparation of Chitosan nanoparticles and its effect on detached rice leaves infected with *Pyricularia grisea*

Appu Manikandan, Muthukrishnan Sathiyabama*

Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

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ABSTRACT

The aim of the present study was to prepare chitosan nanoparticles to evaluate their effect on protection of rice plants from blast fungus. Nanoparticles were prepared using the ionic gelation method by the interaction of Chitosan and sodium tripolyphosphate. The particle size, polydispersity index, zetapotential and structure was confirmed by DLS, FTIR, TEM and XRD. The Chitosan nanoparticle was evaluated for suppression of rice blast fungus (*Pyricularia grisea*) under the detached leaf condition. It is evident from our results that chitosan nanoparticle have potential in suppressing blast disease of rice which can be used further under field condition to protect rice plants from the devastating fungus.

1. Introduction

Rice (*Orysa sativa* L.) is an important food crop feeding more than half of the world's population [1]. A qualitative and quantitative loss due to major fungal disease (blast) caused by *Pyricularia grisea* has been reported [2,3]. Use of fungicides to control this disease has been practiced generally. However, the excessive and indiscriminate use of these chemicals may result in environmental pollution, ecological imbalance in soil and ailing effects on human health [4]. Moreover, the excessive use of chemical agents can cause development of resistance in phytopathogenic fungi.

Nanotechnology focuses on applications in the field of medicine in the management of microbial infection [5], in cancer treatment [6] etc. Research from these fields facilitate the development of plant protecting chemicals, and precision farming techniques [7] and opens up potential applications in agriculture in improving the existing crop management techniques. Nanomaterials have been utilized in agriculture mostly in crop protection due to their size-dependent qualities, high surface- to volume ratio and unique optical properties. Among the nanoparticles, metal-based nanoparticles were widely used in crop protection [8]. But the possible environmental toxicity due to the unpredicted nature of metal-based nanoparticle questioned their application in crops [9,10]. These issues raised interest to search for bio-based nanomaterial in crop protection. Nanoparticles prepared from natural sources possess advantages such as availability from replinishable resources, biocompatibility, biodegradability and ecological safety.

Chitosan based nanoparticles are preferably used worldwide for various applications owing to their biodegradability, high permeability, non-toxicity to human and cost-effectiveness [11]. [12] reported that chitosan based nanoparticle have antifungal activity towards some phytopathogenic fungi under in vitro condition. Our previous study showed that β-glucan nanoparticle inhibit the growth of *Pythium aphanidermatum* under in vitro condition [13]. If a natural polymer-based nanoparticle could be developed to control crop diseases, a more sustainable agricultural system could be formed. Therefore, the present study aims to use a biobased nanoparticle (chitosan nanoparticle- a renewable resource) to protect rice plants from the blast fungus.

2. Materials and methods

2.1. Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared based on the ionotropic gelation between chitosan and sodium tripolyphosphate (TPP). Chitosan was dissolved at 0.5% (w/v) with 1% (v/v) acetic acid and pH was adjusted to 5.0. TPP was dissolved in water at a concentration of 0.25% (w/v). Formation of chitosan nanoparticle was aided by the addition of TPP to chitosan solution (1:3) under magnetic stirring. The colloidal suspension was centrifuged at 10,000 G for 10 min.

* Corresponding author. Tel.: +91 4312407061; fax: +91 4312407045.
E-mail address: sathiyabamam@yahoo.com (M. Sathiyabama).

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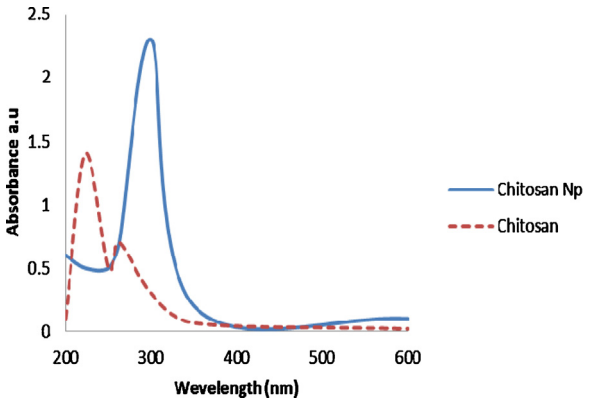

Fig. 1. UV–visible spectrum of synthesized chitosan nanoparticle.

The precipitate was washed twice to remove unreacted substance and then freeze-dried.

2.2. Characterization of chitosan nanoparticles

UV–visible spectra were recorded using a Shimadzu UV–visible 1800 spectrophotometer for the confirmation of nanoparticle formation. The structural features of chitosan nanoparticles were performed in a Nicolet 560 FTIR Spectrometer in a range between 400–4000 cm^{-1} using a KBR pellet technique. Particle size analysis was performed by dynamic light scattering (DLS). The charge on the surface of the particles was characterized by measuring the zeta potential of the suspension using a zetasizer (Malvern Instruments, U.K). The size and morphology of the chitosan nanoparticles were examined by HRTEM (JEOL model 1200 EX). X-ray diffraction studies were performed with an X-ray diffractometer (Rigaku Ultima III XRD) with Cu Kα1 radiation to determine the structure of the sample. The X-ray source was operated at 40KV and 40 mA. Diffraction intensity was measured in the reflection mode at a scanning rate of 2/min for 2θ = 10–70°.

2.3. Determination of antifungal activity

The antifungal activities of nanoparticles (0.05, 0.075, 0.1%, w/v) on spore germination of *P. grisea* were tested. Spore suspension (1.0×10^5 spores/ml) of *P. grisea* was prepared aseptically from 10day old culture. 50 μl of spore suspension and 50 μl of nanoparticle at above mentioned concentrations in aqueous were taken on glass slides (Borosil, Mumbai) in 6 replicates. Conidial suspensions incubated in water served as control. All the treatments were maintained at 28 ± 1 °C for 60 h and the observations were made under the microscope (Optika Microscope XDS-2, Italy). Antifungal activity of chitosan nanoparticle against *P. grisea* was also evaluated by disc diffusion method.

2.4. Effect of chitosan nanoparticle on disease development using detached leaves

The effect of chitosan nanoparticle on the suppression of leaf blast of *O. sativa* caused by *P. grisea* was studied on detached leaves. Leaves (from 30d old plants) of *O. sativa* (Andhra Ponni) was washed thoroughly in running tap water for 3 min and with sterile dist. H_2O twice for 2 min each. They were placed on sterile petri plates lined with moist filter paper. The leaves were treated with chitosan nanoparticle solution (0.1% (w/v), 500 μl/leaf) with a painting brush (Camlin Ltd, Bombay, India) and incubated at 25 ± 2 °C for 24 h. Leaves treated with sterile distilled water served as control. After 24 h incubation, the control and chitosan nanoparticle treated leaves were challenged with a spore suspension (1×10^5 spores/ml) of *P. grisea* (500 μl/leaf) using a painting brush. They were maintained at 25 ± 2 °C with 100% humidity for 10 days to monitor the disease development.

3. Results and discussion

The preparation of chitosan nanoparticle is based on an ionic gelation interaction between positively charged chitosan and negatively charged tripolyphophate at room temperature [14].

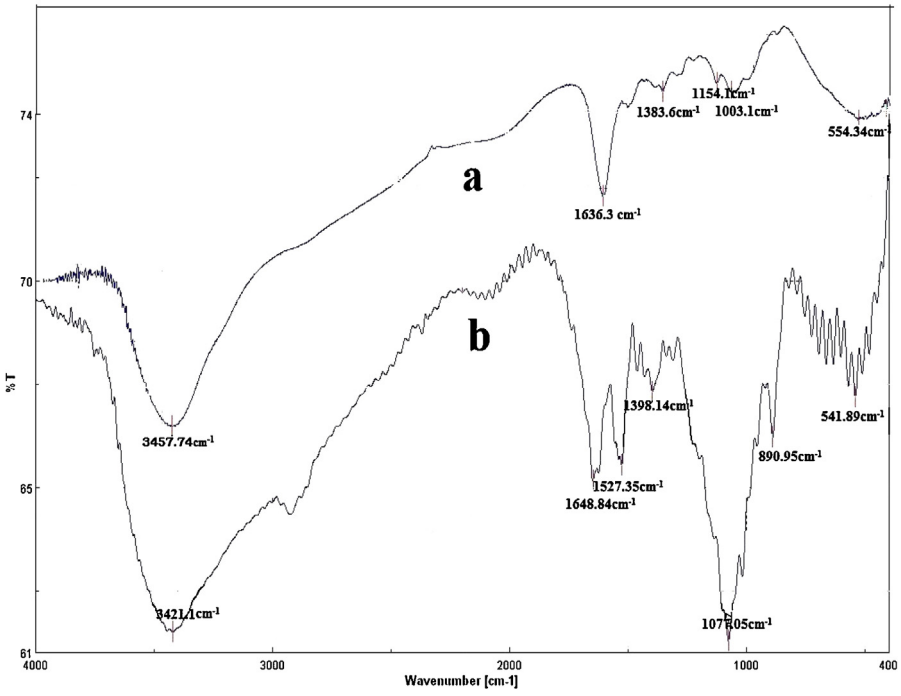

Fig. 2. FT-IR spectrum of chitosan (a) and prepared chitosan nanoparticle (b).