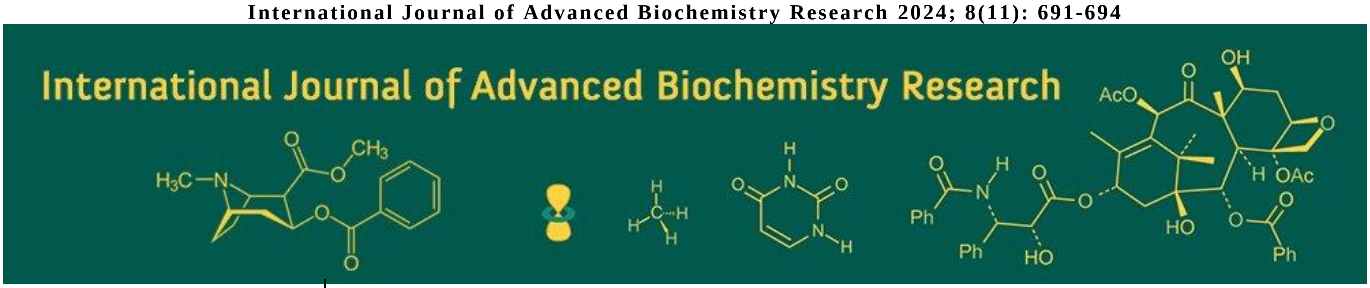

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Synthesis and efficacy of chitosan copper nanoparticles in mitigating *Fusarium oxysporum* infection in soybean

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Abstract

In the present study, chitosan-copper nanoparticles (Cht-Cu-NPs) were successfully synthesized using aqueous leaf extract of *Moringa oleifera*. The synthesized Cht-Cu-NPs were characterized by UV-visible spectroscopy (UV-Vis), particles size by Dynamic light scattering (DLS) and Zeta potential. The formation of nanoparticle was observed by the peak at 520 nm using UV-Vis spectroscopy. The average particle size of the nanoparticle as determined by DLS was about 176 nm, while the zeta potential was +13 mV. The antifungal activity of the synthesised Cht-Cu-NPs was analysed against the *Fusarsium oxysporum* in soybean crop. Cht-Cu-NPs exhibited significant antifungal properties and stability, making them promising for agricultural applications, particularly in managing fungal pathogens. This eco-friendly method cuts down on harmful chemicals, saves money, and boosts biological action. By using copper's germ-killing power, chitosan's protective traits this approach offers a long-lasting option to replace chemical fungicides for dealing with fungal diseases in crops.

Keywords: Chitosan, copper nanoparticles, antifungal activity, fusarium oxysporum, sustainable agriculture

Introduction

Fungal diseases such as *Fusarium* cause major threat to soybean (*Glycine max*) crop. Wilting is the major challenge caused by the *F. oxysporum* and farmers often use chemical sprays, but long-term use chemical-based fertilizers affects the environment, builds up resistance, and leaves toxic traces. This creates a need to find lasting, eco-friendly ways to fight plant diseases effectively and efficiently. Nanotechnology is a fast-emerging field in modern research, involving the synthesis of nanoparticles whose properties exhibit a sea of difference from those of their bulk counterparts. This is due to their size, distribution, and morphology. (Olszak-Przybyś *et al.*, 2023, Zhang *et al.*, 2024) [8, 9]. Plant extract based especially *M. Oleifera* chitosan-copper nanoparticles (Cht-Cu-NPs) fight off *Fusarium oxysporum* in soybeans. Chitosan, a biopolymer from chitin, has its own germ-fighting powers. It is eco-friendly bio-degradable, bio-adaptable molecule. Because it has a positive charge, chitosan can mix with fungi's charged cell walls to break them down and kill fungal cells. Chitosan nanoparticles help metal nanoparticles like copper last longer and work better when they're wrapped up inside. This makes them great choices to protect plants. This plan uses copper's germ-killing effects, chitosan's protective traits. It offers farmers a green choice instead of chemical fungicides (Alsamhary *et al.*, 2024) [10].

Copper and its compounds are among the various noble metals that have been investigated; their antibacterial qualities have made them useful since ancient times. There is no doubt that copper nanoparticles could improve wheat output and growth. Copper has a greater potential fungicidal effect at the nanoscale than copper salt (Kasana *et al.*, 2017) [12]. Many pathogens, including *Salmonella typhi, Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Klebsiella pneumonia*, are successfully inhibited by copper nanoparticles and are employed in food packaging applications (Ivanova *et al.*, 2024) [13].

Chitosan is a biodegradable and biocompatible polymer which also possesses broad antifungal properties. Combining chitosan with copper has been demonstrated to have antibacterial properties and promote plant development by increasing antioxidants and defence enzymes. It is necessary to streamline the synthesis and broaden the studies on copper nanoparticles based on chitosan for use against biotic stresses and ultimately helps to improve yield and production.

Chitosan copper nanoparticles (Cht-Cu-NPs) have caught scientists' attention due to their strong germ-killing powers. These tiny particles have a big surface area and release metal ions, which break down the structures of microbes. Scientists have come up with a fresh way to make these nanoparticles using plant leaf extract, especially *M. oleifera* leaf extract which is full of polyphenols and acts as a natural reducer (Ermini *et al*., 2021) [14]. Methods based on plant extracts cuts down on harmful chemicals, saves money, and boosts the environment stability.

Plant extracts as natural reducing agents. *Moringa* leaf extract has a high content of polyphenols making it work well to reduce and form chitosan-copper nanoparticles (Cht-Cu-NPs). This eco-friendly method cuts down on harmful chemicals, saves money, and boosts biological action.

This research sets out to create Cht-Cu-NPs with *Moringa* oleifera leaf extract and test how efficacy to fight against the fungus *Fusarium oxysporum* in soybean. By using copper's germ-killing power, chitosan's protective traits, and Moringa's natural ability to reduce, this approach offers a long-lasting option to replace chemical fungicides for dealing with fungal diseases in crops.

Materials and Methods

Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium hydroxide (NaOH) and acetic acid (CH COOH) and chitosan MIDC, Wagle Industrial Estate, Thane (West), Maharashtra, India.

Preparation of leaf extract

The leaves of *Moringa oleifera* were washed thoroughly under running tap water to remove any foreign and dust particles after which they were shade dried. Aqueous extract of the plant was prepared by taking about 3 g of the dried leaves in a 100 mL distilled water, the mixture was heated at 80°C for about 30 minutes. After cooling it down, it was filtered with the help of Whatman filter paper no.1 and the filtrate was used as the plant extract for the subsequent synthesis of the desired nanoparticles (Maheo *et al*. 2022) [5].

Synthesis of Chitosan-copper nanoparticle (Cht-Cu-NPs)

Chitosan-copper nanoparticle (Cht-Cu-NPs) were synthesized using *Moringa oleifera* and chitosan following a modified method by Jayaramudu *et al*. (2019) [2]. Chitosan (1.5 g) was dissolved in 100 mL of 1.5% acetic acid at 60°C. Separately, 1.25 g of $CuSO_4 \cdot 5H_2O$ was dissolved in 100 mL water. 60 mL of the $CuSO_4$ solution was mixed with 20 mL of the plant extract and stirred for 30 minutes. Then, 20 mL of 0.6 M NaOH was added to adjust the pH to ~9, followed by 20 mL of chitosan solution. The mixture was stirred for 3 hours at 70 °C, with a colour change from blue to greenish-black. A control was prepared without chitosan. The solution was filtered, washed with distilled water, and dried at 80 °C for characterization.

Characterization of Chitosan copper nanoparticles

UV-visible spectra were recorded using (Systronics AU 2701 Double Beam) UV-visible spectra for the confirmation of NP formation. 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 Anton paar@Litesizer DLS 500.

Determination of antifungal activity

A disk diffusion method was used to assay the antifungal activity of synthesized Cht-Cu-NPs. against *Fusarium oxysporum* in potato-dextrose agar media at various concentrations i.e, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm. For comparison, a control dish with 300 ppm of native chitosan to check the antifungal activity. The plates were incubated for 72 hours at 37 °C and zones of inhibition were measured.

Results and Discussion

UV-Visible Spectroscopy

Peaks at 270, 380, and 520 nm were seen in the UV-visible spectroscopy data, indicating that the chitosan-copper nanoparticles (Cht-Cu-NPs) were successfully formed. The chitosan molecule's π-π* transition is linked to the peak at 270 nm, whereas the copper ion's n-π* transition is linked to the peak at 380 nm. The copper nanoparticles' surface plasmon resonance (SPR) is associated with the peak at 520 nm. These results are consistent with earlier research that reported the synthesis of chitosan-copper nanoparticles using plant extracts and validate the formation of Cht-Cu-NPs (Manikandan, A., & Sathiyabama, M. (2015)) [11].

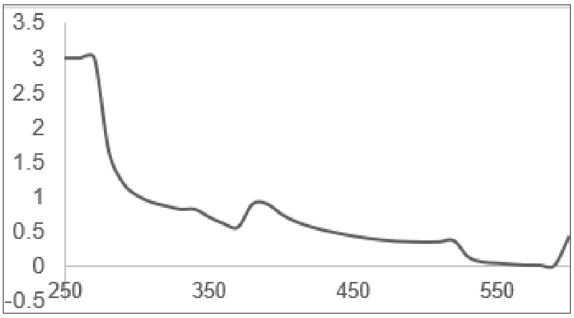

Fig 1: UV-Vis spectrum of Cht-Cu-NPs

Where k = 0.89, λ is the wavelength of x-rays (1.5405 Å), β is the full width at half maximum (FWHM), θ is the diffraction angel (Bragg) and D is the particle diameter size.

$$D=\frac{k\lambda}{\beta \cos^{\theta}}$$

(Qazi *et al*., 2009) [15]

Dynamic Light Scattering (DLS) and Zeta Potential

The characterization of Chitosan copper nanoparticles (Cht-Cu-NPs) confirms their nanoscale dimensions and stability, highlighting their potential for broad applications. DLS analysis revealed that the Cht-Cu-NPs have an average particle size of 176 nm, firmly within the nanometer range, ensuring optimal properties for bioavailability and cellular interaction.

Further validation was provided by zeta potential measurements, which exhibited a positive potential of +13 mV. This value not only confirms the surface charge but also indicates the colloidal stability of the nanoparticles in suspension, reducing the likelihood of aggregation over time. The positive charge is attributed to the copper ions interacting with the amine groups of chitosan on the nanoparticle surface (Vanti *et al*., 2020) [16].

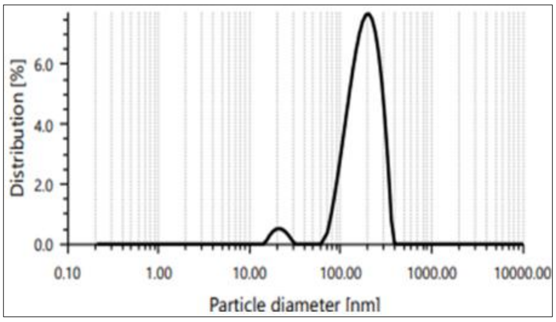

Fig 2: Particle Size of Cht-Cu-NPs

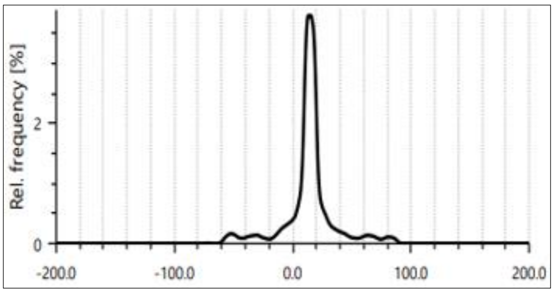

Fig 3: Zeta Potential of Cht-Cu-NPs

Characterization of Cht-Cu-NPs confirms that the successful formation of nanoparticle through Uv-Visible spectroscopy (520 nm), Dynamic light scattering (176 nm) and Zeta potential (+13 mV).

In vitro antifungal activity assay

The antifungal activity assay demonstrated a dose-dependent inhibitory effect of chitosan copper nanoparticles (Ch-Cu-NPs) on *Fusarium oxysporum* growth. The mean percentage inhibition increased progressively with higher concentrations, ranging from 35.06% at 50 ppm to a maximum of 49.40% at 300 ppm, highlighting the enhanced antifungal efficacy at elevated doses. This result indicates that Cht-Cu-NPs are more effective in limiting fungal growth at higher concentrations, potentially due to increased interactions between the nanoparticles and fungal cell walls.

A comparative analysis revealed that chitosan alone, at an equivalent concentration of 300 ppm, achieved only 39.20% inhibition, which is significantly lower than the 49.40% inhibition observed with Cht-Cu-NPs as given in Table 1.1 and Fig 4. This suggests that the functionalization of chitosan with copper ions significantly enhances its antifungal properties. Copper ions may contribute to the generation of reactive oxygen species (ROS) and disrupt essential enzymatic functions within the fungal cells, thereby amplifying the antifungal action.

The control group, with a mean radial growth of 6.33 cm and 0% inhibition**,** demonstrated the natural growth of the fungal pathogen in the absence of any treatment, thereby underscoring the effectiveness of both Ch-Cu-NPs and chitosan in suppressing fungal proliferation (Ibarra-Laclette *et al*., 2022) [17].

The observed differences in antifungal activity were statistically significant, as confirmed by an F Test value of 2.66 and the Critical Difference (CD) at 5% level. This affirms the reliability and reproducibility of the experimental results.

Table 1: Concentration and mean radial growth of *F. Oxysporium* after treating with nanoparticle

Concentration	Mean Radial Growth(cm)	Mean % Inhibition
50	4.11	35.06
100	3.96	37.47
150	3.65	42.37
200	3.56	43.74
250	3.40	46.3
300	3.20	49.4
300(Chitosan)	3.85	39.2
Control	6.33	0
F Test		2.66
SE(m)±		6.67

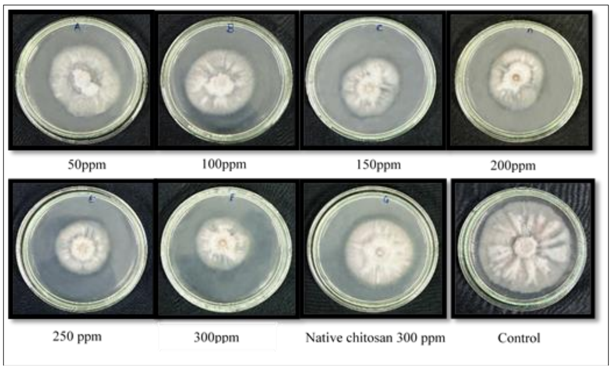

Fig 4: *In-vitro* inhibitory potential of Cht-Cu-NPs against *fusarium oxysporum*

Furthermore, the stability and positive surface charge of Cht-Cu-NPs (+13 mV) likely contribute to their strong antifungal properties, promoting better adhesion to fungal membranes. These findings suggest that Cht-Cu-NPs hold significant promise as a potent antifungal agent in agricultural practices, offering a dual benefit of plant protection and growth enhancement. Future studies could explore their mechanism of action, including ROS generation and disruption of fungal cell integrity, to further elucidate their potential. (Gordon & Martyn, 1997; Khan *et al*., 2017; Khan *et al*., 2019; Ravi *et al*., 2020) [1, 3, 4, 7].

Conclusion

Stable chitosan copper nanoparticles with a nanoscale size and positive zeta potential have been successfully synthesized using *M. oleifera* leaf extract. The synthesized NPs were characterized by UV-Vis, DLS and zeta potential. The main role attributable to the chitosan and copper both acts against the bacterial and fungal pathogens. Our results indicate the potential of Cht-Cu-NPs for combating pathogenic microorganisms. Cht-Cu-NPs exhibit significant antifungal properties and stability, making them promising for agricultural applications, particularly in managing fungal pathogens like *Fusarsium oxysporum*. Nanoparticles synthesized from this method are eco-friendly and might less affect the environment and be a promising antimicrobial material.

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