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Efficacy of Synthesized Nanoparticles from *Pseudomonas fluorescens* on the Mycelial Growth Suppression of *Ustilaginoidea virens* Causing False Smut of Rice

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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

Rice is one of the most important food grain crops grown throughout the world as well in India. The present study aimed to evaluate the antifungal efficacy of different synthetic nanoparticles derived from *Pseudomonas fluorescens* and standard fungicide (copper oxychloride @ 2500ppm) on the radial mycelial growth of *Ustilaginoidea virens*, the causal agent of false smut disease in rice, under *in vitro* conditions. Fourteen treatments, including control (T14) and standard fungicide (T13), were assessed at 5th, 7th, and 10th days of incubation. The findings showed notable variations between treatments in suppressing the mycelial growth of *U. virens* of the formulations tested, treatment T3 showed the least mycelial growth (6.32 mm, 13.35 mm, and 18.57 mm on days 5, 7, and 10, respectively) and had the highest percentage of inhibition (54.97%) compared to the control, with T7 (53.37%) and T2 (51.96%) closely following. The fungicide at 2500 ppm achieved 47.74% inhibition, outperforming SiO_2NPs but remaining less effective than Ag NPs and ZnO NPs. In comparison, the control treatment (T14) exhibited the highest mycelial growth (22.57 mm, 31.50 mm, and 41.24 mm) with no restrictions. These results indicate that synthesized nanoparticles from *Pseudomonas fluorescens* have significant antifungal activity against *U. virens* and may act as effective eco-friendly substitutes for chemical fungicides in controlling false smut disease in rice.

Keywords: Nanoparticles; synthesis; mycelia growth; P. fluorescens; U. virens.

1. INTRODUCTION

Rice is one of the most important food grain crops grown throughout the world as well in India and is a staple food for majority of the world's population. The crop is cultivated from 6ft below sea level to 2700 ft above sea level in Himalayas (Pathak *et al.,* 2020). Rice is cultivated under diverse ecologies, ranging from irrigated to rain-fed and upland to lowland and deep water system (Kumar *et al.,* 2014). In the year 2022-23, rice covers around 165.04 million ha of area worldwide with production is 787 million tons (Anonymous, 2022). India ranks first in area of cultivated rice followed by China and second in the production of rice following China. In India, 130.29 million tons of rice was produced from an area of 46.38 million hectares in the year 2022 with yield of 28.09 q/ha. In U.P., rice is cultivated in an area of 5.70 million ha which is highest among states in India and it holds second position in production (15.27 million tons) next only to West Bengal with yield of 26.79 q/ha (Anonymous, 2022-23). Agricultural statistics at a glance. Among diseases, fungal diseases like, blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia solani*), brown spot (*Helminthosporium oryzae*), bakanae disease or foot rot (*Gibberella fujikuroi*), sheath rot (*Sarocladium oryzae*), leaf scald (*Microdochium oryzae*), narrow leaf spot (*Cercospora* oryzae), leaf smut (*Entyloma oryzae*), udbatta disease (*Balansia oryzae*) and false smut of rice (*Ustilaginoidea virens*) *etc*., bacterial diseases such as bacterial leaf blight (*Xanthomonas oryzae* pv*. oryzae*) and bacterial leaf streak (*Xanthomonas oryzae* pv*. oryzicola*), viral disease such as rice tungro (*Rice tungro bacilliform virus* and *Rice tungro spherical virus*) are the more prevalent and destructive under Indian condition. Among all these diseases, false smut (*Ustilaginoidea virens*), is of the most emerging devastating disease causing significant damage of rice yield and quality worldwide (Abbas *et al*., 2014, Brooks *et al.,* 2010, Ashizawa *et al.,* 2011, Ladhalakshmi *et al.,* 2012 and Bashyal *et al*., 2020). False smut disease was first reported from Tirunelveli in Tamil Nadu state (Cooke, 1878). Recently, *Claviceps oryzae sativae*(Hashioka, 1971), and the new name has been given *i.e.Villosiclava virens* as the teleomorphic stage (Tanaka *et al.,* 2008). The False smut pathogen (*U. virens*) infect the plant during floweringstage where an individual healthy grains converts firstly into yellowish orange togreen velvety spores which later turns into greenish black in colour (Baite *et al.,*2014 and Tanaka *et al.,*2016). The fungus mainly attacks the stamen filaments of rice at the booting stage and grows intercellularly in host tissues (Hu *et al.,*2014 and Yong *et al.,* 2016). In general, few grains of apanicle are affected or sometimes may be several. Present-day strategies for managing the disease primarily depend on chemical fungicides such as Copper oxychloride, Carbendazim, and Captan; however, their extensive use raises serious concerns regarding environmental safety and human health (Nimse *et al*., 2025).These issues have driven scientists to investigate sustainable and environmentally friendly approaches to disease control, with particular attention on botanical extracts and nanotechnology as potential alternatives. Nanotechnology a new emerging and interesting field of science is currently applied in many areas. It has great impact on application in the field of agriculture and biotechnology. These nanoparticles have renewed a great interest towards alternative methods of prevention and control of plant diseases being widely used in the field of agriculture. Nanotechnology can be termed as the fabrication, characterization, exploration and application of nanosized (1-100nm) materials for the development of science. It deals with the study of extremely minute structures and the prefix "nano" is a Greek word which means "dwarf of miniature" (Siddqui *et al*., 2015).Nanoparticles are materials that range between 1 to 100 nanometers (nm), it's have the potential to be directly applied as foliar spray for protection against several pathogens, such as fungi, bacteria and viruses *etc*. In the era of climate change, global agricultural systems are facing numerous, unprecedented challenges. In order to achieve food security, advanced nano- technology is a tool for boosting crop production and assuring sustainability. Nanotechnology helps to improve agricultural production by increasing the efficiency of inputs and minimizing relevant losses (Shang *et al.,* 2019).

The synthesis of nanoparticles using beneficial microbes, such as *Pseudomonas fluorescens*, is an emerging and eco-friendly approach in nanobiotechnology and plant disease management. This method combines the natural bioactive properties of *P. fluorescens*—a well-known plant growth-promoting rhizobacterium (PGPR) with the unique characteristics of nanoparticles, leading to highly effective, sustainable, and targeted disease control strategies. This approach utilizes bioactive compounds such as phenols, flavonoids, terpenoids, and proteins to reduce metal ions into nanoparticles while providing natural capping and stabilization (Arya, 2010). Silver and zinc nanoparticles synthesized through green methods demonstrate broad-spectrum antifungal activity, enhanced plant defense responses, and minimal toxicity to beneficial organisms (Kim *et al*., 2007). Baker *et al*. (2017) examined nano-agroparticles for sustainable agriculture and plant disease management, highlighting the antimicrobial properties of silver, zinc oxide, and titanium dioxide nanoparticles against plant pathogens.

2. MATERIALS AND METHODS

2.1 Experimental Site

The present investigation was carried out during 2023-24 and 2024-25 in the Biocontrol Lab and laboratory of Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, 208002 (Uttar Pradesh).The following work was conducted during the experiment described as below:-

2.2 Collection of Disease Samples

The false smut samples (infected panicles bearing smutted balls) were collected in air tight polythene bags from Student's Instructional Farm (S.I.F) of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.).Diseased samples were stored at 20-25ºC (Singh 1997). The infected grains were separated from panicles and store in air tight glass vials.

2.3 Isolation and Purification of the Pathogen (*Ustilaginoidea virens*)

The false smut balls were surface sterilized by dipping in 1% sodium hypochlorite solution for 1–2 minutes followed by 70% ethanol wash for 1-2 minute and finally repeated washing (2-3 times) with sterilized distilled water. The smut balls were then dried between two sterilized filter papers. The outer portion of dark powdery mass of spores was teased out into small pieces which were then placed over the media and incubated at 27±2°C. To avoid bacterial contamination streptomycin @ 100 ppm was added in the medium at luke warm stage before pouring into Petri plates. Since it is a slow growing fungus, the culture was often contaminated during incubation with fast growing saprophytes, so hyphal tip method was used for sub culturing of the fungus in PSA (Potato Sucrose Agar) slants or Petri plates in order to get the pure culture of the fungus. The culture was periodically transferred to fresh media during the study. All the work of isolation and transfer of fungal inoculum were carried out in laminar airflow to avoid contamination.

2.4 Maintenance of the Cultures

The pure culture was maintained on potato sucrose agar (PSA) medium at 27°Cin BOD incubator throughout the studies. Whenever the need arises, the fungus was transferred to fresh slants. The cultures were stored in refrigerator at 4^0C for further studies.

2.5 Bio-Efficacy Test

Silver, zinc oxide and Silicon dioxide nanoparticles 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 (Schmitz, 1930). The different concentrations of nanoparticles (100, 200, 300 and 500 ppm) were tested and radial growth of *Ustilaginoidea virens* was measured in mm. To compare the efficacy of nano particles against pathogen in *in-vitro* conditions, the per cent growth inhibition over control was calculated by (Vincent, 1947).

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

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

2.6 Treatment Details

T_1= Synthesized Ag NPs from *P. fluorescens* @ 100ppm, T_2= Synthesized Ag NPs from *P. fluorescens* @ 200ppm, T_3= Synthesized Ag NPs from *P. fluorescens* @ 300ppm, T_4= Synthesized Ag NPs from *P. fluorescens* @ 500ppm, T_5= Synthesized ZnO NPs from *P. fluorescens* @ 100ppm, T_6= Synthesized ZnO NPs from *P. fluorescens* @ 200ppm, T_7= Synthesized ZnO NPs from *P. fluorescens* @ 300ppm, T_8= Synthesized ZnO NPs from *P. fluorescens* @ 500ppm, T_9= Synthesized SiO_2 NPs from *P. fluorescens* @ 100ppm, T_{10}= Synthesized SiO_2 NPs from *P. fluorescens* @ 200ppm, T_{11}= Synthesized SiO_2 NPs from *P. fluorescens* @ 300ppm, T_{12}= Synthesized SiO_2 NPs from *P. fluorescens* @ 500ppm, T_{13}=Standard fungicide copper oxychloride @ 2500ppm and, T_{14}= Control (untreated).

2.7 Statistical Analysis of Data

The experiment was conducted incompletely randomized design (CRD) *in vitro* under lab conditions. 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:

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

3. RESULTS AND DISCUSSION

3.1 Efficacy of Synthesized Silver (Ag), Zinc Oxide (ZnO) and Silicon Dioxide (SiO_2) Nanoparticles (NPs) from *Pseudomonas fluorescens* and Fungicide at Different Concentrations on Radial Mycelial Growth of *Ustilaginoidea virens* (*in vitro*)

The efficacy of different synthesized nanoparticles (silver, zinc oxide and silicon dioxide) at different concentrations (100, 200, 300 and 500 ppm) against pathogen (*U.* virens) was determined *in vitro* through Poisoned Food Technique (Schmitz, 1930). The findings shown in table-1 indicated that notable differences were recorded across all treatments on the 5th, 7th, and 10th days of incubation. The untreated control (T14) exhibited the greatest radial mycelial growth of 22.57 mm, 31.50 mm, and 41.24 mm on the 5th, 7th, and 10th day, respectively. Conversely, the minimum mycelial growth was recorded in the treatment containing silver nanoparticles (Ag NPs) at 300 ppm (T3), exhibiting growth measurements of 6.32 mm, 13.35 mm, and 18.57 mm on the respective observation days. This treatment also achieved the maximum percentage of inhibition over control (54.97%), demonstrating the most potent antifungal activity against *U. virens*. of the ZnO nanoparticle treatments, ZnO NPs at 300 ppm (T7) exhibited mycelial growth of 7.52 mm, 13.64 mm, and 19.23 mm on the 5th, 7th, and 10th day, respectively, showing a 53.37% inhibition rate, which was second only to Ag NPs at 300 ppm. Silver nanoparticles at 200 ppm (T2) and ZnO NPs at 200 ppm (T6) showed significant effectiveness with inhibition rates of 51.96% and 50.41%, respectively. Silicon dioxide nanoparticles (SiO_2 NPs) showed comparatively lower effectiveness, with SiO_2 NPs at 300 ppm (T11) leading to 51.29% inhibition, followed by SiO_2 NPs at 200 ppm (T10) showing 39.33% inhibition, whereas the least inhibition (37.15%) occurred at 500 ppm (T12). The standard fungicide, copper oxychloride @ 2500 ppm (T13), inhibited mycelial growth by 47.74%,

Table 1. Efficacy of synthesized Silver (Ag), Zinc oxide (ZnO) and Silicon dioxide (SiO_2) Nanoparticles (NPs) from *Pseudomonas fluorescens* and fungicide at different concentrations on radial mycelial growth of *Ustilaginoidea virens* (*in vitro*)

Treatments	Radial mycelial growth (mm)			Percent inhibition over control (untreated) after 10 days
	5th days	7th days	10th days	
T_1	10.92	15.93	22.31	45.90
T_2	8.15	13.92	19.81	51.96
T_3	6.32	13.35	18.57	54.97
T_4	10.55	15.54	21.76	47.24
T_5	11.82	16.63	23.55	42.89
T_6	8.94	14.32	20.45	50.41
T_7	7.52	13.64	19.23	53.37
T_8	11.52	16.23	22.92	44.42
T_9	12.11	17.05	24.32	41.02
T_{10}	12.63	17.41	25.02	39.33
T_{11}	9.72	14.74	20.09	51.29
T_{12}	13.21	17.81	25.92	37.15
T_{13}	10.25	15.12	21.55	47.74
T_{14}	22.57	31.5	41.24	0
C.D. at 5%	0.488	0.630	1.314	-
SE(m)	0.168	0.216	0.451	-
C.V.	2.601	2.249	3.348	-

which was lower than that of Ag NPs @ 300 ppm and ZnO NPs @ 300 ppm but still significantly higher than most other treatments. Statistical analysis revealed that all treatments differed significantly from the control at the 5% level of significance. Overall, the results that the silver nanoparticles (Ag NPs) @ 300 ppm showed the highest inhibitory effect on the radial mycelial growth of *Ustilaginoidea virens*, followed closely by ZnO NPs @ 300 ppm. The result was supported by the experimental findings of Kim *et al*. (2012) who reported significant mycelial growth suppression using antifungal effects of silver nanoparticles (Ag NPs) against plant pathogenic fungi, using concentrations of 10, 25, 50, and 100 ppm. Susanta Banik (2017) also observed greater activity of copper nanoparticles compared to controls, but less than Ag NPs, consistent with previous research on CuNP-induced growth suppression in plant pathogens. These findings indicate both nanoparticles are statistically effective, but Ag NPs are superior under tested conditions.

4. CONCLUSION

The present research highlights that the nanoparticles synthesized from *Pseudomonas fluorescens* exhibit notable antifungal activity against *Ustilaginoidea virens*, which is responsible for false smut disease in rice. Among the treatments evaluated, silver nanoparticles (Ag NPs) at 300 ppm demonstrated the greatest inhibition of mycelial growth, with zinc oxide nanoparticles (ZnO NPs) at the same concentration following closely. Silicon dioxide nanoparticles (SiO_2 NPs) exhibited moderate antifungal effects. The results indicate that biosynthesized Ag and ZnO nanoparticles can act as efficient, environmentally friendly substitutes for traditional fungicides in controlling false smut disease in rice, encouraging sustainable and safe agricultural practices.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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