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Antifungal activity of silver nanoparticles against *Rhizoctonia solani* Kühn

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Abstract

Sheath Blight caused by *Rhizoctonia solani* Kühn is the second most devastating disease in rice. Managing the causal agent becomes difficult as it produces sclerotia that survive even in adverse environmental conditions. Silver nanoparticles (AgNPs) are increasingly recognized for their strong antimicrobial properties. The study aimed to assess the antifungal effect of silver nanoparticles at different concentrations against *R. solani* Kühn under *in vitro* condition using poisoned food technique. The statistical analysis revealed a high significant variation among treatment means from 3 days until 7 days of incubation. Based on the results, the ability of AgNPs to suppress the growth of *R. solani* Kühn improves with increasing nanoparticle concentrations, indicating a concentration-dependent inhibitory effect.

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Introduction

Rice is the most essential staple food in the Philippines, supplying nearly half of the population's daily calorie needs (Mamiit *et al*., 2021). With the growing global population, maintaining consistent and successful rice production is essential to meet rising food demands. However, this goal faces challenges from various biotic factors, including the significant threat posed by *Rhizoctonia solani* Kühn, a soil-borne pathogen responsible for Sheath Blight Disease in rice.

After the rice blast, sheath blight stands as the second most crucial fungal disease-causing substantial yield loss in rice (Pan *et al*., 1999). Under conditions favorable for disease development, on average, it is estimated that sheath blight causes a reduction of about 6% in rice production in Asia, though localized losses can be as high as 50% (Singh *et al*., 2019). In the Philippines, a yield reduction due to *R. solan*i Kühn obtained of 3.59 to 27.01% during the wet season and 3.16 to 28.62% during the dry season was observed by Dilla (1993). At the beginning of the infection, *R. solani* Kühn causes green-gray, water-soaked lesions on the sheath near the base of rice plants or close to the waterline in the paddy field. As the disease progresses, the lesions turn grayish-white at the center and dark brown at the edges. Over time, the lesions may merge and cover the entire sheath, causing it to become yellow, wilted, and eventually rot and die (Taheri and Tarighi, 2011).

To combat this disease, numerous rice growers have embraced synthetic fungicides as their primary tool for disease management. Although these chemical interventions do provide benefits in controlling diseases, their excessive use comes with notable adverse effects. The extensive utilization of synthetic fungicides escalates the potential hazards linked to fungicide residues, which can be ingested by humans. Studies have demonstrated that the heavy application of synthetic fungicides could hasten the development of fungicide resistance, leading to the emergence of new races and epidemics in agricultural ecosystems (Delmas *et al*., 2017; McDonald *et al*., 2019). Therefore, there is a need to shift towards an eco-friendly approach to address the environmental and health concerns associated with the current practice. One of which is utilizing biosynthesized silver nanomaterials, which are poised to have a promising impact in controlling fungal diseases and noted with low toxicity and environmental-friendly approach (Pantidos and Horsfall, 2014).

Silver has long been acknowledged as a non-toxic and safe inorganic substance with antibacterial and antifungal properties, a legacy that spans centuries. In particular, silver, when employed in the form of nanoparticles, exhibits significant potential in a broad array of biological applications. One of the intriguing potential applications of silver lies in its role in managing plant diseases. Silver exhibits a remarkable capacity to inhibit microorganisms through a variety of mechanisms (Clement and Jarrett, 1994). This unique property opens up the possibility of using silver as an alternative approach to control a wide range of plant pathogens. In doing so, it offers a safer alternative when compared to the use of synthetic fungicides (Park *et al*., 2006).

Although many studies have successfully utilized silver nanoparticles as antifungal agents, limited research has explored their efficacy against *R. solani* Kühn. Hence, this study aimed to evaluate the efficacy of silver nanoparticles on *R. solani* Kühn isolate from rice.

Materials and methods

Source of silver nanoparticles

Silver nanoparticle was synthesized using *Aspergillus niger* Tiegh with an average size of 68-99 nm in a dark brown colloid physical form. The *A. niger* Tiegh inoculum was obtained from an infected onion bulb, and the pure culture was grown on Potato Dextrose Agar (PDA). The silver nitrate ($AgNO_3$) reagent used in the synthesis was purchased from Scharlab S.L., Spain, ensuring high analytical grade quality. The nanoparticles were synthesized following a modified protocol, with the resulting colloid visually confirmed by a color change from light to dark brown.

Collection of diseased plants

The diseased plant of rice showing typical symptoms of sheath blight was collected at Dabong-dabong, Valencia City, Bukidnon, Philippines. The leaves were cut into pieces and surface sterilized using sodium hypochlorite then washed with distilled water three times and air dried.

Isolation of pathogen

The surfaced-sterile leaves were grown in Potato Dextrose Agar and incubated for five days under room temperature. Pure culture was obtained from sub-culturing the original isolates and kept for future use.

Pathogenicity testing of organism

To confirm that the suspected pathogen was responsible for the disease, pathogenicity test was done. The isolate from diseased plants was revived on PDA plates. Mass production of the inoculum was done using a sterilized rice hull. Mycelial plug was added into a sterilized rice hull and incubated for 6 days. The basal portion of rice seedling was added with a five gram of a thoroughly mixed rice hull and inoculum. The inoculated plant was covered with cellophane and incubated until the symptom is manifested in the sheaths or until sclerotia appears. From the inoculated plants, the pathogen was re-isolated and was compared from the original isolate.

Experimental design and treatments

The *in vitro* study was laid out using a Complete Randomized Design (CRD) with seven treatments replicated three times and sub-replicated three times. The treatments used were the following: Treatment 1 - Negative Control (Water only) Treatment 2- Positive Control (Fungicide) Treatment 3- 100ppm of synthesized AgNPs Treatment 4- 200ppm of synthesized AgNPs Treatment 5 -300ppm of synthesized AgNPs Treatment 6- 400ppm of synthesized AgNPs Treatment 7- 500ppm of synthesized AgNPs.

In vitro Assay of silver nanoparticles against R. solani

The poisoned food technique was done to evaluate the antifungal potential of silver nanoparticles against *Rhizoctonia solani* Kühn. In a melted potato dextrose agar, a 1mL of silver nanoparticles at different concentrations was added. The media were hardened and a 6mm mycelial plug was added into the center of the plates. The plates were incubated at room temperature and the antifungal activity was observed at 7 days.

The percent inhibition rate of *Rhizoctonia solani* Kühn by AgNPs was measured using the modified formula described by Lamsal *et al.* (2011).

Inhibition Rate (%) = {(R-r)/R} ×100

Where; R= Average diameter growth of fungi in the control plate

r = Average diameter growth of fungi in AgNPs treated plate

Statistical analysis

The data was analyzed using Analysis of Variance (ANOVA) and the differences among the treatment means was determined using Tukey's HSD test.

Results and discussion

Antifungal activity against Rhizoctonia solani

A poisoned food method was used to evaluate the antifungal activity of biosynthesized silver nanoparticles against *Rhizoctonia solani* Kühn. The percent growth inhibition of *R. solani* Kühn applied with silver nanoparticles is presented in Table 1 and Fig. 1 while Fig. 2 depicts the varying degrees of antifungal activity of silver nanoparticles against *R. solani* Kühn at 3, 5, and 7 days after incubation.

The statistical analysis revealed a high significant variation among treatment means from 3 days until 7 days of incubation.

At 3 DAI, the highest percent growth inhibition was observed in Treatment 2 (+) with a mean 100%, followed by Treatments 7, 6, and 5, which had means of 68.9%, 61.8%, and 56.1%, respectively. Treatment 6 showed no significant difference compared to Treatments 5 and 7, while Treatments 1, 3, and 4 were also not significantly different from one another. However, Treatment 2 was significantly different from all other treatments.

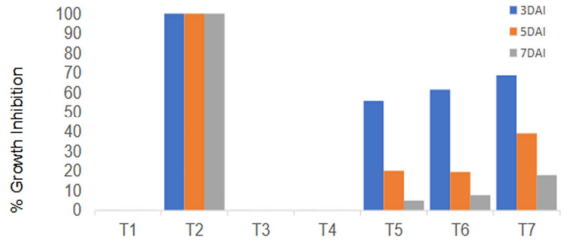

Fig. 1. Percent mean growth inhibition of *Rhizoctonia solani* Kühn applied with biosynthesized silver nanoparticles after 3, 5, and 7 days of incubation.

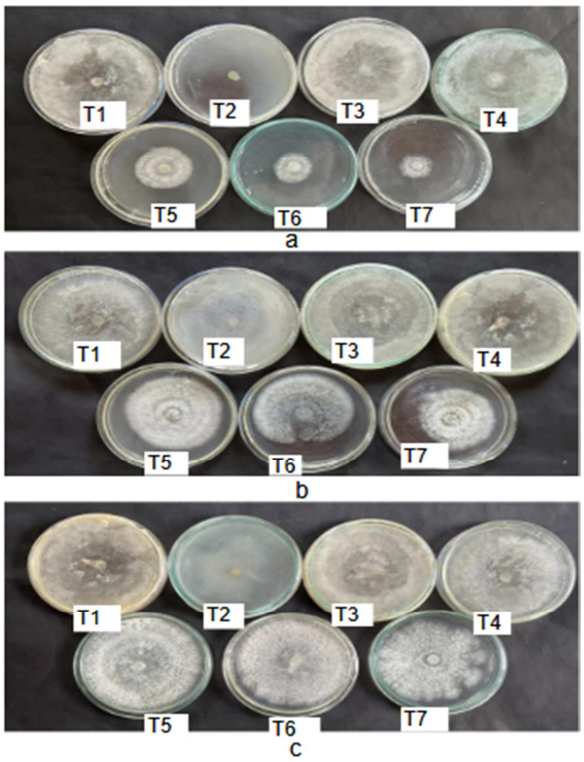

Fig. 2. *In vitro* assay of biosynthesized silver nanoparticles against *R. solani* Kühn after (a) 3 days, (b) 5 days and (c) 7 days after incubation

At 5 days after incubation, the highest percent inhibition was still observed in Treatment 2 (+) remains at 100%. This was followed by Treatments 7, 6 and 5 having means of 39.1&, 19.6%, and 20.2%, respectively. Treatments 1, 3 and 4 obtained no inhibition at all and showed no difference among treatment means. Treatment 5 and 6 also showed no variation while treatment 2, and 7 showed significant difference among treatment means.

Table 1. Mean percent diameter growth (mm) of *R. solani* Kühn applied with biosynthesized silver nanoparticles after 3, 5, and 7 days of incubation

Treatment	Days after incubation		
	3	5	7
Treatment 1- Water (-)	0.0^{d}	0.0^{d}	0.0^{e}
Treatment 2- Nativo (+)	100^{a}	100^{a}	100^{a}
Treatment 3- 100 ppm AgNP	-1.7^{d}	0.0^{d}	0.0_{e}
Treatment 4- 200 ppm AgNP	0.0^{d}	0.0^{d}	0.0^{e}
Treatment 5- 300 ppm AgNP	56.1^{c}	20.2^{c}	5.0^{d}
Treatment 6- 400 ppm AgNP	61.8^{bc}	19.6^{c}	7.7^{c}
Treatment 7- 500 ppm AgNP	68.9^{b}	39.1^{b}	17.9^{b}
F-test	**	**	**
CV%	7.0	6.3	6.2

This means a column with the same letter is not significantly different at 5% level based on Tukey's HSD test.

** - highly significant

At 7 days after incubation, Treatment 2 (+) continued to show the highest percent inhibition at 100%. This was followed by Treatments 7, 6 and 5 with the percent inhibition means of 17.9%, 7.7%, and 5.0%, respectively. While Treatments 1, 3, and 4 remains no inhibition at all.

Discussion

Based on the observations of the current study, the treatments that were applied with the least concentrations of silver nanoparticles showed no effects against *R. solani* Kühn. After 3 days of incubation, treatments 3, and 4 manifested the same growth patterns with Treatment 1 (-) with no application at all. There was aerial growth of mycelia were observed in the plates. In contrast, the higher concentrations like Treatments 5, 6 and 7 exhibited slower mycelial growth. The plates were not fully covered with mycelia and no aerial growths were observed.

Similarly, the current study is in accordance with Soltani Nejad *et al.* (2016) where the highest percentage of mycelial growth inhibition was observed in higher concentration of silver nanoparticles when applied against *R. solani* under *in vitro* conditions.

Elamawi and Al-harbi (2014) silver nanoparticles showed remarkable antifungal properties, effectively targeting *R. solani* Kühn, the pathogen responsible for sheath blight in rice. Additionally, previous studies have confirmed the efficacy of AgNPs against other rice fungi, including *Bipolaris sorokiniana* and *Magnaporthe oryzae*.

Furthermore, Islam *et al.* (2024) highlights the potential of AgNPs as a sustainable and efficient alternative for controlling rice sheath blight disease, providing a safer option in light of environmental concerns linked to traditional fungicides.

According to Gopinath *et al.* (2013) and Sondi *et al.* (2004), the inhibitory effects of AgNPs are attributed to several mechanisms; binding to the cytoplasmic membrane, causing damage to the cell membrane, leading to the formation of pits on the cell surface and altering cell wall permeability. Additionally, AgNPs have been shown to disrupt key cellular processes, including respiration, DNA replication, and cell division, ultimately resulting in loss of cell viability and cell death. It is possible that small-grained AgNPs not only damage the cell membrane but are also internalized into the cell via endocytosis. Once inside, they may disrupt intracellular organelles and protein functions, leading to alterations in fungal morphology as well as various metabolic and signaling pathways (Foldbjerg *et al.*, 2015).

Conclusion

Silver nanoparticles (AgNPs) synthesized using *Aspergillus niger* Tiegh have demonstrated significant antifungal activity against *Rhizoctonia solani* Kühn. The effectiveness of AgNPs in suppressing the growth of *R. solani* Kühn increases with higher nanoparticle concentrations, suggesting a dose-dependent inhibitory effect.

Recommendation(s)

The study emphasizes the potential of biologically synthesized silver nanoparticles as an eco-friendly and efficient alternative for managing plant diseases caused by *R. solani* Kühn. The study further recommends increasing the volume of silver nanoparticles which would be effective in controlling against *R. solani* Kühn. It is also recommended to test their antifungal activity against diverse fungal pathogens of different crops to confirm their broad applicability and effectiveness.

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