American Journal of Botany and Bioengineering
https://biojournals.us/index.php/AJBB
ISSN: 2997-9331

Using Nanotechnology to Control Penicillium Digitatum

Emad Abd Atia
Department of Biology, College of Science, Al Muthanna University, Samawah, Iraq

Received: 2024 19, Dec
Accepted: 2025 28, Jan
Published: 2025 28, Feb

Annotation: The application of nanomaterials to control several types of microbial species, such as agricultural pathogens and laboratory contamination, can lead to the preparation of new effective fungicides for the control of fungi. This research includes the preparation of silver nanoparticles (AgNPs) using green leaves of Allium cepa. One milliliter of AgNO3 solution was added to 99 milliliters of plant extract. After 45 minutes, the color of the solution became brown, indicating the formation of AgNPs. Several concentrations (0 ppm, 50 ppm, 100 ppm, 250 ppm, and 500 ppm) were prepared. One milliliter of each concentration was added to Petri plates before adding the PDA media. The antifungal action of silver nanoparticles produced through natural plant extracts was explored against Penicillium digitatum. The concentration of 500 ppm AgNPs showed the maximum antifungal action against Penicillium digitatum, with an inhibition rate of 68.46%, while the concentration of 250 ppm exhibited 53.85% inhibition. The lowest antimicrobial action was observed at 50 ppm, with an inhibition rate of 23.07%. Higher concentrations showed greater inhibition compared to lower concentrations, which can be attributed to the increased number of AgNPs at higher concentrations, allowing for broader coverage and better interaction with the fungal surface. The higher particle density enables more effective contact with and compaction of the fungal hyphae. Finally, it can be concluded that

AgNPs are a viable substitute for other chemical formulations used in agriculture and the food industry as fungicides.

Keywords: Silver nanoparticles; Penicillium digitatum; Antifungal activity.

1. Introduction

Nanotechnology has gained widespread popularity in recent years, being extensively used in science and technology to develop new materials at the nanoscale (1 nm - 100 nm). It has found applications in various fields such as healthcare, environmental pollution cleanup, medical imaging, and military purposes. Among the different types of nanoparticles, metallic nanoparticles—particularly those made of gold, iron, and silver—have shown significant potential in biomedical applications due to their large surface area and unique biological properties (Hui Wen et al., 2023; Albrecht et al., 2006).

Silver nanoparticles (AgNPs) have garnered particular interest due to their broad antimicrobial activity and relatively low side effects. Historically used as a disinfectant, silver has seen renewed interest in recent years, especially with the rise of antibiotic-resistant bacteria (AbdelRahim et al., 2017). AgNPs are particularly effective in interacting with bacterial cell membranes, altering signaling pathways, and generating oxidative stress, which leads to bacterial death (Kim et al., 2012).

Various methods, including electrochemical reduction, photochemical reduction, heat evaporation, and biological approaches, are employed for synthesizing silver nanoparticles. However, as documented in the literature, these techniques tend to be costly and often involve the use of hazardous chemicals, posing risks to both the environment and biological systems (Verma and Mehata, 2016). Recently, the use of plant extracts for silver nanoparticle synthesis has gained significant attention due to its eco-friendly nature. Plant extracts serve as both reducing and capping agents in nanoparticle synthesis. Extracts from plants like Moringa oleifera (horseradish), Azadirachta indica (neem), Trigonella foenum-graecum (fenugreek), and Citrullus colocynthis (bitter apple) have been successfully used in the production of metal nanoparticles (Verma et al., 2016).

Penicillium digitatum is a widespread plant pathogen known as green rot, which infects citrus fruits and causes significant economic losses, especially post-harvest (Qiya Yang et al., 2019). This fungus can only infect citrus fruits after the fruits are exposed to mechanical wounds, leading to the entry and fixation of fungal spores as a result of their contact with the wounded tissues, and thus the symptoms of citrus soft rot appear (Wang et al., 2015).

This study aims to explore the potential use of biogenic silver nanoparticles synthesized from plant extracts in controlling the growth of Penicillium fungi, providing an eco-friendly alternative to conventional antifungal methods.

Materials and methods

2-1: Experience Tools:

Test tubes, electric mixer, petri dishes, pipette, Bunsen burner, tandard Flask and Cylinder, centrifuge device, A piece of filter cloth, Sterile forceps and a laboratory spoon (with a flat end), balance, water path, Incubator, Autoclave.

2-2: Method of work:

2-2-1: Preparation of medium

It was prepared in the biological lab in Biology department in college of science in Almuthanna university. It was taken the grams above of peeled potatoes stood cut into pieces and boiled in water. Then used filter for extract the boiling potatoes. The other compound dissolved in potato extract and Adding 1000 ml via addition distilled water. The final mixture was disinfected at 1.1 kg cm-2 pressure for 20 min.

2-2-2: Isolation of the fungus

Penicillium digitatum was isolated from orange fruits after collecting them from local markets and leaving them in a humid place for seven days. Transferred the fungus to PDA plates that were sterile. They stayed held at 27.1°C for a week to promote the growing of the fungus.

2-2-3: Prepare the extract:

Green onion leaves are collected from the local market. The damaged leaves have been removed, the green leaves which are clean from the effects of insect and microbial injuries collected. The leaves were washed with distilled water for three repeat, and after removing the impurities, the leaves were cut and dried in the oven with a degree of 50 ° C for 3 days. Then it was cut by the mixer. Take the weight of 30 grams of dry leaves and add 450 ml of distilled water. Then heat the solution at a temperature of 90 ° C to ensure the evaporation of water to get 100 ml. After the solution was cooled, the solution was filter with filter paper.

2-2-4: Preparation of AgNPs

The silver nitrate solution was prepared by adding 6 grams of silver nitrate to 100 ml of distilled water after the cylinder was wrapped to be dark to prevent the oxidation of the silver nitrate and was heated with a degree of 60 with stirring.

Take a volume of 10 ml of silver nitrate solution and added to 90 ml of green onion solution. The samples were put for 45 minutes with continuous stirring at the room temperature to ensure that the nanoparticles are obtained. The nanoparticles of the extract are separated by using the centrifuge at 3000 rpm for 10 minutes. Washed and repeated for three times to ensure the best percentage of the purity of the nanoparticles.

1 gram of silver nitrate was taken in 1000 ml of distilled water to get 1000 parts per million. different concentrations were prepared (0, 50, 100, 250 and 500) parts per million and according to the calibration law (N1 x v1 = n2 x v2) (Chemiasof, 2011).

One ml of all concentrations was added to Petriplates separately before add the PDA. The medium was dispensed in sterilized plates. 5 mm of mycelial were collected from the fungal culture and added to the center of medium which are poisoned by Nanoparticles and keep in incubator at 27±1° C to 7 days. Used the scale to meager the growth of fungi in poisoned Petriplates and comparison with control Petriplates for each concentration.

The documented growth distance of the cotton plant's stem as a means of measuring the inhibited zone. The size of the colony of the fungus in the controlling plate stayed likewise documented. The percentage of growth reserve of the fungus was estimated by means of the formula proposed via Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where, I = per cent inhibition , C = growth in control, T = growth in treatment.

2-2-5: Ultraviolet-Visible Spectroscopy Analysis (UV)

To confirm the biogenetics synthesis of Ag nanoparticles, the UV-visible spectroscopic analysis was used. The sample was dissolved in a deionized water. The range of wavelength was between 200 and 900.

3: Results and Discussions

The specific investigation on the biosynthesis of silver nano-particles via natural plant extracts like *Allium cepa* is mentioned in this article. The ions of silver were converted into nanoparticles when they were added to the natural plant extract of *Allium cepa*. After 45 minutes of the reaction, it was noticed that the solution's color changed from yellow to a bright yellow and then to a dark brown, which suggested that silver nanoparticles were forming. AgNPs absorption spectrum showed bands at 211–314 nm in the UV region. The result 3of UV spectrum aligned with those reported by (Gupta, 2017). The maximum absorption peaks occurred at 211 nm (Fig 1).

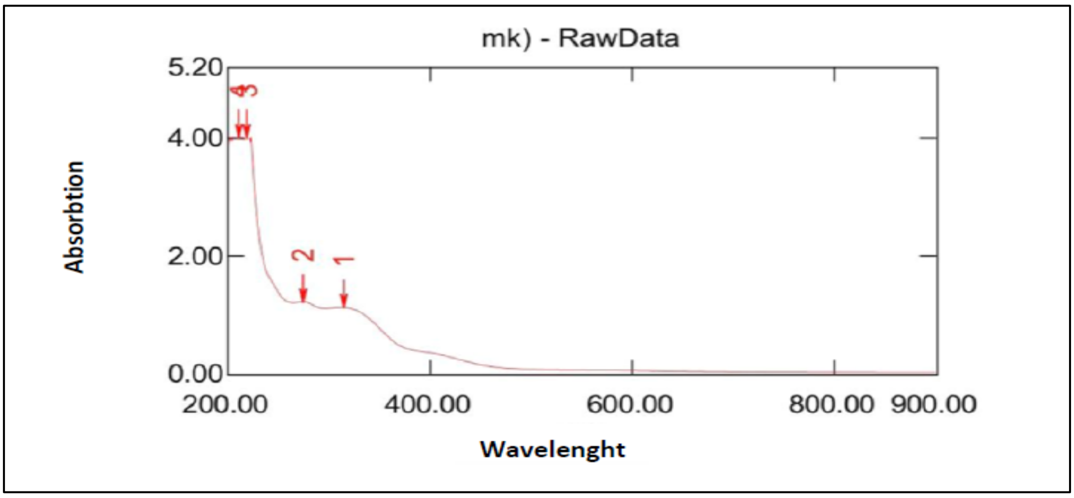

Figure (1): Ultraviolet-visible spectroscopy for Ag NPs

The observation revealed that the decrease in the number of Ag+ ions ensued external of the cell. (Mengqin Xu, *et al.*, 2023; Nestor *et al.*, 2008).

Many researchers have documented the creation of nano-particles with plant extracts for the purpose of biosynthesis. The synthesis of spherical silver nanoparticles by means of a purposely designed composite derived from henna, the extraction of this compound from the leaves of henna at ambient conditions (Najla, *et. al.*, 2023; Kasthuri *et al.*, 2009). By means of green tea as a decreasing agent and stabilizer, gold and silver nano-structures were created in water at room temperature by Nestor *et al.*, 2008. According to the literatures, the synthesis of AgNPs by plant extracts obtained from green components such as live alfalfa, lemongrass bouillies, geranium leaves, and other plants have been achieved . (Torresdey *et al.*, 2003; Shankar *et al.*, 2003b, 2005).

In this work, The antifungal activity of silver nanoparticles derived from natural plants was evaluated against *Penicillium digitatum*. The percentage of inhibition of the diameter of the well with solution of the silver nanoparticle is listed in Table 1. The particles of silver that were produced by the green leaves of *Allium cepa* were exhibited a good antifungal activity against *Penicillium digitatum*, this was followed by particles of concentration 500ppm with inhibition (68.46%) and particles of concentration 250ppm (53.85%). The less effective particles of silver were produced with a concentration of 50ppm (23.07%). The silver nanoparticles demonstrated an effective anti-microbial property that was greater than other concentrations in regards to the larger concentration that had the greatest effect, while the others had a smaller effect. This would be attributed to the high concentration of the solu tion that was able to cover and compact the fungal hyphe (Fig 2).

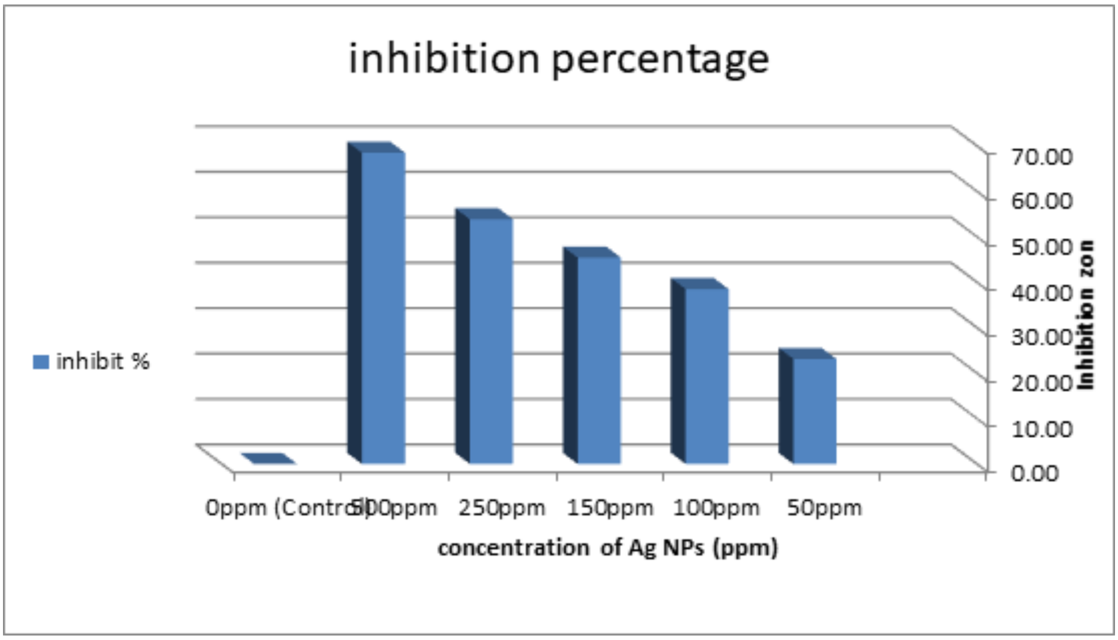

Fig 2: Evaluation of Silver nanoparticles against *Penicillium digitatum*

Reports on the mechanism by which silver ions inhibit microorganisms have demonstrated that, following treatment with Ag+, the DNA lost its ability to replicate, this led to the inactivation of the expression of the ribosomal subunit proteins as well as other proteins and enzymes complicated in the manufacture of ATP (Fuad Ameen, *et. al*., 2023). Other hypotheses have been proposed that concern the effect of Ag+ on enzymes that are membrane-bound, such as those involved in the respiratory chain (Farzaneh Afkhami, et. al., 2018), (Najla A. Alshaikh, et. al., 2023). Overall, AgNPs exhibited a potent anti-fungal effect on the various species of fungi that were tested in vitro. This effect was attributed to the destruction of membrane integrity, which led to the conclusion that AgNPs have a significant anti-fungal capacity. Further research is necessary for field-oriented applications.

Table 1: Evaluation of Silver nanoparticles *against Penicillium digitatum*

concentration of Ag NPs (ppm)	growth (mm)	inhibit %
50ppm	50	23.07692308
100ppm	40	38.46153846
150ppm	35.5	45.38461538
250ppm	30	53.84615385
500ppm	20.5	68.46153846
0ppm (Control)	65	0
mean	40.16666667	38.20512821
S.Em=	0.29	
CV=	1.43	

Conclusion

The silver nanoparticles have been created by the green leaves of *Allium cepa*, and they're used as an antifungal in this research, the potential of AgNPs to regulate the behavior of fungi like *Penicillium digitatum* is demonstrated. This outcome can lead to the progress of new environmentally responsive and operative fungicides for the treatment of Fungi. The biologically created silver nano-particles could have an important role in medical science due to their efficient antifungal properties. finally, we can state that AgNPs are a viable substitute for other chemical formulations that are used in the medical, agriculture and food industries as fungicides.

Declaration of Conflict Interest

The authors declare that they have no conflict of interest.

Data availability statement

All data and information are available in the manuscript.

Funding

Non applicable.

References

1. AbdelRahim, K., Mahmoud, S. Y., Ali, A. M., Almaary, K. S., Mustafa, A. E. Z. M.A., and Husseiny, S. M. 2017. Extracellular biosynthesis of silver nanoparticles using Rhizopus stolonifer. Saudi J. Biol. Sci. 24, 208–216. doi: 10.1016/j.sjbs.2016.02.025
2. Chemiasof 2011. Guide To Preparation of Stock Standard Solutions First Edition, (May) , pp. 22–61.
3. Farzaneh Afkhami, Parisa Forghan, James L. Gutmann, and Anil Kishen, 2023. Silver Nanoparticles and Their Therapeutic Applications in Endodontics: A Narrative Review.Pharmaceutics, 15(3),715; https://doi.org/10.3390/pharmaceutics15030715
4. Fuad Ameen, Ali A. Al-Homaidan, Ahmed Al-Sabri, Abobakr Almansob and Saleh AlNAdhari, 2023. Anti-oxidant, anti-fungal and cytotoxic effects of silver nanoparticles synthesized using marine fungus Cladosporium halotolerans. Applied Nanoscience volume 13, pages623–631
5. Gupta, R. K., Kumar, V., Gundampati, R. K., Malviya, M., Hasan, S. H. and Jagannadham, 2017. Biosynthesis of silver nanoparticles from the novel strain of Streptomyces Sp. BHUMBU-80 with highly efficient electroanalytical detection of hydrogen peroxide and antibacterial activity. J. Environ. Chem. Eng. 5, 5624–5635. doi: 10.1016/j.jece. 09-029
6. Hui Wen, Huanbin Shi, Nan Jiang, Jiehua Qiu, Fucheng Lin and Yanjun Kou, 2023. Antifungal mechanisms of silver nanoparticles on mycotoxin producing rice false smut fungus. iScience 26, 105763.
7. Kasthuri, J., Veerapandian, S. and Rajendiran, N., 2009. Biological synthesis of silver and gold nanoparticles using apiin as reducing agent. Colloids Surf. B: Biointerf. 68, 55–60.
8. Kim, S. W., Jung, J. H., Lamsal, K., Kim, Y. S., Min, J. S., and Lee, Y. S. 2012. Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology 40, 53–58. doi: 10.5941/MYCO.2012.40.1.053
9. M. A. Albrecht, C. W. Evans and C. L. Raston, 2006, Green chemistry and the health implications of nanoparticles, Green Chem., 8, 417–432
10. Mengqin Xu, Changjin Wang, Weidong Huang & Haibing Yu, 2023. Synergistic antifungal effect of silver nanoparticles combined with mancozeb against Botryosphaeria dothidea. Inorganic and Nano-Metal Chemistry 53(4): 421–427.
11. Najla A. Alshaikh, Kahkashan Perveen and Ali H. Bahkali, 2023. Effect of silver nanoparticles alone and in combination with fluconazole on Candida albicans. Journal of King Saud University - Science 35(1):102399.
12. Nestor, A.R.V., Mendieta, V.S., Lopez, M.A.C., Espinosa, R.M.G., Lopez, M.A.C.and Alatorre, J.A.A., 2008. Solventless synthesis and optical properties of Au and Ag nanoparticles using Camiellia sinensis extract. Mater. Lett. 62, 3103–3105.
13. Qiya Yang , Xin Qian , Solairaj Dhanasekaran , Nana Adwoa Serwah Boateng , Xueli Yan , Huimin Zhu , Fangtao He , Hongyin Zhang , 2019. Study on the Infection Mechanism

of Penicillium Digitatum on Postharvest Citrus (Citrus Reticulata Blanco) Based on Transcriptomics. Microorganisms .7(12):672. doi: 10.3390/microorganisms7120672

14. Shankar, S.S., Ahmad, A. and Sastry, M., 2003b. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog. 19, 1627– 1631.
15. Shankar, S.S., Rai, A., Ahmad and A., Sastry, M., 2005. Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings. Chem. Mater. 17, 566–572.
16. Torresdey, J.L.G., Gomez, E., Videa, J.R.P., Parsons, J.G., Troiani, H.and Yacaman, M.J., 2003. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir 19, 1357–1361.
17. Verma A, Mehata MS. 2016. Controllable synthesis of silver nanoparticles using neem leaves and their antimicrobial activity. J Radiation Res Applied Sci. 9(1):109–115.
18. VINCENT J.M., 1947, Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 159:850–850.
19. Wang M., Sun X., Zhu C., Xu Q., Ruan R., Yu D. and Li H., 2015. PdbrlA, PdabaA and PdwetA Control distinct stages of conidiogenesis in sssPenicillium digitatum. Res. Microbiol. 166:56–65. doi: 10.1016/j.resmic.2014.12.003.