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Research Article

Copper-Chitosan Fused Nanoparticles as A Best Possible Remedy Against Fungal Phyto-Pathogens

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Abstract

Copper (Cu) is known to have antibacterial and antifungal properties; also it is non-toxic to mammals. These properties thus make Copper nanoparticles (Cu-NP) synthesis an attractive area. A number of methods for producing Cu-NPs have been developed using both physical and chemical approaches, which involves elevated temperatures, inert atmospheres, large amount of surfactants and organic solvents. However, the major limitations in the synthesis of CuNPs are their ease of oxidation to CuO or Cu2O during preparation and storage. Therefore, alternative methods have been developed to synthesize CuNP in the presence of polymers and surfactants as stabilizer. There are some recent reports on a successful synthesis of Cu-NPs in aqueous solution, using chitosan which involve additional reducing and stabilizing agents at various steps that are associated with environmental toxicity. In green synthesis method, preparation of Cu-NPs, colloidal stability in aqueous media using chitosan, a biopolymer as a reducing and capping agent. The choice of chitosan as a stabilizer of the Cu-NPs is because of its ability to chelate metals, which makes a perfect material for metal NP synthesis. The synthesis of NPs using polymer has been promising due to their ease of processing, solubility, biocompatibility, less toxicity and also because of the possibility of controlling the growth of the resulting NPs. These NPs generated are strongly attached to the chitosan due to the chemical bond between the electron rich nitrogen present in the amino groups of the polymer and copper. The use of biopolymer as capping and or reducing agents represents an environmentally friendly alternative to hazardous organic solvents. The present study suggests the utility of Copper-chitosan fused nanoparticles as an effective fungicidal agent against phytopathogens. The results showed that, these particles are effective at three sprays at an interval of every 7 days and at thrice a month frequency. The suggested sprays showed the significant fungicidal action to the crop against wide variety of phytopathogens.

Keywords: Copper-Chitosan Nanoparticles; Antifungal Potential; Non-Toxic; Biodegradable; Phyto-Pathogens

Introduction

The nanoparticles in today's era are extremely useful as these are significant in different fields of medical and pharmaceutical sciences. There are different methods available for the production of copper nanoparticles via chemical and biological processes. However, different limitations come into existence during the formation of copper nanoparticles due to oxidation [1]. Thus an attempt has been made to develop copper nanoparticles using chitosan as a natural biopolymer, capping and reducing agent. These nanoparticles cannot get oxide during formation and storage. The synthesis of will be efficient because of ease of processing, solubility, biocompatibility, less toxicity and also for stabilization. Chitosan can coordinate with cuprous ions before reduction to gain stabilization after formation of fused nanoparticles. The reduction of these ions takes place further, coupled with the oxidation of the hydroxyl groups. The studies demonstrated that hydrophilic side chains of chitosan play a fundamental role in stabilization and dispersion of NPs, preventing their agglomeration. When Cu salts dissolve in acidified chitosan solution, Cu ion binds to the polymer chains via amino groups. The reduction of these ions takes place further, coupled with the oxidation of the hydroxyl groups. Green syntheses of copper-chitosan nanoparticles were found to be effective for antibacterial potential [2].The studies have already described the synthesis, characterization and antimicrobial properties of copper nanoparticles [3-7].

Materials and Methods

Copper-chitosan nanoparticles synthesis

In a modified method, about 50 ml of 0.75% (w/v) chitosan solution was prepared using 0.1% acetic acid (in distilled water) solution and 25 ml of 100 mM copper sulphate was then added to it and stirred on magnetic stirrer at 70°C for about 12 hours. After the said time, the content was centrifuged at 10,000 rpm for 20 minutes to generate the pellet/precipitate. The precipitate was again and again collected from the former mixture by centrifugation till the liquid supernatant doesn't results pellet deposition. The pellet/precipitate collected was dissolved in acetone (80%, v/v). Finally, the precipitate was dissolved in water, dried under vacuum overnight and stored [8].

Characterization of fused copper-chitosan nanoparticles via UV-VIS spectrophotometer

The UV-VIS spectrum of the fused copper-chitosan nanoparticles was generated by using Systronics UV-VIS spectrophotometer. The absorption maximum was determined, by quantification of the absorbance at different wavelength ranges.

Characterization of fused copper-chitosan nanoparticles via FT-IR

The fused copper-chitosan nanoparticles formed were characterized by FT-IR spectra using computerized FT-IR spectrometer (Perkin Co., Germany) in the range of 400 - 4000 cm-1 by the KBr pellet technique.

Antifungal potential of copper-chitosan nanoparticles

The modified method of determination of antifungal potential of copper-chitosan fused nanoparticles was determined against different fungal phytopathogens viz. *Curvularia lunata, Microsporum gypseum, Aspergillus flavus, Fusarium oxysporum, Rhizoctonia solani, Penicillium expansum, Fusarium monoliforme* and *Trichoderma viride* by well diffusion method. The inhibition of mycelia growth of respective fungal strain by the nanoparticles will be described as antifungal potential of copper-chitosan fused nanoparticles. The antifungal potential was determined by pre- inoculating the pathogenic fungus in the liquid media and after solidification, boring the sterilized media with sterilized borer followed by filling the well with 50 µl of nanoparticles solution.

Results

In the present study, it was found that, copper-chitosan nanoparticles showed absorption maxima at 510 nm (Figure 1). The FT-IR spectra showed spectra from 4400 cm^{-1} to 400 cm^{-1} (Figure 2). The spectra showed the polymeric nature of fused copper-chitosan nanoparticles. The antifungal potential of copper-chitosan nanoparticles was determined against the selected fungal pathogens. The results showed the significant antifungal activity against *Curvularia lunata, Microsporum gypseum, Aspergillus flavus, Fusarium oxysporum, Rhizoctonia solani, Penicillium expansum*, *Fusarium monoliforme* and *Trichoderma viride* (Figure 3). The results were found to be very effective as the nanoparticles showed significant antifungal potential against the selected fungal strains. The antifungal activity of fused copper-chitosan nanoparticles was found to be effective at 50 µl and 10 ppm concentration.

Figure 1: UV-VIS spectra of copper-chitosan nanoparticles.

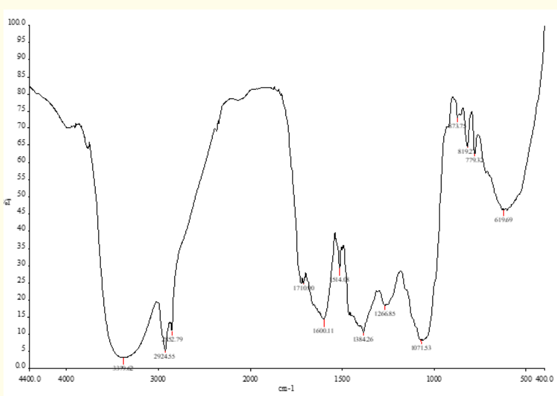

Figure 2: FT-IR spectra of fused copper-chitosan nanoparticles.

Figure 3: Antifungal potential of fungal phytopathogens.

Discussion and Conclusion

The fused copper-chitosan nanoparticles are found to be a strong antifungal agent against the fungal phyto-pathogens. The previous studies [9-13] has elaborated the antifungal properties of chitosan based nanoparticles against different phyto-pathogenic fungi. The fused nanoparticles will be utilized as the basis to explore the utility of such active ingredients in fungicidal formulations. Since these nanoparticles are naturally synthesized, there is no harm to the crop and are biodegradable, thus leaving no toxicity. Furthermore studies are being carried out in order to study their effect against multiple fungal pathogens on wide diversity of crops.

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Conflict of Interest

The authors declare no conflicts of interest.

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