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Biosynthesis And Characterization Of Silver Nanoparticles Using Azadirachta Indica (Neem) Leaves Extract And Post-Harvest Applications On Fruits

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Abstract:
The secretion of phytochemicals as reducing agents from Azadirachta indica can be used for synthesis of metal nanoparticles from metals salts. One of the ecofriendly and reliable processes for manufacturing silver nanoparticles, AgNPs , shows significant milestones in the field of nanotechnology. Biosynthesis is more preferable than chemical synthesis method since it is cost effective, sustainable towards environment, no pollution to soil or environment, improvises environmental conditions and human health safety. Thus, the biosynthesis of silver nanoparticles using neem leaves extract was carried out in present study. The process was carried out by treating aqueous silver nitrate solution with neem leaf extract under controlled conditions. The results obtained by reduction of silver ions to silver nanoparticles with enzyme substrate complex principle was confirmed by colour change under sunlight due to oxidation methods with techniques such as UV visible spectroscopy, Transmission electron microscopy (TEM). The UV visible spectra of green synthesized silver nanoparticles from Neem extract was scanned between the ranges of 450-500 nm. Under TEM analysis, the nanoparticles observed were of 2-60nm, which was scattered, dispersed, spherically shaped. Antifungal activity was conducted in this study to check the effect of silver nanoparticles against Aspergillus niger . Post harvest applications on fruits was studied in this study to check the effects of shelf life period of fruits after application of silver nanoparticles solution from neem leaf extract.

Keywords: *Azadirachta indica, Neem leaf extracts, Silver nanoparticles (AgNPs), TEM, UV analysis, Green synthesis, characterisation, Antifungal activity, Aspergillus niger, post harvest fruits*

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I. Introduction:

Nanoparticles are referred to with a particle having dimensions from 1-100nm. Being special in size they contribute to special antimicrobial properties. Silver demonstrates immense promise for being an effective contender among nanoparticles showing effective agents against microbial growth. Being cost effective, sustainable towards the environment, reduced toxicity and reduced soil pollution are the benefits of AgNPs synthesized biologically. Additionally, silver nanoparticles have special attention towards being the exception of being antibacterial (Banerjee et al., 2014; Velusamy et al., 2015) and antifungal (Kim et al., 2012; Jafari et al., 2015) These nanoparticles contribute to their growing fascination surrounding by its low toxicity, biocompatibility and eco-friendly nature . For generating nanoparticles, this uniqueness leads to green synthesis as a promising process. Using various plant parts like leaf, roots, stem, and microbes such as bacteria, fungi and yeast can be included to produce nanoparticles in green synthesis techniques. This method offers advantages in contrast to chemical and physical synthesis methods in terms of both cost effectiveness and environmental sustainability. (Nangare and Patil, 2020). Currently, there have been biosynthesis methods utilized by plants extracts have been studied from *Pelargonium graveolens*, *Azadirachta indica*, *Cinnamomum,* camphor, Lemongrass, *Medicago sativa* which has gained considerable interests as alternative methods (Verma and Mehta, 2016). We have made an attempt on the green synthesis of AgNPS using *Azadirachta indica* leaf extract. Synthesis of various nanoparticles such as gold, silver, copper, zinc, leaf extract of *Azadirachta indica* plays a vital role as stabilizing agents. The leaf extracts show presence of phytochemicals such as flavonoids and terpenoids which acts as both reducing agents and capping agents that stabilizes the nanoparticles. With the techniques of enzyme substrate complex, the phytochemicals from leaf extract of Azadirachta indica, triggers the silver salt into silver nanoparticles (Verma and Mehta., 2016) . The formation of silver nanoparticles takes place

as leaf extract which acts like reducing agents without any help of chemicals (Lalitha et al., 2013). The formation of silver nanoparticles (AgNPs) takes place by treating aqueous silver nitrate solution with air dried neem powder and making leaf extract by boiling in distilled water under controlled conditions. The formation of silver ions to silver nanoparticles can be confirmed by colour change from yellow to brown-black indicating the formation of AgNPs (Ghazali et al., 2022). Hassanin et al., 2021 used neem leaf extract as reducing and stabilizing agents for nanoparticles by synthesising $AgCo_3O_4$ nanoparticles with different weight ratio of AG/CO_3O_4 and the results were identified with techniques such as Transmission electron microscopy (TEM), X-ray diffraction (XRD) , Scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) and UV-visible spectrophotometry. Zanige and Khan (2021) studied the synthesis of silver nanoparticles using aqueous extract of neem leaves with 1Mm of silver nitrate salt ($AgNO_3$). According to Saravanan (2021) *Azadirachta indica* leaf extract was treated with silver nitrate salt ($AgNO_{3)}$ and was identified as AgNPs using FTIR, SEM, TEM, XRD. In this study, silver nanoparticles (AgNPs) were synthesized with a biosynthesis method of neem leaf extract as the core ingredient. The results obtained from AgNPs were characterized by using different techniques such as TEM analysis, UV spectroscopy analysis to study the crystal formation of nanoparticles.

II. Materials And Methods:

Culturing and preparation of plant extract:

This study was conducted during October 2025 in the Biological Laboratory, Mumbai. To study the biosynthesis of silver nanoparticles, neem leaves were collected from local street side trees from Mumbai areas. The collected leaves were first thoroughly washed with tap water to remove the dirt. The leaves were later sundried for 5 days under the sunlight. These leaves were later crushed and made into a powdered form. 10 grams of leaves powder was measured in 100 ml distilled water at 85^{o}C for a duration of 25 minutes, resulting in the extraction of phytochemicals from the leaves. This mixture was further filtered with Whatman filter paper No. 1 into a conical flask to obtain the clear extract. To maintain its purity and prevent contamination, the leaf extract solution was stored in the refrigerator at 4^{o}C for further subsequent utilisation in future experiments.

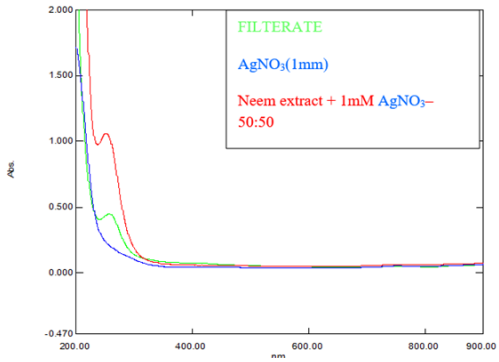
Biosynthesis of Silver Nanoparticles:

To begin with synthesis of silver nanoparticles (AgNPs), 1mM of an aqueous solution of silver nitrate ($AgNO_3$) was prepared (0.086g in 500ml distilled water). The leaf extract of Neem which had reducing properties was mixed with the 1mM concentration of silver nitrate solution in different concentrations of 1:9ml and introduced in a conical flask. This solution was now kept under sunlight to observe the changes. As the reaction progressed, there was noticeable colour change observed in the solution which gradually became darker from a range of yellow to dark brownish black colour. Which indicated the visual confirmation of synthesis of silver nanoparticles due to its oxidation reaction under sunlight. After every 5 minutes the solution was run under UV spectra analysis machine to check the peak which formed due to synthesis of nanoparticles against the solution of neem extract and silver nitrate.

III. Characterization Of Silver Nanoparticles:

UV Visible Spectra Analysis:

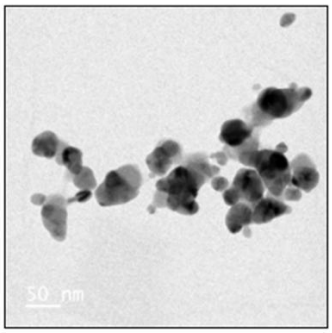
To confirm the formation of nanoparticle synthesis, the sample after keeping under sunlight was ran through UV visible spectra analysis after every 5 minutes. The reading of absorbance was recorded within the range of 200-500nm, using UV-Visible spectrophotometer (Hitachi U2910 spectrophotometer, Japan). This technique helped to record the quantification of changes in absorbance and gave insights into reduction of silver ions during the process of silver nanoparticles. (Renugadevi and Ashwini, 2012) . The peak at which nanoparticles solution was observed was at 265nm.

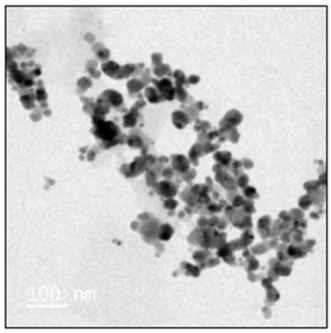


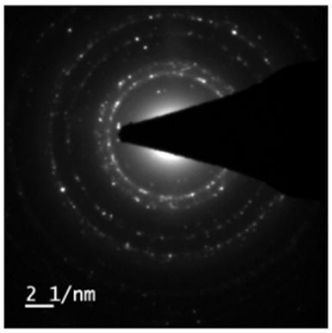
UV Analysis of Neem leaf extract, 1mM of AgNO3 & AgNPs solution

Transmission Electron Microscope (TEM)

To further confirm the shapes and size and surface morphology of nanoparticles, TEM analysis was carried out. The sample was run through TEM from IIT, Powai. The silver nanoparticles observed under TEM analysis were globular, spherical in shape with smooth surface morphology. The diameter of these AgNPs seen in TEM images ranges from 2-60nm and are mono scattered in nature. The average size of nanoparticles observed was 50nm.



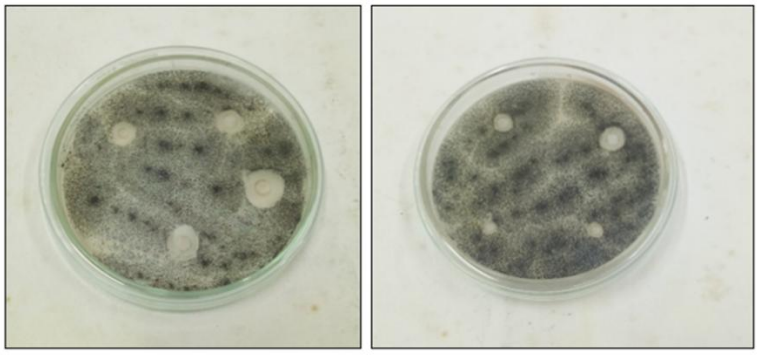




Antifungal activity of AgNPs:

The AgNPs from neem leaf extract was assessed to check the antifungal activity by using agar well diffusion method against *Aspergillus niger*. Potato dextrose agar was made under sterile conditions and was cultured with a swab loaded with fungal suspension on it. Wells were made on this agar plates using a sterile cork borer and 100µl of concentrations of AgNPs was introduced to each well to compare results. 1st well had 10ppm of AgNPs solution, 2nd well had 20 ppm of solution and 3rd well had 50ppm of solution. Well in the center had control which was used as the negative control. The plates after infusion of AgNPs solution was further kept under room temperature 30^{o}C fro 24 hours to study the effect of AgNPs against fungi *A. niger*

It was observed that the growth after 24 hours, 50ppm showed the greater zone of inhibition as compared to the other concentration of AgNPs solution. Several studies demonstrated that the morphology of the nanoparticles influences the antimicrobial activity. The particles of nanoparticles small is the size, greater it provides surface volumes which later creates extensive contact for interaction of silver ions with targeted pathogen(Wan Nur Atiqah Wan Shamsudin)



Antifungal activity against *A.niger* on AgNPs

Post Harvest Effects Of Application Of Nanoparticles On Fruits:

The nanoparticle solutions were sprayed on peeled fruits to test their effects on the shelf life of fruits. One control orange with no applications was considered as control and three oranges were sprayed with different concentrations of nanoparticle solution and kept in room temperature 30 degree for 5 days to check the effect the shelf life period of nanoparticles solution on fruits. Observations were noted regarding their appearances on different days. It was observed that on 5th day, samples sprayed with 10 ppm and 20 ppm concentrations of nanoparticles released pungent odour and had changes in their shapes, while the fruits sprayed with 50 ppm concentrations of nanoparticles were healthy.

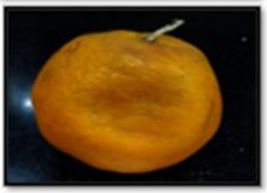
Sample name	Concentration of nanoparticles	
Orange	Control	
	10ppm	
	20 ppm	
	50 ppm	

Table 4.6.1: Application of AgNPs – ***flavus*** **on (orange) at different concentrations compared to control**

IV. Conclusion:

The AgNPs biosynthesized from neem leaf extract are environmentally sustainable, cost effective, and simple. This study suggests that synthesized nanoparticles can be used as antifungal agents on fruits and vegetables. The average size of nanoparticles exhibit up to 50nm which serves excellent antifungal activities against *Aspergillus niger*. In current times there has been a genuine need in developing methods for the synthesis of nanoparticles that are ecofriendly, time conserving, cost effective and simple .We can conclude that the method we applied in synthesizing silver nanoparticles from neem leaf extract is easier to carry out and satisfies the above mentioned criteria. Also they can be further studied for its application and properties. The successful synthesis of silver nanoparticles using neem leaf extract with silver ions was carried out using different characterization techniques. TEM analysis revealed the presence of silver nanoparticles ranging from 2-60nm and average with size of 50nm.The visible colour change of solution due to oxidation under sunlight from yellow to brownish black showed visual evidence of nanoparticle formation in solution. UV analysis showed wide peak significant absorbance at 265nm.

Future Scope:

Biosynthesis is the trending technology since it holds promise in creating reliable, sustainable solutions and innovative ways across domains. These green synthesized nanoparticles show efficient antimicrobial properties and can be used further as biopesticide in future in agricultural fields to replace harmful chemical pesticides. Its application can be further carried on post and pre harvest fruits application to increase the shelf life periods of fruits. This green nanotechnology serves opportunities for biomedical and agricultural fields for advancing sustainable and impactful solutions that shall benefit the society at large scale.

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