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Production of Silver and Gold Nanoparticles using Trichoderma atroviride for the biological control of Rhizome rot disease in Turmeric plants

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Abstract: *Ecofriently generated silver and gold nanoparticles, known as nanocrystallins, are widely employed in the management of numerous pathogenic bacteria that affect humans, animals, and plants. In light of this, an assessment was conducted to determine whether the suppressive capability of nanoparticles generated by the native isolate Trichoderma atroviride against the fungal pathogen Pythium aphanidermatum, which causes turmeric, could be achieved. Spectrophotometric measurements were made at various wavelengths within the range of 300-700 nm to quantify the gold and silver , Using TSM medium supplemented with an aqueous solution containing 2x10-3 M chloroauric acid and 3x10-4 M silver nitrate, T. atroviride generated nanoparticles. The creation of gold and silver nanoparticles was found to have two strong peaks at 450 and 410 nm, respectively, according to the UV-VIS spectra measurements. Even after two months, it was discovered that these peaks remained rather steady, which was consistent with the stability of nanoparticle formation. The antibiotic activity between T. atroviride and P. aphanidermatum was investigated using the culture filtrate that contained nanoparticles. The findings showed that P. aphanidermatum's growth might be inhibited by the culture filtrate.*

Keywords: *silver and gold nano particles, UV-VIS spectra measurements, pathogenic bacteria.*

INTRODUCTION

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It is becoming clear that using microbes to synthesise nanoparticles is an innovative and environmentally beneficial method. A study that involved screening Rhodo-pseudomonas cappsulata revealed that the bacterium was capable of producing gold and silver nanoparticles with varying sizes and shapes. Therefore, it is crucial to synthesise these nanoparticles in a variety of sizes and shapes for use in the management of plant diseases. An earlier study discovered that inside the cell walls of Bacillus subtilis, Au3+ ions could be reduced to gold nanoparticles with a size range of 5–25 nm. With the use of hydrogen gas, Shewanella globrella alga was discovered to reduce Au3+ ions, generating 10–20 nm gold nanoparticles extracellularly [1]. Nanopartocles were also synthesised intra- and extracellularly using fungi like Verticillium lecanni and Fusarium oxysporum and actinomycetes like Streptomyces, Thermomonospora, and Rhodococus spp. [2]. Silver and gold nanoparticle biosynthesis is still rare, nevertheless. Trichoderma atroviride, a Eukaryote fungus, is identified in this study as one of the ecologically and environmentally significant biocontrol agents frequently employed to control a variety of plant diseases.

Curcuma longa L., a member of the Zingiberaceae family, is a widely grown commercial spice crop in India. When it comes to quality and lack of illness, Indian turmeric is regarded as the best in the world [3]. In southern India's Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu states, Pythium aphanidermatum-caused rhizome rot disease is a major issue for turmeric plantations, and since 2006, the illness has significantly decreased turmeric exports [4]. Soil drenching is recommended as a means of controlling the disease, with contact fungicides such as Mancozeb and systemic fungicides like Carbendazim [5]. Fungicide-soaked soil has an adverse effect on healthy microorganisms as well as the soil itself. Antagonists of bacteria, fungi, and actinomycete species have been used to effectively control a wide range of plant diseases [6, 7]. Nevertheless, there is no evidence on the use of culture filtrate containing Trichoderma atroviride nanoparticles for the biological control of turmeric rhizome rot disease.

INTRODUCTION METHODS

In an Erlenmeyer flask with TSM medium, Trichoderma atroviride (MTCC No.
9641) was cultured for 25 days at 27°C with 200 rpm shaking. Following incubation, sterilised double-distilled water was used three times to wash the mycelial biomass prior
to its extraction from the broth using 500 rpm centrifugation. The harvested mycelial biomass, weighing about 500 mg, was subjected to 50 ml of an aqueous solution of 2x10-
3 M AuCl4 to create gold nanoparticles and 75 ml of an aqueous solution of 3x10-4 M AgNO3 to create silver nanoparticles in an Erlenmeyer flask with 250 ml of medium at
pH 5.5. For three to ten days, the mixture was placed in an orbital shaker set to rotate
between 200 and 250 rpm. In addition to visual inspection of the biomass and solution, the intensity of the solution between 300 and 700 nm was measured using a Hitachi spectrophotometer to monitor the bioreduction of metal ions. Silver nanoparticle synthesis was indicated by the reaction solution's colour changing from pale yellow to purple on the fifth day of incubation and finally turning dark brown by the end of the tenth day. Similar to this, in the case of gold nanoparticles, the reaction solution's colour changed from pale green to dark yellow on the fifth day of incubation. [2].
For this investigation, the culture filtrate containing gold and silver nanoparticles that were biosynthesized by T. atroviride was used. P. aphanidermatum, a seven-day-old test pathogen, was centrally injected into petridishes containing Potato Dextrose Agar (PDA) medium supplemented with 10% above biosolution. The disc size measuring five mm. Following five days of room temperature incubation, the pathogen's radial development was assessed using a metric scale to determine the percentage of inhibition [8]. The control was a pathogen inoculated on PDA plates with culture filtrate free of supplemental nanoparticles. To examine the stability of the nanoparticles with regard to antagonistic potential, the culture filtrate containing the nanoparticles was kept for a maximum of six months.

END RESULTS AND TALK

The results of T. atroviride's generation of silver and gold nanoparticles showed that there were more silver nanoparticles in the culture filtrate when assessed at 410 nm. The generation of gold nanoparticles peaked at 450 nm and then sharply declined after that (Fig. 1). After two months of incubation of the culture filtrate, the amount of biogenesis of both silver and gold nanoparticles remained constant (Fig. 2). Subsequent examination of the nanoparticles revealed that 50–75 nm-sized triangular gold nanoplates account for around 60% of the population of nanoparticles overall. The majority of the remaining nanoparticles, which range in size from 10 to 50 nm, are spherical.

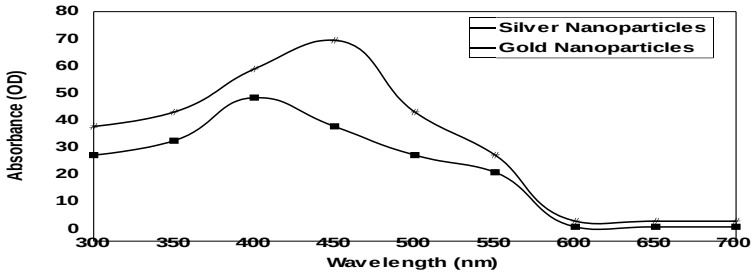

Fig. 1. Biosynthesis of silver and gold nanoparticles by *Trichoderma atroviride*

NADH-dependent enzymes have been shown in earlier research to have a significant role in the production of these two nanoparticles. The bacterium R. capsulata, which has been demonstrated to secrete cofactor NADH- and NADH-dependent enzymes, has been connected to the bioreduction of Au(3+) to Au (0) and the subsequent formation of gold nanoparticles. [1, 2]. The pathogen, P. aphanidermatum, was progressively inhibited from day five to day thirty. After that, the inhibition remained constant over the next two months of incubation (Table 1). Due to the absence of nanoparticles, the pathogen's development was inhibited more effectively than in untreated control plates. On the 30th day of incubation, the growth inhibition of P. aphanidermatum using silver and gold nanoparticles, respectively, was 81.17% and 93.49%. Gold nanoparticles were shown to

be superior to silver nanoparticles that were biosynthesized by T. atroviride among the two nanoparticles examined for the pathogen's growth suppression. Several researchers who examined several phytopathogens reported comparable results [9, 10].

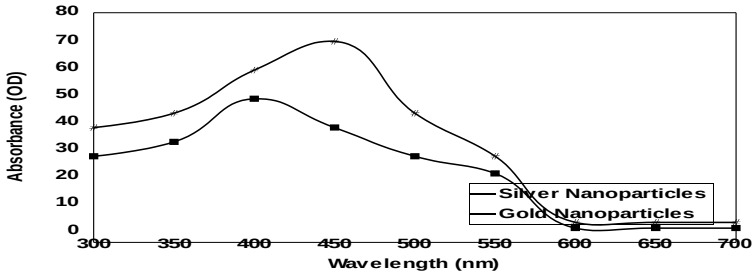

Fig. 2. Effect of different incubation periods on stability of silver and gold nanoparticles by *Trichoderma atroviride*

Table 1. Antagonistic potential of *Trichoderma atroviride* culture filtrate containing silver and gold nanoparticles upon growth inhibition of *Pythium aphanidermatum*

Days	*Trichoderma* culture filtrate containing* after		
	Silver nanoparticle	Gold nanoparticle	Untreated inoculation Control
05	36.43	38.22	20.00
10	54.25	62.76	33.76
15	61.75	70.82	45.82
20	72.00	81.11	56.66
25	76.75	93.42	62.82
30	81.17	93.49	37.66
40	81.17	91.26	28.16
50	80.32	90.67	20.00
60	80.97	91.44	11.44
SE ±	07.47	09.14	06.58
CD at P=0.05**	12.27	14.78	10.96

* Growth inhibition of *Pythium aphanidermatum*
** Significant at 5% level

The antagonist's secretion of diffusible metabolites with low molecular weight may be the cause of this growth inhibition. Furthermore, the reaction between the silver and gold nanoparticles made diffusible metabolites extremely stable for up to two months [11]. The pathogen's growth was greatly suppressed by T. atroviride's antagonistic activity, which was discovered to be caused by the antagonist's colonisation and the participation of wall lytic enzymes secreted by the pathogen [12, 13].

CONCLUSION

In summary, Trichoderma atroviride was able to produce silver and gold nanoparticles in addition to TSM medium, an antibiotic, to combat the fungal infection Pythium aphanidermatum, which is associated with turmeric. To initiate the creation of silver and gold nanoparticles, TSM medium was supplemented with silver nitrate and chloroauric acid, respectively. Spectroscopy was used to measure them at different wavelengths, and two prominent peaks were discovered that correlated with the creation of gold and silver nanoparticles. To demonstrate the stability of the nanoparticles followed by the pathogen's antagonistic activity, these peaks were observed to be quite stable for two to three months. The inclusion of gold and silver nanoparticles in the culture filtrate hindered P. aphanidermatum's in vitro growth.

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