The potential of *Trichoderma* species to remediate silver nanoparticles contamination

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**Abstract.** *Trichoderma* is one of soil fungi that has been widely explored for the removal and recovery of heavy metals. A preliminary experiment was set to investigate the potential of two local species of *Trichoderma*, *T. harzianum* and *T. virens*, to remediate silver nanoparticles (AgNPs) contamination. AgNPs was studied as they have the most commercial applications as consumer products and accumulated in soil. Four levels of AgNPs (0, 100, 200, 300 mg L⁻¹) were used to examine the ability of each species to grow in contaminated media. The growth of *Trichoderma* spp. was observed by measuring the colony diameter and spore production. The results showed that *T. harzianum* was more sensitive to AgNPs than *T. virens* as the colony diameter and spore production reduced significantly. The study indicates that *T. virens* has a potential to remediate AgNPs contamination.

1. Introduction

*Trichoderma* is one of a range of beneficial fungi that live in soil as saprophytes. In particular, *Trichoderma* has the ability to decompose organic matter [1]. Furthermore, *Trichoderma* species also have capability to live on other fungi and this property makes them well known as biocontrol agent against a wide range of plant pathogens [2] [3] [4]. The application of the fungi in bioremediation is well documented. For example, *Trichoderma* spp. are reported as potential metal bioremediation agents in cadmium and nickel polluted agricultural fields [5]. More recent, researchers found that *T. harzianum* is able able to remediate diesel-contaminated sand and degrade hydrocarbon complex in soil [6] [7].

In this study we investigate the potential of two local species of *Trichoderma*, *T. harzianum* and *T. virens* to remediate silver nanoparticles (AgNPs). AgNPs have the most commercial applications as consumer products due to its antimicrobial properties. There are more than 300 everyday products listed such as bed sheet, socks, toothpaste, and towels [8]. The increasing and varied use of AgNPs on consumer products raises the risk of AgNPs release into the environment. The pollutant has toxic effect on beneficial soil microorganisms [9] [10], soil-associated organisms such as red worm *Eisenia fetida* [11], plants [12] and eventually human [13].

In order to be used as bioremediator, the agent must be tolerant of heavy metals. [14] stated that AgNPs at 200 mg L⁻¹ did not affect the growth of *T. harzianum*. So that, in this preliminary experiment, four levels of AgNPs (0, 100, 200, 300 mg L⁻¹) were used to examine the growth of *T. harzianum* and *T. virens* by measuring the colony diameter and spore production.
2. Materials and Methods

2.1. The Effect of AgNPs on T. harzianum and T. virens Colony Diameter

The AgNPs (99.5%) used in the experiment were obtained from Sigma Aldrich. Four levels of AgNPs (0, 100, 200, 300 mg L$^{-1}$ of AgNPs) were added to Potato Dextrose Agar (PDA), before autoclaving at 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 T. harzianum culture was placed in the centre and incubated at 24 °C. The growth of T. harzianum was observed by measuring the colony diameter at 24 hours intervals until control plates were fully covered by mycelia (5-6 days). Silver nanoparticle preparation for T. virens was as above. Control plates were prepared without AgNPs. All experiments were carried out in triplicate.

2.2. The Effect of AgNPs on T. harzianum and T. virens Spore Production

The spores produced by T. harzianum and T. virens were counted on the last day of colony diameter observation day. The spores were harvested by pouring sterile distilled water on the culture. One ml of spore suspension was transferred to a clean 1.5 ml microfuge tube. Dilution was made up to $10^{-3}$. From this, 20 µL of dilution was dropped on the centre of haemocytometer and observed under light microscope. Spores from five random squares (0.04 mm² each square) were counted as the sample. To calculate the number of spore per ml suspension, equation below was used:

$$\text{number of spores counted in 5 squares} \times 25 \times 10^4 \times \text{dilution factor}$$

The number of spores on control plates were also counted. The spore count was carried out in triplicate.

2.3. Statistical Analysis

The data on colony diameter and spore production were statistically analysed for Analysis of Variance (ANOVA) on Minitab 17. Significant differences between mean values were determined using Least Significant Different (P=0.05).

3. Results and Discussion

3.1. The effect of AgNPs on T. harzianum and T. virens Colony Diameter

The colony diameter of T. harzianum and T. virens were observed at 24 hours intervals until the 5th and 6th day, respectively. Figure 1 shows the effect of AgNPs on colony diameter of T. harzianum. A significant effect was indicated on the 3rd day of observation at 200 and 300 mg L$^{-1}$. The impact continued until the 6th day of observation. The lower level of AgNPs, 100 mg L$^{-1}$, inhibited the growth of colony only on the last day of observation. On the other hands, AgNPs at any levels did not give a substantial impact on T. virens colony growth (Figure 2). The study revealed that sensitivity to AgNPs is different among different fungal species. Possible reason for the difference in resistance levels could be the variation in the mechanism of resistance [15]. Eukaryotic organisms, such as fungi, produce enzyme and metabolite to defend themselves when exposed to environmental stress e.g. metallic ion, predator and temperature variation [16] [17]. For example, T. reesei produce enzyme and metabolites for their survival when the mycelium was exposed to silver nanoparticles. In this process the toxic metal ions were reduced to the non-toxic metallic AgNPs through the catalytic effect of the extracellular enzyme and metabolites of the fungus [17].
3.2. The Effect of AgNPs on T. harzianum and T. virens Spore Production

The spore production of both T. harzianum and T. virens were counted at the 6th and 5th day, respectively, after inoculation when the control plates were fully covered by the mycelia. Figure 3 illustrated the effect of AgNPs on T. harzianum spore production. There was a slight decrease on T. harzianum spores production in AgNPs contaminated media at 100 and 200 mg L⁻¹. The highest level of AgNPs, 300 mg L⁻¹, reduced the spores production more than 50% compared to control. On the contrary, AgNPs in growth media, somehow, induced the spore production of T. virens. It can be seen in Figure 4 that the higher the AgNPs levels the more spores produced by T. virens. A study revealed that a fungal strategy to survive in heavy metal polluted environments by accumulating excess metal in the spore cytoplasmis [18]. If it the case, T. virens might produce more spore to catch more AgNPs as a defence mechanism.
Figure 3. Spore production of *T. harzianum* grown on PDA at different levels of AgNPs. The spore was counted at six days after inoculation.

Figure 4. Spore production of *T. virens* grown on PDA at different levels of AgNPs. The spore was counted at five days after inoculation.

4. **Conclusion**

The study indicates that *T. virens* used in this study is more tolerant to AgNPs than *T. harzianum*. The difference tolerant between the species is depend on the individual mechanism of resistance. So that, *T. virens* has a potential as bioremediation agent. However, further study is needed to understand whether *T. virens* able to reduce AgNPs concentration in the growth media.

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