

The potential of silver nanoparticles to control *Rhizoctonia solani* (AG3-PT) growth in vitro

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Abstract. Silver nanoparticles (AgNPs) have been known to have anti-microbial properties and therefore have the potential to be used to control fungal plant pathogens. In this study we investigated the growth of a plant pathogenic fungus, *Rhizoctonia solani* (AG3-PT) in the presence of AgNPs. The effect of AgNPs at two different levels (20 and 50 mg L⁻¹) on hyphal growth and sclerotium production and viability in *R. solani* was investigated. The results showed that at 20 mg AgNPs L⁻¹ *R. solani* hyphal growth was reduced along with the production of sclerotia. The results indicate that AgNPs have the potential to control *R. solani* growth and subsequent development of plant disease symptoms.

Keywords: fungi, plant pathogen, sclerotia

INTRODUCTION

Rhizoctonia solani is an important soil-borne plant pathogenic fungi with a global distribution and a wide host-range. The pathogen is best known to cause “damping-off” and can survive in the soil without a host for many years [1]. A key survival structure for *R. solani* are sclerotia; compact masses of hardened fungal mycelia with 1-3 mm in diameter containing food reserves that are formed in response to stress e.g. unfavourable growth conditions [2]. The broad host-range and the ability to form sclerotia of *R. solani* makes this pathogen very difficult to control.

Silver nanoparticles (AgNPs) range from 1 nm and 100 nm in size and are widely used in industry due to their anti-microbial properties. Common applications include the use of AgNPs for antimicrobial coatings on textiles and electronics to control bacteria [3,4]. The exact anti-microbial mechanism of AgNPs is still not fully understood but studies suggest nanoparticles exert their toxic effects by a variety of mechanisms. For instance, they may

stick to cell surfaces [5], penetrate the cells [6], change cell membrane properties and finally result in DNA damage due to dissolution of Ag ions from the particulate [7]. Conversely, other researcher dispute that damage to DNA may be caused [8]. Other workers propose that silver ions interrupt cellular metabolism and respiration processes [6]. In addition, AgNPs produces reactive oxygen species (ROS), particularly superoxide radical and hydroxyl radical, that damage the cell [8].

The potential of AgNPs to control *Rhizoctonia solani* (AG-5) had been studied [9]. It is known that *Rhizoctonia* species fall into taxonomically distinct groups called anastomosis groups (AGs). *R. solani* is a species complex consisting 13 known AGs which assigned on hyphal interaction base that can be further classified based on the pathogenicity, biochemical and genetic marker. Therefore, *R. solani* from different group (AG3-PT) was studied. Preliminary test showed that AgNPs at 20 and 50 mg L⁻¹ affected the growth of *R. solani* (AG3-PT).

METHODOLOGY

Effect of AgNPs on the growth of *R. solani*

R. solani used was isolated from potato [10] and cultured on PDA for further use. Growth media were prepared by mixing AgNPs powder (20 and 50 mg L⁻¹) with PDA before autoclaving at

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121°C for 15 minutes. Sterile media containing AgNPs were swirled thoroughly before being plated into 90 mm petri dishes. Once the media had set, a 3 mm plug of 7-day-old *R. solani* culture was placed in the centre and incubated at 24°C. The growth of *R. solani* was determined as colony area after five days incubation (when the control plate fully covered by hyphae). The colony growth area was measured using graph paper by drawing the colony area. The number of squares within boundaries was used to calculate the colony area. Control was prepared without agNPs powder.

***R. solani* sclerotia production and germination assays**

The cultures of *R. solani* were incubated at 24°C until sclerotia formed (6 months). The sclerotia were collected by scraping the surface of the media followed by washing with distilled water. Sclerotia were then rinsed with 70% alcohol [9] prior to being oven dried at 50°C for 2 hours to determine total dry weights produced. To test the sclerotia viability, 6 dried sclerotia of approximately similar size ranges were re-grown on a fresh PDA plate (sclerotia was placed approximately 2 cm apart) and incubated at 24°C. A sclerotium was considered to have germinated when any outgrowing hyphae were equal to or greater than the diameter of the sclerotium [11].

Statistical analysis

All data presented are the mean value of three replicates. Values are expressed as means of three replicates $\pm$ standard error (S.E) in each group. All statistical analyses were performed using One-way Analysis of Variance (ANOVA) statistical models on Microsoft Excel 2016. Variance analysis was performed on all experimental data and significant differences ($P < 0.05$) between individual means (three replicates) was analysed using a post hoc Least Significant Difference test.

RESULTS AND DISCUSSION

Effect of AgNPs on the growth of *R. solani*

The presence of AgNPs in growth media decreased the growth rate of *R. solani*. Figure 1 shows that AgNPs at 20 and 50 mg L⁻¹ reduced the colony area of *R. solani* significantly compared to controls (no AgNPs). Interestingly, morphological changes of *R. solani* colonies when treated with AgNPs were observed (Figure 2). Due to these morphological changes, the colony growth was determined as colony area rather than radial growth.

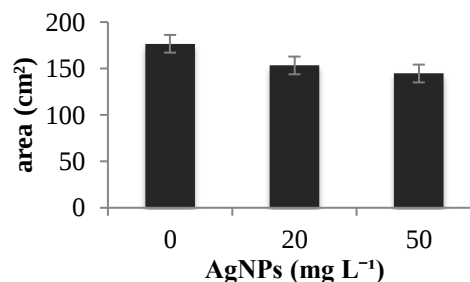


Figure 1. The effect of AgNPs at 20 and 50 mg L⁻¹ on *R. solani* colony area. The data were collected on the 5th day of growth.

The current investigation confirmed that AgNPs have a potential to inhibit the plant pathogenic fungus *R. solani*. Previous study had reported that very low AgNP concentration (7 mg L⁻¹) inhibited hyphal growth of *R. solani* (AG5) due to causing an abnormal shape of the hyphal walls which were then prone to collapse [9]. Similarly, six different strains of *R. solani* (AG-1, AG2-2, AG-5, AG-6, AG-10, and AG-4-HGI) are sensitive to low levels of AgNPs [12]. These findings suggest that sensitivity to silver is likely different between fungal strains of the same species but this should be tested further using similar growth conditions.

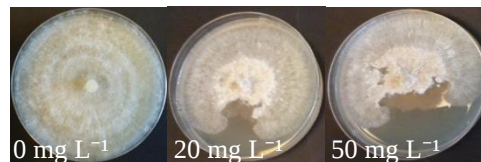


Figure 2. The effect of AgNPs at 20 and 50 mg L⁻¹ on *R. solani* colony area. AgNPs treatments cause morphological change toward *R. solani* colony. The data was collected on the 5th day of growth.

When in contact with heavy metals morphological changes are commonly observed among fungi. For example, morphological changes, including an increasing of aerial hyphae formation and irregular appearance of surface hyphae, in several species of white-rot fungi and *Trichoderma harzianum* were detected when grown on cadmium containing medium [13, 14]. Furthermore, heavy metal affected the morphologies of *T. viride* and *Rhizopus arrhizus* [15]. The phenomenon is potentially a defense mechanism of fungi in the presence of heavy metals.

***R. solani* sclerotia production and germination assays**

AgNPs also showed a potential to control sclerotia production by *R. solani*. Figure 3

shows that the lower level of AgNPs (20 mg L⁻¹) used reduced the dry weight of sclerotia by 89% compared to untreated controls. Interestingly, sclerotia production at 50 mg L⁻¹ of AgNPs appeared to be unaffected. However, the inhibition of AgNPs on sclerotia production did not affect their subsequent germination. This study revealed that sclerotia that formed on AgNP contaminated media able to germinate on fresh PDA (Figure 4).

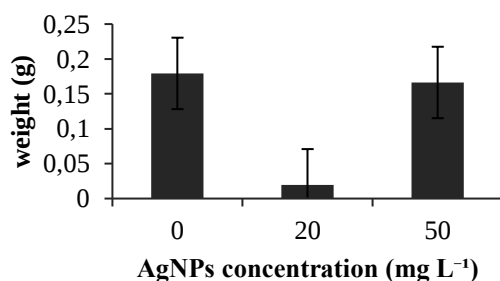


Figure 3. The effect of AgNPs on *R. solani* sclerotia dry weight at 20 mg L⁻¹ 50 mg L⁻¹ AgNPs.

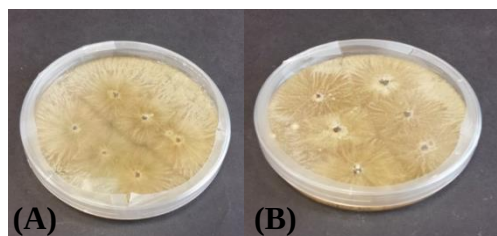


Figure 4. The growth of sclerotia of *R. solani* from AgNPs contaminated media on fresh PDA. (A) 20 mg L⁻¹ of AgNPs and (B) 50 mg L⁻¹ of AgNPs (B)

AgNPs also show a potential to control sclerotia produced by *R. solani*. However, the inhibition of AgNPs on sclerotia production did not affect their subsequent germination on fresh non-contaminated growth media. This finding is convers with previous study that reported that low levels of AgNPs in growth media inhibit sclerotia germination effectively [9]. It is showed that fungi from metal contaminated environments may be able to survive and germinate when conditions become permissible.

CONCLUSION

In summary, AgNPs at 20 mg L⁻¹ have the potential to considerably inhibit the in vitro growth of *R. solani*. However, the viability of sclerotia formed by *R. solani* in AgNP contaminated media did not seem to be affected. It is possible that AgNPs could be used as part of an integrated fungal control strategy.

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