

ANTIFUNGAL POTENTIAL OF COMMERCIAL SILVER NANOPARTICLES AGAINST RICE BLAST PATHOGEN *MAGNAPORTHE ORYZAE*

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Abstract

The rice blast caused by *Magnaporthe oryzae* is one of the major and recurrent threats to sustainable rice production in Bangladesh. To mitigate this problem, the present study was undertaken with the aim to investigate the inhibitory effects of commercially available silver nanoparticles (Ag-NPs) on the growth and development of *Magnaporthe oryzae*, and control of rice blast disease. *In vitro* experiments demonstrated that Ag-NPs significantly suppressed the radial growth of fungal mycelium, with the highest inhibition (83.54%) at a concentration of 250 ppm, while Trooper 75 WP at 400 ppm completely inhibited the fungal mycelial growth. The production of conidia and colony formation were also reduced significantly by Ag-NPs treatment. Furthermore, Ag-NPs exhibited a substantial inhibitory effect on the germination of *M. oryzae* conidia, with complete inhibition observed at concentrations between 200 and 250 ppm. Comparative analyses revealed that the fungicidal effect of Ag-NPs was superior to silver nitrate (AgNO₃), suggesting the augmented activity of the nano properties. The sensitivity of *M. oryzae* to Ag-NPs was determined by calculating EC₅₀ and EC₉₅ values, which indicated a mean EC₅₀ value of 131.5 ppm and a mean EC₉₅ value of 276 ppm. Additionally, *in vivo* experiments showed that preventive and curative treatments with Ag-NPs significantly reduced disease incidence and severity in rice plants infected with *M. oryzae*, with efficacy comparable to that of the fungicide Trooper 75 WP. At higher concentrations of Ag-NPs, preventive treatment was found to be more effective than curative treatment in disease control. These findings highlighted the potential of Ag-NPs as an effective alternative for the management of rice blast.

Keywords: Mycelial growth, conidial germination, EC₅₀, EC₉₅, blast incidence, blast severity.

Introduction

Rice (*Oryza sativa* L.) is the staple food in many countries, including Bangladesh. It plays a vital role in the livelihood of the people of Bangladesh, providing nearly 48% of rural

employment, approximately two-thirds of the total calorie supply, and roughly one-half of the total protein intake of an average person in the country (BRKB 2023). The rice sector contributes one-half of the agricultural GDP and one-sixth of the national income

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in Bangladesh (BBS, 2022). In 2021, rice was grown in 11.70 million hectares of land, yielding 56.94 million tons (FAO, 2022), making it the fourth-highest production in the world. Despite remarkable success in rice production, many formidable challenges still remain. Rice is frequently attacked by a number of insect pests and diseases, leading to significant crop losses (Hossain *et al.*, 2014; Prasher and Sharma, 2022). Farmers lose approximately 37% of their rice crop each year due to pests and diseases (IRRI RKB, 2023). Blast caused by the fungus *Magnaporthe oryzae* Cavara, is considered the most dangerous rice disease and the primary factor limiting rice yield worldwide (Mahmud *et al.*, 2021). This pathogen infects all developmental stages and organs of the rice plant (Le *et al.*, 2010). Outbreaks of the disease are a recurrent problem, and farmers struggle to control the disease in conducive environments. Bangladesh has experienced with several epidemics of blast disease since 1980, and most popular cultivars, both in rainfed and irrigated lowlands, were highly susceptible to blast (Khan *et al.*, 2014). Current methods to combat the disease include resistant cultivars, fungicides, and cultural practices. However, resistance to rice blasts is not very common, and breeding for long-term blast resistance remains a serious challenge due to the emergence of virulent fungal races (Khan *et al.*, 2016). Consequently, fungicides are routinely used to suppress blast disease. However, they are becoming less acceptable due to the possibility of fungicide resistance in the *M. oryzae* population and public concerns about the potential harmful effects of fungicide residues to human health and the environment (Coca *et al.*, 2006). Therefore,

alternative agents are being explored to mitigate the impact of the disease.

Nanoparticles have the potential in combating plant diseases (Beer *et al.*, 2012; Elamawi and Al-Harbi, 2014). Among various nanoparticles, silver nanoparticles (Ag-NPs) are increasingly being used as antimicrobial agents due to their high toxicity to different microorganisms (Min *et al.*, 2005; Jo *et al.*, 2009; Nazeruddin *et al.*, 2014). This suggests the need to assess the biological effects of Ag-NPs on the rice blast pathogen. Previous research has evaluated the antifungal potential of self-assembled Ag-NPs, which have shown inhibitory effects against spore-producing fungal pathogens in rice, including *Bipolaris sorokiniana* and *M. oryzae* (Young *et al.*, 2009; Lamsal *et al.*, 2011; Elamawi *et al.*, 2013; Mishra *et al.*, 2014; Akter, 2019). However, the key challenge in using self-assembled nanoparticles appears to be maintaining a stable and well-defined nanostructure with potent biocidal properties. Liu *et al.* (2021) observed that the particle size distribution and stability of self-assembled nanoparticles obtained from various preparation procedures varied; even when properties such as particle size and crystal phase are quite similar. Different production conditions may result in nanoparticles with variable properties (Tsuzuki, 2009). Moreover, there are limitations to scaling up the large-scale synthesis of self-assembled nanoparticles. In this particular scenario, the use of commercially manufactured nanoparticles with clearly defined structures and consistent activities can be helpful for disease control, as technological advances have made their production more economical. Hence, a study was undertaken to investigate the inhibitory

effects of commercially synthesized Ag-NPs against rice blast caused by *M. oryzae*.

Materials and Methods

Pathogen and plant

A virulent isolate BD576p of *M. oryzae*, which is a differential blast isolate, was used as the test pathogen throughout the study (Khan *et al.*, 2016). The blast-susceptible check variety US2, was employed as the host. Both the blast isolate and the rice variety US2 seeds were obtained from the Plant Pathology Division of the Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

Nanoparticles and fungicide

Commercially synthesized silver nanoparticles (Ag-NPs) procured from CD-Bioparticles (Cat. No. BSP-50-DLS) were used in the study. According to the manufacturer, the particle sizes and shapes were less than 50 nanometers and spherical, respectively. For experimental purposes, nanoparticles of specific concentrations were freshly prepared by dissolving them in sterilized distilled water. Confirmation of Ag-NP formation and plasmonic resonance was conducted by measuring the absorbance of the nanoparticles using UV-Vis spectroscopy (Shimadzu Scientific, Tokyo, Japan). A commercial formulation of the tricyclazole group fungicide, Trooper 75 WP (Auto Crop Care Bangladesh Ltd.), was used as a positive control.

In vitro bioassay of nanoparticles for antifungal activity

An *in vitro* bioassay was conducted to examine the antifungal activity of Ag-NPs against the rice blast fungus *M. oryzae* at concentrations

of 25, 50, 100, 150, 200, and 250 ppm. A stock solution of 500 ppm Ag-NPs was prepared, followed by the preparation of a series of working concentrations obtained through serial dilution with sterilized distilled water. Potato Sucrose Agar (PSA) media amended with each concentration of Ag-NPs was prepared in triplicate. Three PSA plates, each mixed with the reference fungicide Trooper 75 WP at a concentration of 400 ppm, were used as the positive control, while three PSA plates prepared without nanoparticles served as the negative control (Control). Mycelial disks (6 mm in diameter) excised from an actively growing edge of pure cultures of *M. oryzae* were transferred to each of the control and nanoparticle-treated plates. Plates inoculated with *M. oryzae* were incubated at 28°C. The radial growth of each culture was measured at 48-hour intervals, and the evaluation continued until the colony in the control plates reached the rim of Petri plates. The percent growth inhibition (PI) of *M. oryzae* due to Ag-NPs/Trooper compared to the control was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(X - Y)}{Y} \times 100$$

Where,

X = Mycelial growth of the pathogen in the control plates.

Y = Mycelial growth of the pathogen in plates treated with Ag-NPs/Trooper.

The number of conidia produced by each *M. oryzae* culture was estimated by adding sterile distilled water to the culture plates and then harvesting the conidia by scraping them. The resulting conidial suspension was examined under a compound microscope, and the conidia

were counted using a haemocytometer. The number of conidia per mL of solution was then calculated. The experiment was repeated twice.

The antifungal activity of nanoparticles was also evaluated based on new colony formation *in vitro*. Conidia of *M. oryzae* were collected from *M. oryzae* cultures grown on PSA medium and suspended in sterilized distilled water. The conidial suspension was diluted with sterile distilled water to a final concentration of 10^5 conidia/mL and incubated overnight at 25°C in the dark. An aliquot of 500 μ l of the conidial suspension was mixed with an equal amount of Ag-NPs to achieve a final volume of 1 ml. Additionally, a conidial suspension was prepared using sterile deionized water as a control, and another one was mixed with the reference fungicide Trooper 75 WP at a concentration of 400 ppm. All treatments were prepared in triplicates and incubated at 25°C for seven days. Aliquots of 25 μ l from each dilution were spread on PSA plates and incubated at 25°C (FOC 200IL Illuminated Cooled Incubator, Velp Scientifica, Italy) after three days, the number of colonies formed on the plates was counted. This experiment was repeated twice.

Inhibition of conidial germination

Ag-NPs at concentrations of 25, 50, 100, 150, 200, and 250 ppm, as well as Trooper 75 WP at a concentration of 400 ppm, were freshly prepared as described above. Conidia of *M. oryzae* were collected from cultures grown on PSA medium and suspended in sterilized distilled water. The conidial suspension was then diluted with sterile distilled water to achieve a final concentration of 10^5 conidia/ml. The conidial suspension was mixed with

the Ag-NPs solution at a 1:1 ratio, resulting in a final volume of 2 ml in a test tube. Additionally, a conidial suspension was prepared using sterile distilled water as a control, and another one was mixed with Trooper at a 1:1 ratio as a positive control. All treatments were prepared in three replications and incubated at 25°C for 24 hours in the dark. A total of 100 conidia from each replicate were examined under a Zeiss Primo Star microscope (Carl Zeiss AG, Germany) at 100 \times magnification. The number of germinated, ungerminated, and lysed conidia, as well as developmental differences in the appressoria, were evaluated. This experiment was repeated twice.

Comparing the antifungal activity of Ag-NPs and silver nitrate *in vitro*

The inhibitory effect of silver NP and AgNO₃ on fungal growth was examined by measuring hyphal growth and sporulation. The PSA media separately supplemented with 250 ppm of Ag-NPs and same concentration of AgNO₃ were prepared in triplicate. The PSA plates prepared without amendment were taken as controls. Mycelial disks (6 mm in diameter) excised from an actively growing edge of pure cultures of *M. oryzae* was obtained and transferred into each plate of both control and nanoparticles treated plates. Plates inoculated with *M. oryzae* were incubated at 28°C. After colony formation, the radial growth of each culture was measured at every 48 hours interval and the evaluation continued until the colony in the control plates reached the rim of the plates. The percent growth inhibition (PI) of *M. oryzae* due to Ag-NPs over the control was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(X - Y)}{Y} \times 100$$

Where,

X = Mycelial growth of the pathogen in the control plates.

Y = Mycelial growth of the pathogen in the presence of treatments (Ag-NPs/AgNO₃/Trooper). This experiment was repeated twice.

Determination of EC₅₀ and EC₉₅ values and Relative Toxicity Index (RTI) of Ag-NPs

A stock solution of 400 ppm Ag-NPs was prepared, followed by the preparation of a series of working concentrations obtained through serial dilution with sterilized distilled water. Similarly, a stock solution of 400 ppm Trooper 75 WP was prepared and then diluted to generate a series of working concentrations. PSA medium was supplemented with each of these concentrations of Ag-NPs and Trooper, and the mixtures were plated in triplicate. Mycelial disks (6 mm in diameter) were obtained from actively growing pure cultures of *M. oryzae* and transferred to both the control and Ag-NPs-treated plates. The cultures were arranged in a completely randomized design (CRD) and incubated at 28°C. The radial growth of each culture was measured at 48-hour intervals, and the evaluation continued until the colonies in the control plates reached the rim of the plates. The relative growth of mycelia on each plate was calculated by comparing the diameters of colonies treated with Ag-NPs or Trooper to the non-amended control, following the same method as described above. The collected data were used to plot a dose-response curve. The effective concentration for 50% (EC₅₀) and 95% (EC₉₅) inhibition of mycelial growth for Ag-NPs and the reference fungicide against *M. oryzae* was determined using a

logistic regression model (Li *et al.*, 2015). The experiments were conducted three times under similar conditions, and the mean values of EC₅₀ and EC₉₅ were calculated. The relative toxicity index (RTI) of Ag-NPs compared to Trooper 75 WP was calculated using the following formula:

$$RTI = \frac{\text{Mean EC50 values of Trooper}}{\text{Mean EC50 values of Ag-NPs}}$$

Control of rice blast disease by Ag-NPs

The preventive and curative effects of Ag-NPs on rice blast disease were investigated, with the fungicide Trooper 75 WP used as the positive control and water as the negative control. The experiment was conducted in plastic pots (15 cm × 15 cm) within a nethouse, with 25 pots prepared for each experimental group and 5 pots allocated for each treatment. Pre-germinated seeds (30 seeds/pot) of the rice variety US2 were sown in pots filled with sterile field soil. The seedlings were grown under natural light and temperature conditions.

For the preventive treatment, 21-day-old rice seedlings were sprayed with Ag-NPs at two concentrations: EC₅₀ and EC₉₅. Seedlings treated with Trooper 75 WP at two concentrations: EC₅₀ and EC₉₅, served as the positive controls. Seedlings sprayed with sterilized distilled water were used as the negative control. Conidia of the blast fungus, cultured on PSA media, were harvested by flooding the culture plates with sterile water containing 0.5% Tween 20 and filtered through nylon meshes. The spore concentration was adjusted to 1 × 10⁵ spores/mL. After 24 hours of treatment with Ag-NPs and Trooper 75 WP, the rice seedlings were inoculated with *M. oryzae* by spraying the spore suspension.

Another experiment with a similar set of treatments was prepared for the curative treatment, where Ag-NPs and fungicide treatments were administered to seedlings 24 hours post-inoculation with *M. oryzae*. After inoculation, the plants were kept in a humid chamber overnight at 28 to 30°C. The seedlings were then returned to the net house. Seven days after pathogen inoculation, ten seedlings were randomly selected per pot to assess the percentage of leaf blast incidence and severity. The number of symptomatic leaves over the total number of leaves was recorded to determine the blast incidence. Disease severity was measured for each plant by recording the percentage of total plant leaf surface showing symptoms, ranging from 0 (no symptoms) to 100 (most severe with necrotic symptoms).

Statistical analysis

The data were analyzed using the Microsoft Excel program and MSTAT8. One-way ANOVA analysis was performed, and differences among means were determined through Tukey's multiple comparison tests ($p < 0.05$).

Results

Characterization of Ag-NPs

The UV-Visible Spectrum was measured to confirm and characterize the nanoparticle properties of the Ag-NPs. The absorption spectra of Ag-NPs exhibited single-band absorption with peak maximum (Surface Plasmon Resonance, SPR) at 413 nm (Fig. 1).

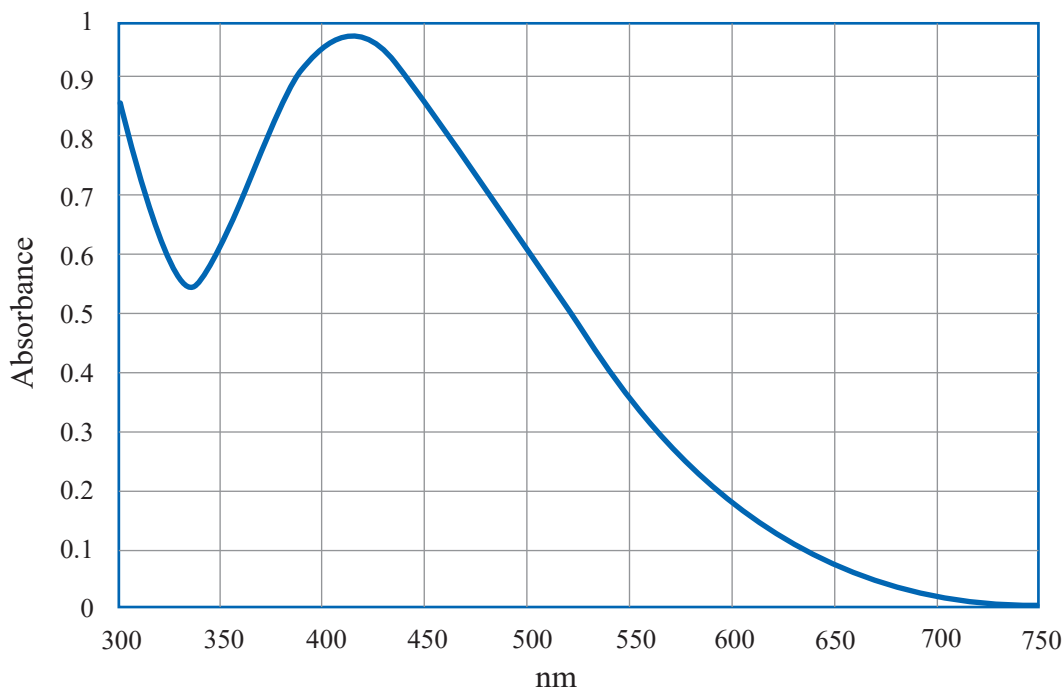


Fig. 1. The UV-Visible absorption spectrum of Ag-NPs.

Effect of nanoparticles on mycelial growth and colony formation of *M. oryzae*

The maximum radial growth of the fungal mycelium (82.78 mm) was observed in culture plates where no nanoparticle and fungicide were added (Control plates) (Table 1). However, culture plates amended with low to high concentrations (25-250 ppm) of Ag-NPs showed significantly reduced mycelial growth of *M. oryzae* compared to control plates. The percent inhibition of mycelial growth by Ag-NPs ranged from 22.02 to 83.54%, where the lowest and highest mycelial growth inhibition of *M. oryzae* was found in culture plates treated with 25 and 250 ppm of Ag-NPs, respectively. Similarly, plates treated with 400 ppm of fungicide Trooper 75 WP completely arrested the fungal mycelial growth, leading

to 100% inhibition of the radial growth of *M. oryzae*. The number of conidia produced by *M. oryzae* was significantly decreased with all concentrations of Ag-NPs applications compared to the untreated control. The production of conidia was reduced by 99.68% in culture media treated with Trooper 75 WP and by 91.20% in media treated with Ag-NPs at 250 ppm concentrations. However, there was no significant difference between the fungicide and the highest concentration of Ag-NPs. Effects of Ag-NPs on the new colony formation of *M. oryzae* were also assayed. Results revealed that colony formation was significantly inhibited by Ag-NPs treatments compared to untreated controls. Among the Ag-NPs concentrations, maximum inhibition (96.42%) was observed at 250 ppm

Table 1. Effect of silver nanoparticles (Ag-NPs) on mycelial growth and colony forming units of *Magnaporthe oryzae* in plate culture assay

Treatments	Concentrations ($\mu\text{g/ml}$)	Mycelial growth (mm)	No. of spores/ml ($\times 10^4$)	Colony formation/plate
Ag-NPs	25	64.54 \pm 1.89b* (22.02%)**	7.67 \pm 0.52b (25.02%)	27.00 \pm 1.00b (25.68%)
Ag-NPs	50	58.03 \pm 1.50c (29.89%)	4.93 \pm 0.18c (51.79%)	23.67 \pm 0.84c (34.84%)
Ag-NPs	100	46.11 \pm 1.34d (41.96%)	3.27 \pm 0.17c (68.07%)	19.00 \pm 0.58d (47.70%)
Ag-NPs	150	37.28 \pm 1.11e (54.96%)	2.03 \pm 0.12d (80.13%)	12.00 \pm 0.33e (66.97%)
Ag-NPs	200	24.65 \pm 1.19f (70.22%)	1.16 \pm 0.09e (88.66%)	4.33 \pm 0.27f (82.33%)
Ag-NPs	250	13.62 \pm 0.81g (83.54%)	0.90 \pm 0.06f (91.20%)	1.67 \pm 0.24g (96.42%)
Trooper 75 WP	400	0.00 \pm 0.00h (100%)	0.03 \pm 0.03f (99.68%)	0.00 \pm 0.00g (100%)
Control (untreated)	-	82.78 \pm 3.49a	10.23 \pm 0.85a	36.33 \pm 1.00a

*The values represent means obtained from three replications ($n=3$). Different letters in the same column indicate a significant difference ($P=0.05$)

** Value in the parenthesis indicates percent decrease over the control

concentrations, which was statistically similar to treatment with Trooper 75WP (100%). These results suggest the potential of Ag-NPs in suppressing rice blast fungus *in vitro*.

Inhibition of conidial germination

On conidial suspension, the inhibition of germination of *M. oryzae* conidia by Ag-NPs was evaluated. After 24 hours of incubation, each treatment significantly decreased conidial germination compared to control (Table 2). The germination rate of conidia in the control suspension (where conidia were dispersed only in water) was 70.97%. Among the germinated conidia, 10.50% produced appressoria, while none exhibited lysis. In contrast, germination of conidia in suspensions treated with varying concentrations of Ag-NPs ranged from 0.0 to 26.23%. Complete inhibition of conidial germination was observed between 200 and 250 ppm of Ag-NPs in the suspension. There had no evidence of appressoria formation in conidia exposed to any concentration of Ag-NPs. However, 5.83 to 25.33% of the ungerminated conidia in the conidial suspension treated with 100

to 250 ppm Ag-NPs exhibited lysis, with the highest lysis occurring at 250 ppm of Ag-NPs. Trooper 75 WP at 400 ppm also completely halted conidia germination, but no conidial lysis was observed.

Comparative study on the effectiveness of AgNO₃ and Ag-NPs against the mycelial growth of *M. oryzae*

A comparative study was conducted to determine whether the observed inhibitory effect of Ag-NPs was due to its nano properties or active ingredient. Culture media were treated with AgNO₃ and Ag-NPs and *M. oryzae* were inoculated. Both AgNO₃ and Ag-NPs showed significantly lower mycelial growth of the pathogen than the control plates, suggesting that the active ingredient Ag itself is fungi toxic (Fig. 2). While comparing the fungi toxicity between AgNO₃ and Ag-Nano, significantly the lowest mycelial growth was observed in plates treated with Ag-NPs (4.23 mm) compared to AgNO₃ treatment (28.75 mm). This suggests that the fungicidal effect of active ingredient Ag was augmented by the activities of Nano properties.

Table 2. Effect of silver nanoparticles (Ag-NPs) on germination and lysis of conidia of *Magnaporthe oryzae* in plate culture assay

Treatment	Concentrations (ppm)	% Conidia germination*	% Appressoria formation	% Lysed conidia
Ag-NPs	25	26.23 ± 1.16b	0.00 ± 00b	0.00 ± 0.00e
Ag-NPs	50	11.23 ± 0.55c	0.00 ± 00b	0.00 ± 0.00e
Ag-NPs	100	5.80 ± 0.89d	0.00 ± 00b	5.83 ± 0.33d
Ag-NPs	150	2.13 ± 1.07de	0.00 ± 00b	12.83 ± 0.60c
Ag-NPs	200	0.00 ± 0.00e	0.00 ± 00b	18.17 ± 1.73b
Ag-NPs	250	0.00 ± 0.00e	0.00 ± 00b	25.33±1.30a
Trooper 75 WP	400	0.00 ± 0.00e	0.00 ± 00b	0.00 ± 0.00e
Control	-	70.97 ± 1.58a	10.50 ± 0.76a	0.00 ± 0.00e

*Different letters in the same column indicate a significant difference ($P=0.05$)

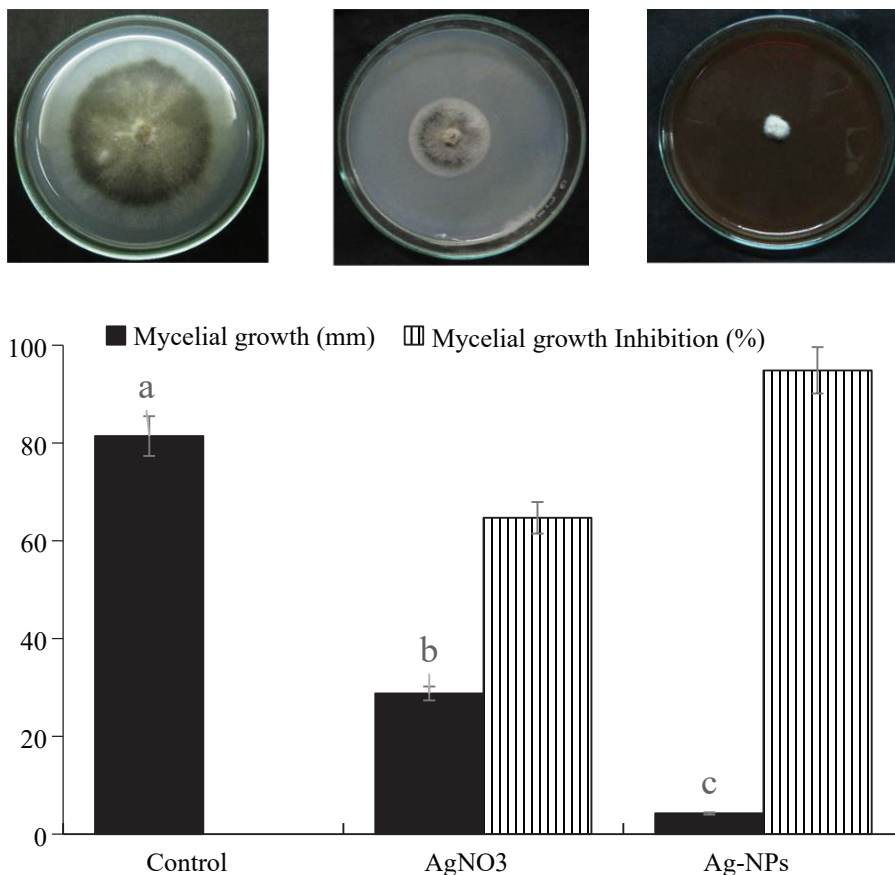


Fig. 2. Effect of silver nitrate (AgNO₃) and silver nanoparticle (Ag-NPs) particles on mycelial growth of *Magneportha oryzae*. *M. oryzae* was grown in potato sucrose agar (PSA) supplemented with 250 ppm of Ag-NPs and AgNO₃ for 12 days. The radial growth of *M. oryzae* was measured, and the percent growth inhibition by Ag-NPs and AgNO₃ was calculated.

Assessment of EC₅₀ and EC₉₅

Sensitivity was assessed using discriminatory doses and concentrations for 50% and 95% mycelial inhibition (EC₅₀ and EC₉₅) (Table 3). The EC₅₀ value of Ag-NPs obtained in three experiments ranged from 128.5-136.3 ppm and the mean EC₅₀ value was estimated to be 131.5 ppm (Table 3). Similarly, the EC₉₅ value of Ag-NPs in these experiments ranged from 269.7 to 280.2 ppm, and the mean value was calculated as 276 ppm. On the other hand, the mean EC₅₀ and EC₉₅ values for Trooper 75WP

were 148.20 and 227.23 ppm, respectively. The Relative toxicity index (RTI) value of Ag-NPs, using Trooper 75WP as the standard, was 1.13.

Effects of Ag-NPs on the control of rice leaf blast

Results illustrated in Fig. 3 showed that the non-treated control plants had the highest leaf blast incidence and severity, while all treated plants receiving preventive and curative treatments with Ag-NPs and Trooper showed

Table 3. Effective concentration of silver nitrate nanoparticles (Ag-NPs) and the reference fungicide Trooper 75WP for 50% (EC₅₀) and 95% (EC₉₅) mycelial growth reduction of *Magnaporthe oryzae*

Treatment	Concentration (ppm)*		Relative Toxicity Index (RTI)**
	Mean EC ₅₀ value	Mean EC ₉₅ value	
Ag-NPs	131.50	276.00	1.13
Trooper 75WP	148.20	290.80	-

*EC₅₀ and EC₉₅ were calculated based on the reduction of mycelial growth over untreated control.

**Relative Toxicity Index (RTI) of Ag-NPs was calculated based on the EC₅₀ values of Ag-NPs and the EC₅₀ value of the reference fungicide Trooper 75WP as described in Materials and Methods

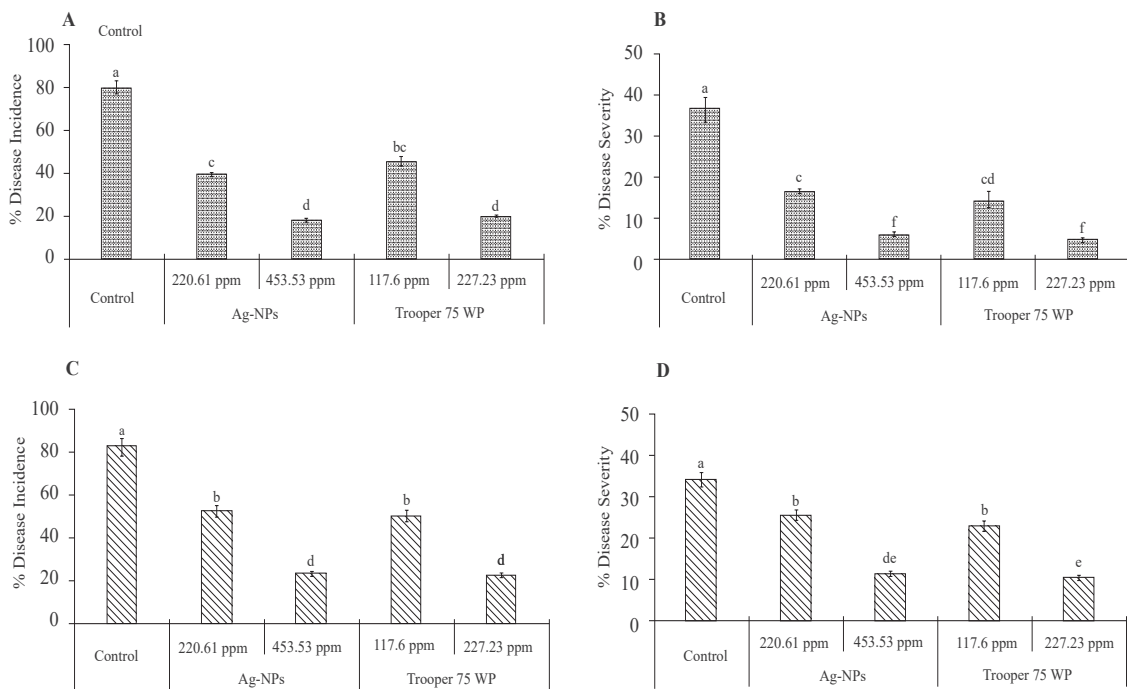


Fig. 3. Effect of preventive and curative application of silver nanoparticles (Ag-NPs) and fungicide Trooper 75 WP on the suppression of rice leaf blast. (A & B) and (C & D) show the percent blast incidence and disease severity on rice seedlings treated with Ag-NPs and the fungicide Trooper 75 WP at EC₅₀ and EC₉₅ concentrations for preventive and curative purposes.

lower disease reactions. The average disease incidence and severity observed in control plants were 79.72 to 82.32% and 34.03 to 36.56 %, respectively (Fig. 3A and 3C). In the preventive experiment, the disease incidence and severity in rice plants sprayed

with Ag-NPs were 17.58 to 39.14% and 5.84 to 16.46%, respectively (Fig. 3A and 3B). In this experiment, the Ag-NPs treatment caused a 43.56 to 75.38% reduction in disease incidence and a 54.98 to 84.03% reduction in disease severity (Fig. 3A and 3B). Regarding

curative effects, the incidence and severity of the disease in rice plants sprayed with Ag-NPs were 39.14 to 52.41% and 11.23 to 25.42%, respectively (Fig. 3C and 3D). Ag-NPs treatment resulted in a 36.33 to 50.90% reduction in disease incidence and a 25.30 to 54.98% reduction in disease severity. In both preventive and curative experiments, the highest reduction in disease was observed in treatments with EC_{95} concentration of Ag-NPs, with preventive treatment proving more effective than curative treatment. On the other hand, blast incidence and severity on rice plants sprayed with Trooper 75 WP did not differ significantly from Ag-NPs in both preventative and curative trials. In the preventive experiment trial, the fungicidal treatment resulted in a 43.56 to 75.38% reduction in disease incidence and a 57.84 to 91.60% reduction in disease severity. However, fungicide applied as curative treatment reduced the blast incidence and severity by 38.97 to 72.64 5% and 32.91 to 69.76 %, respectively. Similar to Ag-NPs, interventions with EC_{95} concentrations of Trooper 75 WP demonstrated the maximum disease reduction in both experiments. These show that applying Ag-NPs as a preventive or curative treatment is as effective as Trooper 75 WP in controlling leaf blast disease.

Discussion

Ag-NPs were tested for antifungal activity against *M. oryzae* in both *in vitro* and inoculation tests on rice plants. The findings of this investigation demonstrate that Ag-NPs have a strong inhibitory effect on *M. oryzae* fungal growth and colony formation. Besides, *M. oryzae* conidia treated with Ag-NPs failed to germinate and produce appressorium. In

many cases, the conidial walls were severely destroyed due to Ag-NPs treatment, a process known as plasmolysis. Previous research also revealed the inhibitory effects of Ag-NPs against *M. oryzae* (Young *et al.* 2009, Elamawi *et al.* 2013; Akter 2019). Ag-NPs are highly reactive because they release nanometer-sized Ag^+ ions (Morones *et al.*, 2005), which effectively penetrate microbial cells (Samuel and Guggenbichler, 2004). Ag-NPs continue to have an effect on transport processes, in particular, ion efflux (Morones *et al.* 2005). The ion efflux dysfunction can result in the fast accumulation of silver ions, disrupting cellular activities such as metabolism and respiration, by interacting with molecules (Elamawi *et al.*, 2013). Ag^+ ions are also known to generate reactive oxygen species (ROS) through their reactivity with oxygen, which are harmful to cells and cause damage to proteins, lipids, and nucleic acids (Hwang *et al.*, 2008). Therefore, the lysis of *M. oryzae* conidia induced by Ag-NPs may not be solely attributable to the disintegration of the hyphal wall, but rather to oxidative damage and diverse cellular effects of Ag^+ on conidia.

EC_{50} and EC_{95} (the effective concentrations to cause inhibitions by 50 and 95%, respectively) are commonly used to express the fungi toxic potency of any chemical control agent (Li *et al.* 2015). Different methods, such as linear regression of mycelial growth inhibition vs. logarithmic concentration, are commonly used to calculate EC_{50} and EC_{95} values (Chen *et al.* 2013). In this investigation, the EC_{50} and EC_{95} values for Ag-NPs against *M. oryzae* were slightly lower than those for the fungicide Trooper 75WP. Furthermore, the RTI value was somewhat slightly higher than one. Based on these EC_{50} , EC_{95} , and RTI values, Ag-NPs

appeared to be as effective as Trooper 75 WP in suppressing the growth of *M. oryzae*. The incidence and severity of leaf blast disease significantly decreased when Ag-NPs were applied either before or after inoculation at EC₅₀ and EC₉₅ values. The disease-suppressive effect of Ag-NPs was comparable to that of the fungicide Trooper 75 WP. Foliar diseases caused by *M. oryzae* are usually transmitted by its asexual conidia (Khan *et al.* 2016). Germ tubes are formed when the conidia adhere to a plant surface and begin to multiply (Tucker and Talbot, 2001). Conidia germinate, and the resultant germ tubes penetrate plant surfaces within 24 hours at high humidity (>100% relative humidity) and warm temperature (25°C) (Howard and Ferrari 1989). A 24-hour time lag between inoculation and the onset of Ag-NPs' antifungal activity supports the notion that direct contact between Ag⁺ and spores or germ tubes is essential to prevent disease progression (Young *et al.*, 2009). The observed direct effect of Ag-NPs on the mycelial growth and germination of conidia of *M. oryzae* suggests the possible mechanism of the antifungal activity of Ag-NPs. At seven days post-inoculation, the antifungal efficacy of Ag-NPs was still evident, indicating that Ag-NPs may have entered the plant cell wall to prevent the spread of the disease. No signs of phytotoxicity were observed in Ag-NPs-treated rice plants. Namasivayam and Chitrakala (2011) have reported that Ag-NPs have non-toxic or weak phyto and ecotoxic effects on plant growth, seedling emergence, soil properties, microflora, and fauna in the phyllosphere. Therefore, Ag-NPs might successfully combat rice blast disease and protect against harmful infections.

Conclusion

In conclusion, our study provides compelling evidence for the effectiveness of Ag-NPs in combatting rice blast disease caused by *M. oryzae*. The direct impact of Ag-NPs on mycelial growth, sporulation and conidial germination suggests a promising mechanism for their antifungal activity. Moreover, their efficacy as preventive and curative treatments and lack of phytotoxicity make Ag-NPs a potential candidate for environmentally friendly disease control strategies in rice cultivation. However, follow-up field studies could be done to evaluate the duration of Ag-NP efficacy against blast and other diseases that threaten rice production while evaluating their effect on rice yield. Moreover, studies should be undertaken to determine the best way to combine Ag-NPs with fungicides. It is also vital to monitor the effects of using Ag-NPs in the field on the environment and human health. The registration and labelling of Ag-NPs as fungicides for crop protection may depend heavily on these data.

Acknowledgments

The authors express their sincere gratitude for the research grants received to conduct this study from the University Grants Commission, Bangladesh, through the Research Management Committee (RMC) of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

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