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Synthesis Of Silver Nanoparticles And Standardization Of Dose/Concentration Against Selected Plant Pathogenic Fungal Species

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ABSTRACT

Bio silver nanoparticles were generated in a lab using Bacillus pseudomycoides MT32 supernatant, and their optimal growing conditions were investigated. The manufactured nanoparticles were characterized by a battery of seven cutting-edge analyzers. The absorption peak in the UV-visible spectra was located around 420 nm. According to results from Transmission Electron Microscopy (TEM), the generated SNPs typically had a diameter of 25 to 43 nm. The particles, as determined by powder X-ray diffraction (XRD), are crystalline, have a spherical shape, and vary in size from 32 to 86 nm. Based on in vitro antifungal testing, the synthesized SNPs had the potential to inhibit, to varying degrees, eleven economically important plant pathogens. The MIC and MFC values reported for these SNPs ranged from 70 to 90 and 75 to 100 g/l, respectively, however these values varied depending on the fungus utilized. Bacillus pseudomycoides MT32's SNPs have antifungal action, which may help with a variety of issues related to agricultural productivity and animal nutrition. Plants are being considered as a viable and inexpensive solution for the biological generation of silver nanoparticles. At 100 ppm silver nanoparticles, C. gloeosporioides did not grow in vitro, although it expanded by 44.50 1.14 mm in the absence of the nanoparticles.

KEYWORDS Nano fungal**,** TEM, silver nanoparticles, XRD

INTRODUCTION

Human and animal safety must be prioritized in the development of biological control agents. Commercial biological control products now face challenges include losing efficacy between in vitro and in vivo testing and adapting to new environments. Now is the moment to use nanotechnology to create biogenic silver, zinc, copper, gold, and iron biopesticides that are both more effective and less likely to survive in the environment. Agricultural biocontrol has been demonstrated to benefit from fungitoxic metabolites, which include antibiotics and other bioactive compounds. Under the right circumstances, fungi may manufacture a wide variety of extracellular enzymes, including xylanases, cellulases, mannanases, proteases, glycosyl hydrolases, chitinases, and glucanases. Much interest has been shown in metallic nanoparticles derived from biological sources due to their compatibility with the principles and goals of green chemistry.

Technology developments have made the production of nanosized silver particles more cost-effective, increasing their popularity. Zinc oxide and silver nitrate have been the starting point

for several efforts to create nano-based antifungal chemicals for the efficient control of plant pathogenic fungi such Fusarium oxysporum, Penicillium digitatum, Alternaria citri, Alternaria alternative, and Aspergillus niger. Similarly, copper nanoparticles were produced and shown to have potent antifungal activity against a panel of plant pathogenic fungi responsible for agricultural illnesses. There is a rising need for the creation of eco-friendly procedures that do not include the use of harmful chemicals in the synthesis protocols. Many advantages may be gained by adopting green synthesis methods instead of conventional ones that rely on chemical agents that have been related to environmental damage.

LITERATURE REVIEW

Rabab M. Elamawi et.al (2018) A "microbial nanotechnology" capable of fast nanoparticle manufacturing at scale would need a very effective biosynthesis mechanism. The effectiveness of nanoparticle biogenesis was studied by varying physical factors such fungal biomass concentration, temperature, incubation duration, and agitation. Silver nanoparticles (AgNPs) were produced, then their stability and antifungal activity were evaluated. The optimal conditions for the formation of the silver nanoparticles were established to be 10 grams of fungal biomass, a reaction temperature of 28 degrees Celsius, a 72-hour incubation time with no shaking, and no agitation throughout the incubation period. When silver nanoparticles develop, they take on a brownish color. Under ideal circumstances, maximum absorption is seen at a wavelength of 385 nm, as measured by UV-vis spectroscopy. Researchers used TEM and discovered that spheres had formed with consistent sizes, with a mean of 10 nm. The FTIR spectrometer identified up bands at 1634.92 and 3269.31 cm1. The Z-average size was confirmed to be 24.43 and the PdI value to be 0.420 using dynamic light scattering (DLS). A single peak with a zeta potential of 19.7 mV was observed. This biosystems method resulted in AgNPs that were reasonably stable for up to 2 months after synthesis.

Alananbeh, Kholoud et.al (2017) The Kingdom of Saudi Arabia recognizes the use of wastewater for agricultural purposes. The current study set out to do just that by isolating, identifying, and quantifying fungal species from a variety of waste water sources in Al-Madinah Al-Munawwarah, Saudi Arabia, and then figuring out what kind of nanoparticles would be most effective against the most common of them. Both A. niger and A. terreus were exposed to silver nanoparticles in two different forms (rod and cube) and four different concentrations (0.0, 100, 10, and 1 g/ml). Both Aspergillus species clearly showed a dose-dependent decrease in growth as the silver nanoparticle concentration rose. In comparison to A. niger, A. terreus showed more suppression. Concentrations of 1, 10, and 100 g/ml were tested, and there was no discernible difference between them. When compared to cube-shaped nanoparticles, rod-shaped ones grew more slowly. Silver nanoparticles might be used as an antifungal material, but there are other factors to take into account first.

Xi-Feng Zhang et.al (2016) Among the many metallic nanoparticles with biological uses, silver nanoparticles (AgNPs) stand out as among the most important and intriguing. When it comes to nanoscience and nanotechnology, nanomedicine is where AgNPs really shine. While several noble metals have been put to use, researchers have zeroed in on AgNPs for their promise in cancer diagnostics and treatment. In this study, we explore the physical, chemical, and biological approaches of synthesizing AgNPs. We also go through ways to characterize AgNPs

and their attributes. We also go into detail on the mechanism of AgNPs' anti-cancer activity and their many other bio-applications. We also cover therapeutic strategies and obstacles for employing AgNPs in cancer treatment. Finally, we look at the potential future of AgNPs.

Mousa A. Alghuthaymi et.al (2015) When it comes to controlling plant diseases, nanotechnology may provide environmentally benign and sustainable options. Fungi are employed as bio-manufacturing units since they are both eco-friendly and simple to operate in comparison to other bacteria. Silver nanoparticles may be manufactured in large quantities thanks to the non-pathogenic nature of particular fungal species and the ease with which they can be produced and handled. The capacity to produce silver nanoparticles has been recently investigated in a wide variety of fungus. Researchers have also documented the mycosynthesis of a wide variety of other nanoparticles. The use of nanotechnology in plant pathology is only getting started. In agriculture, for instance, nanopesticides, nanofungicides, and nanoherbicides are all seeing widespread application. Using sophisticated nano-delivery systems that can be remotely activated and monitored, farmers of the future may be able to reduce the amount of fungicides and pesticides they use. Gene transfer facilitated by nanoparticles might help make crops more pest- and disease-resistant. In order to create inexpensive strategies for the synthesis and recovery of nanoparticles, this study presents a complete examination of the function of fungi in the synthesis, the mechanism involved in the synthesis, and the influence of varied situations on the reduction of metal ions. The effects of nanotoxicity on plant communities and soil microbes, as well as their applicability in plant disease management, have also been addressed at length.

S. Iravani, et.al (2014) Scientists are interested in silver nanoparticles (NPs) because of their peculiar properties. Methods for the production of silver NPs have been reported to range from laser ablation and gamma irradiation to electron irradiation and chemical reduction to photochemical procedures and microwave processing, and even biological synthesis. This page provides a synopsis of the physical, chemical, and biological methods for producing silver nanoparticles. Therefore, this review paper seeks to consider the present and future of the aforementioned methodologies, focusing on their advantages and disadvantages for various industrial settings.

METHODS

Bacterial Isolation and Selection

Soil samples were sealed in sterile polythene bags for the trip to the lab. After diluting the soil sample with sterile saline solution (0.9% w/v), Two days at 30 degrees Celsius were spent spreading out nutritional agar (NA) to recover the bacterial isolates. Shaker incubator conditions of 100 rpm and 30°C were used for 48 hours with the culture flasks. Primary screening results were recorded after 48 hours, while secondary screening results were recorded after 24 hours. The selected microbe was chosen based on the intensity of the acquired color and the turbidity of the culture flasks, which indicated healthy development. Both visual inspection and absorbance measurements with a UV - Vis spectrophotometer EL were used to track the progress of the bio reduction in the turbid flasks.

Preparation of Bacterial Cell Free Extract

Separate inoculations of a single bacterium strain were made in 250 ml conical flasks with 50 ml of sterile (NB). pH 7 was used as the growth medium, with the incubation temperature set at 30 degrees Celsius and the agitation speed set to 160 revolutions per minute in a shaker incubator. The NO3 reductase enzyme required for the extracellular production of SNPs was extracted from the supernatant.

Biosynthesis of Silver Nanoparticles

In order to biosynthesize SNPs, the tested culture was exposed to 1 mM aqueous solutions of filtered, sterilized AgNO3 for 20 hours at 37 degrees Celsius in 250 mL conical flasks containing 20 mL of supernatant from the bacterial cultures. Next, the flasks containing the reaction mixture were heated to 30 degrees Celsius for 24 hours while being shaken at 160 revolutions per minute. Along with the experimental samples, a pair of control flasks were also produced and incubated. The first group used a supernatant devoid of AgNO3, whereas the second used AgNO3 alone.

Identification Bacterial Isolate

Bergey's manual of systematic bacteriology was used to identify the microbe based on its morphological, biochemical, and physiological characteristics; then, the previously identified samples were double-checked using the cutting-edge and precise method of matrix-assisted laser desorption ionization time of flight (MALDI TOF) mass spectrometry.

Extraction and Purification of Silver Nanoparticles

Incubating the tested organism in NB at 30 degrees Celsius for 40 hours with agitation speed of 140 rotations per minute and pH of 6.5 resulted in the maximum yield of SNPs. The SNPs were separated after the mixture was ultra-centrifuged three times for 20 minutes at 4oC and 17,000 rpm. To remove any trace of biological material, the supernatant was resuspended in sterile deionized water. In a hot air oven set to 60 degrees Celsius, the solution was dried for 12 hours after cleaning. The SNPs powder was reconstituted with 10 ml of deionized water, the mixture was maintained on a sonicator to break up any clumps and allow for further characterization.

In vitro experiments have revealed that O. kilimandscharicum-produced silver nanoparticles are effective against the fungi Fusarium oxysporum and Colletotrichum gloeosporioides. Both F. oxysporum and C. gloeosporioides were cultured on potato dextrose agar (PDA) for three days at 25 °C. Silver nanoparticles generated in the lab were tested in vitro using varying amounts of plant extract and temperatures. Additional testing was done with silver nanoparticles manufactured at 25, 50, 75, and 100 ppm with the optimal concentration of plant extract and heating temperature. Petri dishes with a 90-mm diameter were filled with a medium containing silver nanoparticle. Petri dishes were filled with uniformly sized agar plugs infected with fungus. Agar plugs with fungal infections were utilized to inoculate untreated culture medium. The plates were analyzed after 7 days of incubation at 25 °C. After 4 and 7 days of incubation, radial development of the fungal colonies was observed.

RESULT

The characteristics, size, shape, and stability of the metal NPs were significantly impacted by the experimental growth circumstances. Learning how to design a biosynthetic process that allows one to regulate morphology, characteristics, stability, and size is a hot topic. In the beginning, color shifts were used to gauge how far along the reaction mixture in the turbid flasks was. Within a day of inoculation, the reaction mixture changed color from pale yellow to brown, indicating that the chosen bacterial extract had catalyzed the manufacture of SNPs. When incubated under the same circumstances, the control treatments show no change in color. It is widely established that metal NPs in water take on a brownish yellow hue due to the activation of surface Plasmon trembling. The color shift is due to the activation of surface plasmon trembling, which is unique to silver nanoparticles. Dipole oscillations resulting in surface Plasmon vibrations occur when a visible electromagnetic field is joined to the collective oscillation of transmission electrons.

Figure 1: Color change indicates the creation of SNPs.

pH Levels

We chose to optimize at five distinct pH levels: 5.5, 6.0, 6.5, 7.0, and 7.5%. After incubation, the UV-Vis values were taken that are shown in Figure (2). The optimal pH for silver nanoparticle production by B. pseudomycoides MT32 was determined to be 6.5. A significant absorbance record was seen at a low pH, and several studies have documented a rise in SNP production at this pH. Reduced production and decreased absorbance were seen at higher pH values, which may be due to the NO3 - reductase enzyme becoming deactivated as conditions got more alkaline.

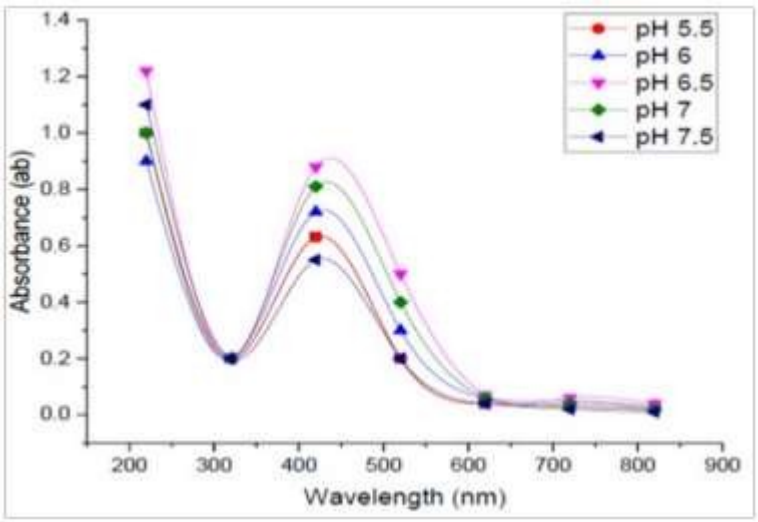

Figure 2: UV- Vis spectra of different pH values used during SNPs biosynthesis.

Greenish yellow coloration in the solution and mirror-like lighting on the walls of the Erlenmeyer flask were found to indicate that the used bacteria approved of the bio production of SNPs in the interaction mix. Third example: a change in color denotes the presence of a new SNP.

Figure 3: Color change indicates the creation of SNPs biosynthesis.

Antifungal Study In vitro

The antifungal activity of the SNPs generated by B. pseudomycoides MT32 at 5 different concentrations was investigated using PDA media and 10 fungus species typically found in Egyptian agriculture and generating various difficulties in crop output, animal nutrition, cleanliness, and performance.

Table 1: Inhibitory effect of the produced SNPs against the tested fungi

NO	Test pathogen	Growth diameter (Cm)								
		*PDA Only	**PDA+ AgNO3	Concentration of SNPs (µg/ ml)						
				10	30	50	70	90	MIC	MFC
1	*Aspergillus flavus*	9	8	7	5	4	2	0	85	90
2	*Aspergillus niger*	9	8	7	5	3	1	0	80	85
3	*Aspergillus tereus*	8	7	6	5	3	1	0	80	85
4	*Penicillum notatum*	8	7	6	4	2	0	0	70	75
5	*Rhizoctonias olina*	9	8	7	6	4	2	1	95	100
6	*Fusarium solani*	9	8	8	6	5	2	0	90	95
7	*Fusarium oxysporum*	9	8	7	5	3	1	0	75	80
8	*Trichoderma viride*	9	8	8	7	5	2	1	95	100
9	*Verticillium dahliae*	8	8	7	5	3	2	0	75	80
10	*Pythium spinosum*	9	8	8	6	3	1	0	75	80

In Fig. 4a. Silver nanoparticles produced with varying amounts of O. kilimandscharicum plant extract exhibited distinct inhibition rates. At 30% plant extract concentration, 66.5% inhibition was detected against F. oxysporum, but at 50% plant extract concentration, only 28.3% inhibition was seen. Silver nanoparticles produced with varying amounts of plant extract inhibited C. gloeosporioides growth by 18.4-48.7%. The results demonstrate that the concentration of plant extract affects the rate of inhibition. The highest effective concentration of plant extract for synthesizing silver nanoparticles and inhibiting infections in vitro was 30%.

In Fig. 4b, we see the antifungal activity of silver nanoparticles generated at various temperatures (RT, 40, 60, and 80 °C) for 10 min using extract of O. kilimandscharicum. F. oxysporum was inhibited by 28.7% on PDA at a concentration of 50 ppm when the sample was prepared at room temperature. The proportion of inhibition was found to be higher in heated samples. At 40 °C, 62% inhibition was shown for F. oxysporum on PDA, whereas at 60 °C, 47% inhibition was observed, and at 80 °C, 47% inhibition was observed. C. gloeosporioides grew differently depending on the temperature of the sample preparation. Nanosilver, when present at a concentration of 50 ppm, was shown to inhibit growth by 22% in samples created at room temperature without heating them. Heated samples had a greater inhibitory impact than those kept at ambient temperature. The percentage of inhibition was found to be highest for nanosilver generated between 40 and 80 °C, ranging from 28% to 46%. Nanosilver's antifungal impact was more pronounced in heated samples compared to room temperature controls. When treated at 60 °C for 10 minutes, nanosilver produced using O. kilimandscharicum extract showed the greatest inhibitory activity against both fungi.

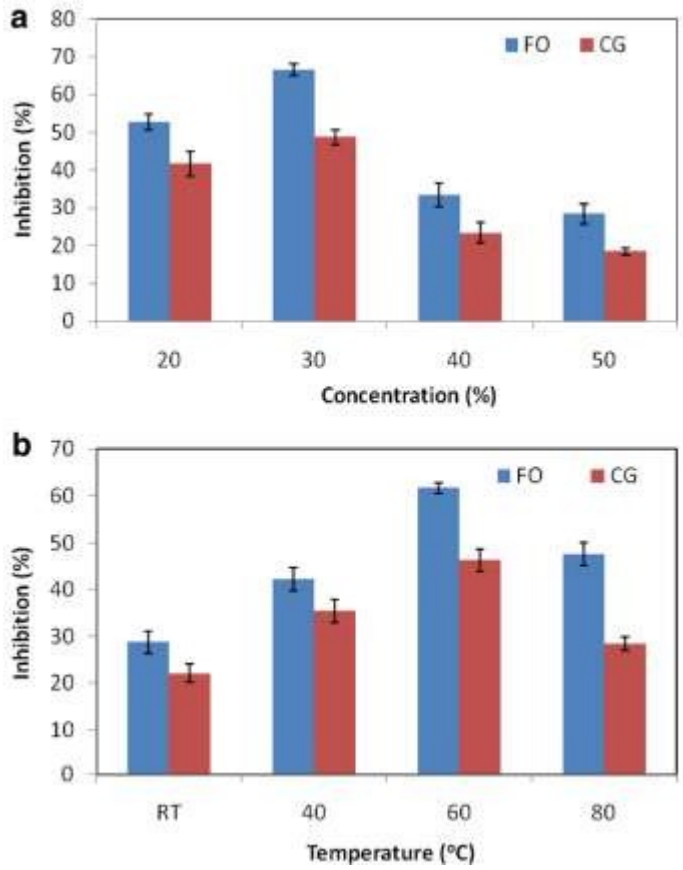

Fig. 4 Antifungal activity of silver nanoparticles

The effectiveness of silver nanoparticles synthesized at room temperature against plant-harming fungus, bacteria, etc. was evaluated after incubation for 24 hours at 30 percent O. kilimandscharicum plant extract and heating at 60 degrees Celsius for 20 minutes. In vitro tests were performed using varying quantities of silver nanoparticles. The effectiveness of silver nanoparticles as antifungal agents against the plant disease Fusarium oxysporum was investigated. After incubation at ambient temperature for 24 hours or after heating at 60 °C for 20 minutes, radial development was assessed at days 4 and 7 (Table 2).

Table 2 Antifungal activity of silver nanoparticles against Fusarium oxysporum

Treatment	Radial growth (mm)	
	4 days	7 days
AgNP-RT25 ppm	23.00b	45.50b
AgNP-RT50 ppm	14.75d	38.25c
AgNP-RT75 ppm	0.00e	0.00e
AgNP-RT100 ppm	0.00e	0.00e
AgNP-H25 ppm	25.00b	46.75b
AgNP-H50 ppm	19.50c	40.25c
AgNP-H75 ppm	12.50d	28.50d
AgNP-H100 ppm	0.00e	0.00e
Control	44.50a	67.75a
SE ($N=4$)	1.14	1.15
5% LSD	3.34	3.37

CONCLUSION

Based on the results of this investigation, it seems that B. pseudomycoides MT32, a soil isolate, is an efficient SNPs producer, with optimal growing conditions including an incubation temperature of 30o C and a pH of 6.5 for the growth medium. Depending on the strain of fungus utilized, the MIC and MFC values reported were between 70 and 90 and 75 and 100 g/l, respectively. Biosynthesized silver nanoparticles were very effective against both the fungi and bacteria that were used in the study. The strongest antibacterial activity was found in silver nanoparticles produced with 30% leaf extract.

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