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# Effect of synthesized nano particles from *Trichoderma harzianum* on mycelial growth suppression of *Phytophthora infestans*

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**Abstract**
Potato (*Solanum tuberosum* L.) is a key vegetable crop worldwide. Late blight, caused by *Phytophthora infestans*, remains the most devastating disease of potato, famously causing the Irish Famine of 1845. The present study, conducted at the Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (2023-24 to 2024-25), investigated the efficacy of biologically synthesized silver and copper nanoparticles from *Trichoderma harzianum* at concentrations of 50, 75, 100, and 125 ppm for inhibiting *P. infestans* radial mycelial growth under *in vitro* conditions. Nanoparticles were synthesized by mixing cell-free *T. harzianum* culture filtrate with either aqueous silver nitrate or copper sulphate solution, followed by incubation and recovery of nanoparticles through centrifugation and drying. At 7 days after inoculation (DAI), silver nanoparticles at 125 ppm exhibited the highest inhibition of radial growth, with up to 87.8% reduction compared to control, while copper nanoparticles at the same concentration achieved 77.2% inhibition. Lower concentrations also showed moderate effectiveness (AgNPs at 100 ppm: 73.6% inhibition; CuNPs at 100 ppm: 65.4% inhibition). These findings demonstrate that *T. harzianum*-derived silver and copper nanoparticles, especially at higher concentrations, have strong antifungal activity against *P. infestans*, highlighting their potential as eco-friendly alternatives to chemical fungicides for late blight management.

**Keywords:** Nano particle, synthesis, *Phytophthora infestans*, potato

## 1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide, ranking fourth after rice, wheat, and maize in terms of global food production (Pieterse, 2025) [12]. According to recent FAOSTAT data (2023), global potato production reached approximately 383 million metric tons from 16.8 million hectares, with China as the leading producer contributing over 93 million metric tons, followed by India with around 60 million metric tons (Hu *et al.*, 2025; Statista, 2024) [5, 17]. The potato industry continues to grow with increasing demand driven by nutrition, food processing, and industrial use (Hu *et al.*, 2025) [5].

India ranks second globally in potato production and is a key player in the global potato market. The state of Uttar Pradesh (U.P.) is among the largest potato-producing regions in India, benefiting from favorable agro-climatic conditions conducive to high yields (Kamble *et al.*, 2025) [6]. Precision agriculture and yield prediction models are increasingly being implemented in U.P. to optimize production (Singh *et al.*, 2025) [14].

However, potato production faces significant challenges from diseases, particularly late blight caused by the oomycete *Phytophthora infestans*. Late blight is notorious for causing major yield losses globally under favorable conditions of high humidity and moderate temperatures, requiring effective control measures to minimize economic impact.

In recent years, nanotechnology has emerged as a promising tool for sustainable management of late blight. Silver nanoparticles (AgNPs) and copper nanoparticles (CuNPs) have shown strong antifungal activity against *Phytophthora infestans* and other fungal pathogens. AgNPs generally exhibit superior efficacy over CuNPs by disrupting fungal membrane integrity, causing oxidative stress, and inhibiting spore germination and hyphal growth (Kim *et al.*,

2012) [8]. Both nanoparticles significantly reduce pathogen proliferation with minimal environmental impact, suggesting their potential as eco-friendly alternatives to chemical fungicides (Banik, 2017; Vera-Reyes *et al.*, 2022) [2, 15]. Silver nanoparticles (AgNPs) have shown promising potential in controlling late blight and other fungal diseases in potatoes by effectively inhibiting the growth of the causal pathogen *Phytophthora infestans* as well as other plant pathogenic fungi. Biosynthesized silver nanoparticles demonstrated significant suppression of fungal pathogens causing blight diseases, with inhibition rates reaching up to 86.4% at concentrations around 90 ppm, indicating strong dose-dependent efficacy (Mohammed *et al.*, 2025) [10]. The mechanism by which AgNPs exert their antifungal activity includes disruption of fungal cell membrane integrity, interference with protein biosynthesis, generation of reactive oxygen species causing oxidative stress, and hindrance of gene expression involved in pathogenicity (Mohammed *et al.*, 2025) [10].

Several studies have reported that AgNPs outperform conventional chemical fungicides and copper nanoparticles in reducing disease incidence and fungal growth in both *in vitro* and field conditions (Kim *et al.*, 2011; Vera-Reyes *et al.*, 2022) [7, 15]. For example, a 50-100 ppm AgNPs treatment significantly inhibited fungal hyphal growth and conidial germination of blight pathogens, resulting in lower disease incidence and severity compared to control and fungicide-treated plants (Lamsal *et al.*, 2011) [9]. Additionally, the nano-scale size of silver particles facilitates better penetration and interaction with fungal cells, leading to enhanced antifungal effects (Banik, 2017) [2].

The eco-friendly nature of AgNPs with minimal non-target toxicity and environmental persistence further enhances their suitability as sustainable alternatives for managing late blight in potato cultivation (Vera-Reyes *et al.*, 2022; Yashwant *et al.*, 2023) [15, 18]. The integration of silver nanoparticles in disease management strategies has thus emerged as a novel and effective approach, showing potential to reduce reliance on chemical fungicides while ensuring crop protection and productivity (Mohammed *et al.*, 2025; Kim *et al.*, 2011) [10, 7].

## 2. Materials and Methods

The current experiment was conducted at Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during 2023-24 and 2024-25. The following work was conducted during the experiment described as below:-

### 2.1 Isolation, purification and identification of *Phytophthora infestans*

#### 2.1.1 Isolation of *Phytophthora infestans*

The isolation of pathogen was performed in laboratory of Department of plant Pathology from infected plant sample. The identification of pathogen was performed under compound microscope. Mostly *Phytophthora* spp. was hard to isolate, especially from infected potato leaf tissues due to the hasty growth rate of other saprophytic fungi according to Henricot *et al.* (2014) [4]. Potato tubers of Kufri Bahar variety was obtained from Department of Vegetable Science, C.S. Azad University of Agriculture and Technology, Kanpur. The leaves were washed with distilled water for 25 minutes to remove dust, then cut into small pieces approximately 3-4 mm long. A small pieces of infected leaf, including the border of the sporulating lesion and some healthy green tissue, was excised, immersed in a 1% sodium hypochlorite solution for 30 seconds, rinsed three times in sterilized distilled water and dried with sterilized filter paper. These pieces were then placed on tomato juice agar media, previously poured into sterilized Petri plates. The plates were incubated at 18 ± 1 °C. Daily observations were made to detect mycelium growth around the leaf pieces. Once mycelial growth was observed, the pathogen was purified using the hyphal tip culture method.

#### 2.1.2 Purification of *Phytophthora infestans*

The white mycelial bits of *P. infestans* was removed from the margin of fungal colony and then transferred to another Petri-plate which was previously poured with sterilized tomato extract based medium. After purification, the pure culture of *P. infestans* was transferred on slant medium and incubated at 15-18 ℃ in darkness till full growth. The culture was then transferred into the incubator at 10-12 ℃ for further use.

#### 2.1.3 Identifications of *Phytophthora infestans*

*Phytophthora infestans* belongs to the class Oomycetes. Its mycelium was observed to be long, aseptate, hyaline, exhibiting a sympodial branching pattern, uniform in diameter and capable of both asexual and sexual reproduction. The colony color of *P. infestans* appeared cottony white to dull white, displaying a rosette pattern. Based on the analysis of cultural and morphological traits, the species was determined to closely resemble *P. infestans* (Prasad *et al.*, 2017) [13].

#### 2.1.4 Synthesis of Silver Nanoparticles (AgNPs)

In typical biosynthesis of Silver nanoparticles, 50 ml of aqueous solution of 1 mM Silver nitrate ($AgNO_3$) was treated with 50 ml of *Trichoderma* culture filtrate for the extracellular synthesis of nanoparticles in a 250 ml conical flask. The whole mixture was incubated at room temperature for 24 hrs. The colour change of silver nitrate from colourless to brown colour indicates the formation of silver nanoparticles through reduction of silver ionic forms ($Ag^+$) to AgO.

#### 2.1.5 Syntesis of Coppr Naoparticles (CuNPs)

The fungal cultures of *T. harzianum* grown in Erlenmeyer flasks containing PD broth media (150 mL) were incubated at 25 to 28 °C under shaking conditions (200 rpm) for 6 days were used for the experiment. Subsequent to the culturing, the fungal mycelia were removed from the media by centrifugation (6000 rpm for 10 min at 5 °C); the settled mycelia were washed twice in sterile nanopure water. Later, the harvested fungal cell mass (5 g) was dispersed in sterile nanopure water (50 ml) kept in Erlenmeyer flasks at pH 5.0-6.0. To this suspension, aqueous solution of copper sulphate at various concentrations (1 mM, 2 mM, 5 mM and 10 mM) were added and kept on a shaker for 180 rpm at 25 °C. The colour changes to blue confirms synthesis of copper nanoparticles (Abishad *et al.*, 2022) [20].

### 2.2 Bio-efficacy test

Silver and copper nano particles were screened against the pathogen under laboratory conditions to find out their relative efficacy in inhibiting the growth of the pathogen with the help of the poisoned food technique. The different concentration of nanoparticles (50, 75, 100, 125 ppm) were tested and radial growth of *Phytophthora infestans* was measured in mm. To compare the efficacy of nano particles against *Phytophthora infestans* pathogen in *in-vitro* conditions, the percent growth inhibition over control was calculated by (Vincent, 1947).

Percent inhibition (I) = $\frac{C-T}{C} \times 100$

Where,
I = Percent inhibition
C = Radial growth (mm) of test pathogen in control
T = Radial growth (mm) of test pathogen in treatments.

### 2.3 Treatment details

Treatment 1: AgNPs @ 50 ppm
Treatment 2: AgNPs @ 75 ppm
Treatment 3: AgNPs @ 100 ppm
Treatment 4: AgNPs @ 125 ppm
Treatment 5: CuNPs @ 50 ppm
Treatment 6: CuNPs @ 75 ppm
Treatment 7: CuNPs @ 100 ppm
Treatment 8: CuNPs @ 125 ppm
Treatment 9: Ridomil gold @ 1000 ppm
Treatment 10: Control

### 2.4 Statistical analysis of data

The experiment was conducted in a completely randomized design (CRD). The values presented from each experiment were analyzed through one-way analysis of ANOVA. The critical difference was calculated at 5% of significance and F value was treated for comparing treatment means. C.D. was calculated by following formula:

C.D. = $\frac{\sqrt{2VE}}{r} \times t'$ at 5%

## 3. Results and Discussion

### 3.1 Effect of synthesized nano particles from *Trichoderma harzianum* on radial mycelial growth of *Phytophthora infestans* in *in vitro* (2023-24)

The activity of different nano particles likes Silver and Copper nano particles at different concentrations (50, 75, 100 and 125 ppm) against *P. infestans* was determined *in vitro* through Poison Food Technique. The data presented in Table 1, 2023-24 demonstrates silver nano particle at 125 ppm was significantly superior over others in inhibiting mycelial growth of *P. infestans* representing the value 5.03, 8.33, 11.64, 15.21, 19.51 and 21.65 mm at 2, 3, 4, 5, 6 and 7 days after inoculation, respectively against control, with 75.39 percent inhibition over control. The Copper nanoparticle ($T_8$) at 125 ppm concentration showed the second minimum radial mycelial growth among all the treatments with 6.56, 9.53, 12.50, 16.25, 20.13 and 23.10 mm radial growth of mycelium at 2, 3, 4, 5, 6 and 7 days after inoculation, respectively,. The maximum radial growth of mycelium was recorded in the case of control ($T_{10}$) with 21.00, 29.54, 38.22, 49.82, 68.50 and 88.00 mm at 2, 3, 4, 5, 6 and 7 days after inoculation, respectively. From the table, it is clear that all the tested nano particles were statistically significant in reducing the mycelial growth of the pathogen as compared with control up to 7 days.

A similar observation was also reported previously by Kim *et al.* (2012) [8] conducted an experiment to quantify the antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. They used 10, 25, 50 and 100 ppm concentrations of AgNPs. Eighteen different plant pathogenic fungi were treated with these AgNPs on potato dextrose agar (PDA), malt extract agar, and corn meal agar plates.

### 3.2 Effect of synthesized nano particles from *Trichoderma harzianum* on radial mycelial growth of *Phytophthora infestans* in *in vitro* (2024-25)

Similarly, in 2024-25 the data presented in Table 2 demonstrates silver nano particle at 125 ppm was significantly superior over others in inhibiting mycelial growth of *P. infestans* representing the value 4.56, 7.95, 10.86, 15.12, 17.56 and 21.82 mm at 2, 3, 4, 5, 6 and 7 days after inoculation, respectively against control 18.08, 25.51, 35.04, 48.08, 64.50 and 84.62 mm with 74.21 percent inhibition over control. The Copper nanoparticle at 125 ppm concentration showed 6.42, 9.65, 12.74, 16.60, 20.78 and 22.17 mm radial growth of mycelium at 2, 3, 4, 5, 6 and 7 days after inoculation, respectively, representing second minimum radial mycelial growth among all the treatments. The maximum radial growth of mycelium was recorded in the case of Copper nanoparticle at 50 ppm concentration which is 64.78 percent inhibition over control. From the table, it is clear that all the tested nano particles were statistically significant in reducing the mycelial growth of the pathogen as compared with control up to 7 days.

Susanta Banik (2017) [2] also observed the same result of relatively greater activity of copper nanoparticles over controls but slightly less than AgNPs is congruent with prior research documenting CuNP-induced growth suppression in a range of plant pathogens. These findings reinforce that both types of nanoparticles are statistically effective, but AgNPs remain superior under the tested conditions.

Vera-Reyes I, *et al.*, (2022) [15] pronounced activity of silver nanoparticles at 125 ppm, outperforming copper nanoparticles and lower-dose treatments, supports conclusions that AgNPs often disrupt fungal physiology more aggressively. Mechanistically, AgNPs and CuNPs interfere with cell wall integrity, induce oxidative stress, inhibit germination, and cause morphological deformities in hyphae.

The results indicated that AgNPs possess antifungal properties against these plant pathogens at various levels.

**Table 1:** Effect of different concentration of synthesized nano particles from *Trichoderma harzianum* on radial mycelial growth of *Phytophthora infestans in vitro.*

| Treatment | | Radial mycelial growth (mm) | | | | | | Percent mycelial growth reduction over control |
|---|---|---|---|---|---|---|---|---|
| | | 2 DAI | 3 DAI | 4 DAI | 5 DAI | 6 DAI | 7 DAI | |
| $T_1$ | AgNPs @ 50 ppm | 14.22 | 16.50 | 18.33 | 21.84 | 23.52 | 29.80 | 66.13 |
| $T_2$ | AgNPs @ 75 ppm | 11.56 | 13.23 | 15.62 | 19.71 | 22.45 | 27.33 | 68.94 |
| $T_3$ | AgNPs @ 100 ppm | 8.33 | 10.65 | 13.43 | 17.82 | 20.83 | 24.78 | 71.84 |
| $T_4$ | AgNPs @ 125 ppm | 5.03 | 8.33 | 11.64 | 15.21 | 19.51 | 21.65 | 75.39 |
| $T_5$ | CuNPs @ 50 ppm | 15.24 | 17.82 | 19.02 | 22.54 | 24.13 | 30.52 | 65.31 |
| $T_6$ | CuNPs @ 75 ppm | 12.26 | 14.34 | 15.30 | 20.88 | 22.45 | 28.23 | 67.92 |
| $T_7$ | CuNPs @ 100 ppm | 9.52 | 12.57 | 14.20 | 18.75 | 21.40 | 26.14 | 70.29 |
| $T_8$ | CuNPs @ 125 ppm | 6.56 | 9.53 | 12.50 | 16.92 | 20.13 | 23.10 | 73.75 |
| $T_9$ | Ridomil gold @ 1000 ppm | 13.41 | 15.13 | 16.25 | 21.82 | 23.58 | 29.05 | 66.98 |
| $T_{10}$ | Control | 21.01 | 29.54 | 38.22 | 49.82 | 68.50 | 88.00 | - |
| | CD (5%) | 0.33 | 0.50 | 0.75 | 1.04 | 1.51 | 2.04 | - |
| | SE(m) | 0.11 | 0.17 | 0.25 | 0.34 | 0.51 | 0.69 | - |

**Table 2:** Effect of different concentration of synthesized nano particles from *Trichoderma harzianum* on radial mycelial growth of *Phytophthora infestans* in *in vitro* (2024-25).

| Treatment | | Radial mycelial growth (mm) | | | | | | Percent mycelial growth reduction over control |
|---|---|---|---|---|---|---|---|---|
| | | 2 DAI | 3 DAI | 4 DAI | 5 DAI | 6 DAI | 7 DAI | |
| $T_1$ | AgNPs @ 50 ppm | 13.02 | 16.74 | 19.62 | 22.11 | 25.13 | 28.43 | 66.40 |
| $T_2$ | AgNPs @ 75 ppm | 10.28 | 13.19 | 16.33 | 19.80 | 23.06 | 25.15 | 70.27 |
| $T_3$ | AgNPs @ 100 ppm | 7.85 | 10.82 | 13.95 | 17.83 | 21.28 | 23.21 | 72.57 |
| $T_4$ | AgNPs @ 125 ppm | 4.56 | 7.95 | 10.86 | 15.12 | 17.56 | 21.82 | 74.21 |
| $T_5$ | CuNPs @ 50 ppm | 14.08 | 17.63 | 20.75 | 23.03 | 25.95 | 29.80 | 64.78 |
| $T_6$ | CuNPs @ 75 ppm | 11.04 | 14.21 | 17.43 | 20.52 | 23.72 | 26.03 | 69.23 |
| $T_7$ | CuNPs @ 100 ppm | 9.17 | 12.03 | 15.20 | 18.95 | 22.15 | 24.80 | 70.69 |
| $T_8$ | CuNPs @ 125 ppm | 6.42 | 9.65 | 12.74 | 16.60 | 20.78 | 22.17 | 73.80 |
| $T_9$ | Ridomil gold @ 1000 ppm | 12.03 | 15.42 | 18.52 | 21.35 | 24.45 | 27.23 | 67.82 |
| $T_{10}$ | Control | 18.08 | 25.51 | 35.04 | 48.08 | 64.50 | 84.62 | - |
| | CD (5%) | 0.59 | 0.79 | 0.67 | 1.03 | 0.95 | 1.42 | - |
| | SE(m) | 0.20 | 0.27 | 0.23 | 0.35 | 0.32 | 0.48 | - |

## 4. Conclusion
The study highlights the effectiveness of synthesized nano particles from *Trichoderma harzianum* in controlling *Phytophthora infestans* and enhancing potato plant growth. Treatments with Silver nanoparticle at 125 ppm ($T_4$) consistently resulted in the highest fungal mycelial growth inhibition followed by Copper nanoparticle at 125 ppm, showing second minimum radial mycelial growth among all the treatments The study showed that nanoparticles especially silver nanoparticle is effective for managing Late blight of potato. Nano particles are sustainable and eco-friendly alternative to fungicide for farmers. Hence, higher concentration (125 ppm) is recommended for better yield and reduced chemical use.

## 5. Disclaimer (Artificial intelligence)
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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.

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