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In vitro Efficacy of *Trichoderma harzianum* Nanoparticles Against *Colletotrichum capsici* Causing Fruit Rot of Chilli

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluates the antifungal effectiveness of biosynthesized Trichoderma harzianum nanoparticles against Colletotrichum capsici, the pathogen responsible for anthracnose in chili crops. In vitro efficacy of biosynthesized Trichoderma harzianum nanoparticles were assessed against Colletotrichum capsica causing anthracnose of chilli using Agar well method. All three nanoparticles tested showed significant inhibition of mycelial growth at concentrations of 100, 250, and 500 ppm. The lowest mycelial growth was recorded with CuNP at 250 ppm (64.50 mm), while Zinc sulphate at 250 ppm showed the highest growth (85.00 mm). The zone of inhibition was

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greatest with CuNP at 250 ppm (25.50 mm), indicating strong antifungal activity, followed by AgNP and CuNP at 500 ppm. Overall, CuNP showed the highest zone of followed by Silver and Zinc nanoparticles. These findings suggest that CuNPs could be a promising biocontrol agent for chili anthracnose management, offering an eco-friendly alternative to chemical fungicides."

Keywords: Chilli; Trichoderma harzianum; AgNP; CuNP; ZnNP; Colletotrichum capsica;

1. INTRODUCTION

Chilli (*Capsicum annuum* L.), a prominent member of the Solanaceae family and native to tropical America, has become one of the most widely cultivated vegetables worldwide, especially in Asia, covering over 1.5 million hectares globally. With around 20-27 species, only five are extensively cultivated: *C. annuum*, *C. baccatum*, *C*. *chinense*, C. *frutescens* and *C. pubescens*. India stands as the largest producer, accounting for 36% of global production, with states like Telangana leading in yield and area under cultivation. However, chilli production faces significant threats from pathogens, particularly *Colletotrichum capsici*, which causes anthracnose and can lead to considerable yield losses, sometimes reaching 75%. This fungal disease targets above-ground plant parts, diminishing both quantity and quality (Pandurang et al., 2019, Phal et al., 2023). The genus *Trichoderma*, known for its biocontrol properties, has been recognized since the early 1930s for its ability to combat plant pathogens through mechanisms such as mycoparasitism and antibiotic production. Recently, nanotechnology has emerged as a promising tool in agriculture, allowing for the development of environmentally friendly solutions to enhance crop protection and productivity (Reddy et al., 2019, Sam-On et al., 2024). Metal nanoparticles, particularly those synthesized through green methods using *Trichoderma* spp., have shown significant antimicrobial activity against various pathogens, offering a sustainable alternative to chemical pesticides and may play a crucial role in addressing challenges faced in chilli cultivation and broader agricultural practices (Vincent, 1927, Yadav et al., 2023).

2. MATERIALS AND METHODS

2.1 Biosynthesis of Nanoparticles

For biosynthesis of silver nanoparticles combining 100 ml of an aqueous solution containing 1 mM silver nitrate ($AgNO_3$) with 10g of wet biomass from the *Trichoderma harzianum* fungus. for creating copper nanoparticles entails combining 100 ml of an aqueous solution containing 3 mM copper sulphate ($CuSO_4.5H_2O$) with 10g of wet biomass from the *Trichoderma* species fungus and for creating zinc nanoparticles entails combining 100 ml of an aqueous solution containing 2 mM zinc sulphate ($ZnSO_4$) with 10g of wet biomass from the *Trichoderma* species fungus (Kumar, 2024). Subsequently, the mixture was maintained in a revolving shaker set to rotate at 100 rpm for 72 hours at 28°C.

2.2 Concentration Levels

The efficacy of nanoparticles and culture filtrate of *T. harzianum* were assessed using the “Agar well method” method, by utilizing Potato Dextrose Agar (PDA) as a base culture medium. Silver Copper and Zinc nanoparticles (Table 1) was tested at 250, 500 and 1000 ppm concentration, whereas silver nitrate, copper sulphate, zinc sulphate and *T. harzianum* filtrate tested at 250 ppm concentration. A total of 20 milliliters of sterilized molten PDA medium was poured into 90 mm Petri plates. 5 mL of seeded agar, containing a concentrated test pathogen, was spread on PDA medium. Wells were created using a sterile cork borer and is filled with different concentrations of nanoparticles (100 ppm, 250 ppm and 500 ppm) along with culture

Table 1. Nanoparticle solution (Silver, Copper and Zinc)

Tr. No	Treatments	Tr. No	Treatments
T_1	AgNP @ 100 ppm	T_8	CuNP @ 500 ppm
T_2	CuNP @ 100 ppm	T_9	ZnNP @ 500 ppm
T_3	ZnNP @ 100 ppm	T_{10}	*T. harzianum* filtrate @ 250 ppm
T_4	AgNP @ 250 ppm	T_{11}	Silver nitrate @ 250 ppm
T_5	CuNP @ 250 ppm	T_{12}	Copper sulphate @ 250 ppm
T_6	ZnNP @250 ppm	T_{13}	Zinc sulphate @ 250 ppm

T_7	AgNP @ 500 ppm	T_{14}	Control (Untreated)

filtrate of T. harzianum. The plates then be incubated at a temperature of 27±2 °C.

Inhibition zones of the test fungi were measured after every 24 hours until control plates were fully covered with mycelium.

3. RESULTS

Results (Table 2, Plate 1 and Fig. 1) revealed that, *T. harzianum* culture filtrates treated with 1 mM silver nitrate, 3mM copper sulphate and 2mM zinc sulphate solution @ 100 ppm, 250 ppm and 500 ppm concentrations, respectively and among these *Trichoderma* spp. culture filtrate, silver nitrate, copper sulphate and zinc sulphate @ 250 ppm solution evaluated *in vitro* by Agar well method were exhibited fungistatic antifungal activity against *Colletotrichum capsici* and numerically inhibited its growth, over untreated control.

In this method, mycelial growth was observed minimum (64.50mm) in *T. harzianum* CuNP @ 250 ppm, followed by CuNP @ 500 ppm (70.50mm) and was maximum at Zinc sulphate @ 250 ppm (85.00mm) followed by Silver nitrate @ 250ppm (83.50 mm), Copper sulphate @ 250 ppm (82.50mm), respectively.

The zone of inhibition was recorded highest in *T. harzianum* CuNP @ 250 ppm (25.50mm), followed by AgNP @ 500 ppm (20.00mm), CuNP @ 500ppm (19.50mm), respectively and lowest at Zinc sulphate @ 250 ppm (05.00mm), Silver nitrate @ 250 ppm (06.50mm), Copper sulphate @ 250 ppm (07.50mm), respectively (Table.2, Plate 1 and Fig.1). Among the metal nanoparticles it is found that CuNP is most effective followed by silver and zinc nanoparticles, respectively.

4. DISCUSSION

The outcomes validated the previous research findings of Chowdappa et al. (2014) who studied antifungal activity of chitosan-silver nanoparticle composite against *Colletotrichum gloesporioides* by using agar well method. Composite of chitosan and AgNP containing 0.1 (0.00001%), 1.0 (0.001%) and 10.0 ug/ml (0.001%). Elenany, 2024 After 12 hours of incubation on the glass slide, *C. gloeosporioides* was found to have normal conidial germination in sterilized distilled water with 0.1% (v/v) acetic acid. The outcome demonstrated that, the chitosan-AgNP composite treatment was more successful than its equivalent.

Divya et al. (2017) used agar well diffusion method to screen green nanoparticles of various metals (copper, silver, nickel and magnesium) synthesized from ajwain and neem leaf extracts in inhibiting the mycelial growth and spore germination of *C. musae*. Complete inhibition was demonstrated by Ajwain-Mg NPs, Ajwain-Ni NPs (@ 0.2%) and Neem-Ag NPs (@ 0.1 & 0.2%).

Table 2. *In vitro* evaluation of *Trichoderma harzianum* biosynthesized nanoparticles on fruit rot of chilli by Agar well method

Tr.No.	Treatment at different concentrations	Mycelial growth* (mm)	Zone of inhibition* (mm)
T_1	AgNP @ 100 ppm	74.50	15.50 (23.18) **
T_2	CuNP @ 100 ppm	75.50	14.50 (22.38)
T_3	ZnNP @ 100 ppm	77.00	13.00 (21.13)
T_4	AgNP @ 250 ppm	72.00	18.00 (25.10)
T_5	CuNP @ 250 ppm	64.50	25.50 (30.32)
T_6	ZnNP @ 250 ppm	77.00	13.00 (21.13)
T_7	AgNP @ 500 ppm	80.00	20.00 (26.56)
T_8	CuNP @500 ppm	70.50	19.50 (26.20)
T_9	ZnNP @ 500 ppm	74.50	15.50 (23.18)
T_{10}	*Trichoderma* spp. filtrate @ 250 ppm	77.00	13.00 (21.13)
T_{11}	Silver nitrate @ 250 ppm	83.50	06.50 (14.77)
T_{12}	Copper sulphate @ 250 ppm	82.50	07.50 (15.89)
T_{13}	Zinc sulphate @ 250 ppm	85.00	05.00 (12.92)
T_{14}	Control (Untreated)	90.00	00.00 (00.00)
S.E. ±		**0.54**	**0.60**
C.D. at 1 %		**2.16**	**2.48**

*: Mean of the three replications **Figures in the parentheses are arc sine values

Plate 1. *In vitro* evaluation of *Trichoderma harzianum* biosynthesized nanoparticles on fruit rot of chilli by Agar well method

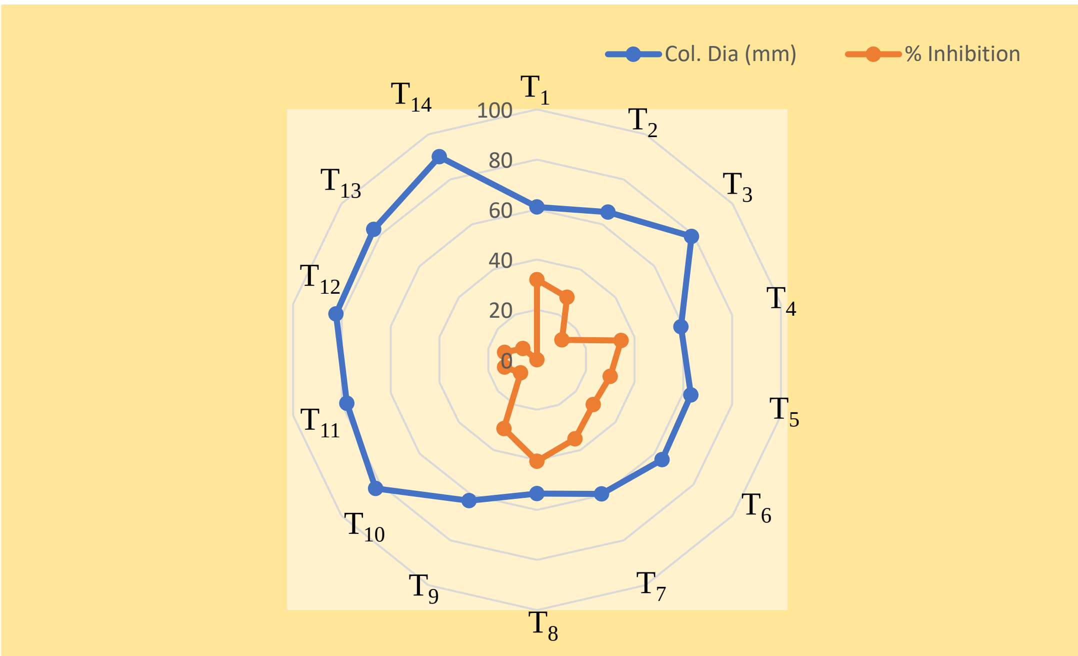

Fig. 1. *In vitro* evaluation of *Trichoderma harzianum* biosynthesized nanoparticles on fruit rot of chilli by Agar well method

Pandurang et al. (2019) evaluated using the well diffusion method, at a concentration of 200 ppm for Cu nanoparticles, the highest zone of inhibition was observed, measuring 28.00±081 mm in diameter. Meanwhile, at a concentration of 30 ppm for CuNPs, the minimum growth inhibition was observed, measuring 9.75±2.06 mm in diameter. The current study's conclusions showed that, fungus *Colletotrichum capsici* is more vulnerable to the effects of copper nanoparticles at greater concentrations.

5. CONCLUSION

The study investigated the *In vitro* antifungal efficacy of biosynthesized nanoparticles from *Trichoderma harzianum* (CuNP, AgNP, ZnNP) against *Colletotrichum capsici,* a pathogen causing anthracnose in chilli. Results showed that copper nanoparticles (CuNP) at 250 ppm were the most effective, resulting in the lowest mycelial growth (64.50 mm) and the highest zone of inhibition (25.50 mm). Silver nanoparticles (AgNP) and zinc nanoparticles (ZnNP) were less effective, with ZnNP exhibiting the least inhibitory effect.

The study concludes that CuNP, followed by AgNP, has the strongest antifungal activity against *C. capsici* among the nanoparticles tested. This supports the potential application of CuNPs in managing anthracnose in chilli, offering a promising biocontrol approach for sustainable agriculture.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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