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Research Article

Green Synthesized Clove Nanoparticles: a novel, potent antifungal innovation

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Abstract

Sheath blight disease in rice is of major concern as it has led to a decrease in the rice crop yield worldwide. Therefore, to combat this particular fungal pathogen using a sustainable alternative, nanomaterials have come into play. This study reports the usage of green chemistry for the synthesis of silver nanoparticles using silver nitrate as the precursor agent and Syzygium aromaticum (clove) extract as the reducing agent and surfactant. The evaluation of the in-vitro antifungal activity was performed on Rhizoctonia solani using various concentrations of silver nanoparticles. Several methods like UV-Vis Spectroscopy and Scanning Electron Microscopy, were employed for characterization purposes. The Scanning Electron micrograph showed that nanoparticles were roughly spherical. The clove silver nanoparticles were highly effective in inhibiting the growth of the pathogen as observed and based on the obtained results, the clove nanoparticles can be investigated further as a probable replacement for synthetic fungicides.

Keywords: Green synthesis, silver nanoparticles, Rhizoctonia solani, plant nano-biotechnology, in vitro antifungal activity.

1. Introduction

A global estimation tells that the total food production will only be sufficient for only sixty per cent of the world population, as by 2050 it is expected to reach over nine billion. This predicted increase in population will also require a significant increase in crop yield to meet the requirements of the advancing global need for food [1]. Rice (Oryza sativa L.) is yet another staple crop in Asian countries and is also considered as the world's most widely consumed cereal crop with the leading producers being China and India). In India, it is grown over a land area of 43.95 million hectares with a production rate of 106.54 million tons [2]. This crop can be grown in any type of soil including alkaline and acidic soils. The major rice-growing states in India are Assam and other northeastern states, Chhattisgarh, Punjab, Orissa, West Bengal, etc. There are several numbers of rice varieties present all over the world among which IR64 is considered the best due to its wide adaptation, early maturity, improved quality, high yield, and disease resistance against brown planthopper (BPH), green leafhopper (GLH), grassy stunt virus and stem borer [3]. Lately, plant pests and pathogens have caused a significant reduction in the production yield of rice, the major pathogen being Rhizoctonia solani (R. solani) which causes sheath blight (ShB) disease-causing nearly fifty per cent damage to the yield [4]. The disease cycle starts with the advancement of abrasion which finally restrains the grain filling. R. solani is challenging to manage owing to the extensive host range of the pathogen even under unfavourable environmental conditions [5]. Strategies for pest management rely heavily on the use of chemical-grade fungicides whose disadvantages outweigh the advantages as many of these fungicides have a harmful effect. Thus, to combat this, an environment-friendly, efficient alternative is required which is adept by nanotechnology.

The field of nanotechnology has implicated great interest and is being increasingly seen as a promising solution for various problems ranging from the areas of medicine to sustainable agriculture. Nanoparticles are formulated from certain bioactive agents like plant materials and microorganisms and several forms of nanoparticles can be prepared like silver (Ag) nanoparticles, copper (Cu) nanoparticles, and silica-based nanoparticles that have significant importance due to their certain properties like low toxicity, biocompatibility, and eco-friendliness.

Recently, there has been a growing inquisitiveness about gold and silver nanoparticles as they present superior characteristics with handy flexibilities [6]. The use of nanoparticles has seen a rapid increase, especially in the use of silver nanoparticles as they are being utilized in various fields like medical, food, healthcare, consumer, and even industrial purposes due to the one-of-a-kind physical and chemical representations.

Because the surface-to-volume ratio of nanoparticles varies greatly, these nanoparticles are unique and can significantly change physical, chemical, and biological properties, so they are overworked for individual applications [7, 8]. To meet the requirements of the AgNP, several methods have been adopted for coalescence purposes. Broadly, the physical and chemical methods after being in use were considered to be expensive as well as hazardous [9]. Among the various synthetic methods used for the synthesis of nanoparticles the biological method of synthesis was deemed fit as a simple, reliable, and green approach that can generate clearcut dimensions and arrangement under revamped circumstances for translational investigations. After much research, the environmentally safe chemistry concept for the synthesis of silver nanoparticles shows an ample amount of promise.

In crop protection, nanotechnology has the potential to revolutionize the way we grow and protect crops. Nanoparticles can be used as delivery systems for pesticides, fertilizers, and other agricultural inputs [10]. They can also be used to enhance plant growth, increase nutrient uptake, and improve soil quality. The use of nanomaterials is widening and some of these include Zinc, copper, and silver nanoparticles which have been studied and tested for their antifungal, and anti-bacterial properties against a variety of pathogens [11].

Among the many nanoparticles in use, silver nanoparticles (Ag-NP) have caught the attention of researchers because of their unique characteristics including chemical stability, high catalytic activity, and anti-fungal efficiency [12].

There are numerous ways for producing nanomaterials mechanically which are not considered to be safe at times. Customarily, the nanoparticle synthesis takes place by putting into use, the three different methodologies which typically include the top-down or bottom-up approaches [13]. In physical terms, nanoparticles were composed by evaporation-condensation by applying the tube furnace at atmospheric pressure [14]. The usual substantial methods included spark discharging and pyrolysis for the synthesis of silver nanoparticles. Some of the advantages included speed and radiation which were in use as the reducing agents with no toxic chemicals in use. However, the detrimental effects included low yield and increased level of energy consumption, contamination of the solvent, and lack of orderly distribution [15]. Chemical method of nanoparticle synthesis incorporates several methods like cryochemical synthesis laser ablation, lithography, electrochemical reduction, laser irradiation, and many more [16]. The downside of these included the excessive use of hazardous chemicals and its effects on living beings and this has prompted researchers to look for safe and effective methods of production. Researchers have come up with the green synthesis method of nanoparticles using fungi, bacteria as well as plant [17]. Various plants that have been explored for the green synthesis of AgNPs include Callistemon lanceolatus Xanthostemon chrysanthus Cassia auriculate Andean blackberry and Ficus panda [18-20].

A plant that has received relatively little attention is Syzygium aromaticum, (L) commonly referred to as the clove [21]. This spice is popular for its aroma due to the presence of eugenol and rich antioxidants belonging to polyphenols, aromatic hydroxy acids, and many more. Several studies performed on clove have found its antiseptic, antibacterial, antifungal, and antiviral properties. Clove is also a beloved antidote for dental disorders and respiratory disorders. sore throat in daily medicines of Asian countries. This spice plant holds particular promise for preparing silver nanoparticles (AgNPs) owing to the phytoconstituents [22].

In this context, the study aimed to synthesize silver nanoparticles using the green synthesis method from Syzygium aromaticum L (clove) extract thereby evaluating the in-vitro antifungal activity of Ag-NPs on the phytopathogenic fungi Rhizoctonia solani known to cause increased damage to rice plants (Oryza sativa L).

2. Material and Methods

2.1. Plant Materials and Chemicals

Clove (Syzygium aromaticum) buds were bought from the local market of Kolkata, West Bengal, India. Culture media (Potato Dextrose Agar) SRL Pvt.Ltd, Petri plates, and silver nitrate present in the laboratory were used. The chemicals in use were of analytical grade with greater than 95% purity.

2.2. Microorganisms

The phytopathogenic fungi selected for the anti-fungal assay was Rhizoctonia solani. The stock culture of the same was obtained from the Department of Plant Pathology, Bidhan Chandra Krishi Vishwavidyalaya (BCKV), Mohanpur, Nadia, West Bengal.

2.3. Clove extract preparation

Clove buds of 25g were measured and thoroughly crushed into a fine powder using mortar-pestle available in the laboratory. It was mixed with 250ml of distilled water and heated at 80°C with continuous stirring for 1-2 hours in a beaker. The mixture was filtered using Whatman's filter paper, No.1, and kept at 4°C for further use. The filtrate obtained portrayed yellowish-orange colour. The whole process is depicted in Figure 1.

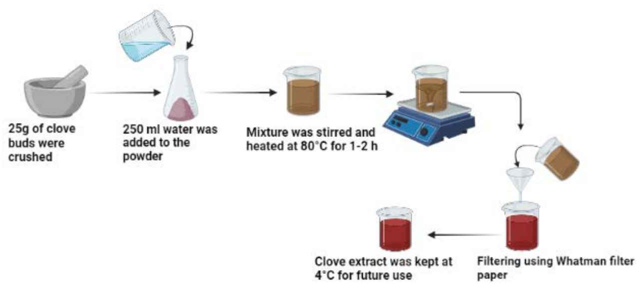

Figure 1: Schematic showing the Syzygium aromaticum (clove) extract preparation.

2.4. Synthesis and extraction of silver nanoparticles using Syzygium aromaticum (clove) extract

The synthesis of AgNPs was carried out by adopting the methodology of [23, 24]. 90 ml of 1 mM $AgNO_3$ solution was prepared and mixed with 10 ml of clove extract in a conical flask. Again, it was heated at 80°C for 2-3 hours with con-

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tinuous stirring. The formation of Ag-NP in conjugation with clove extract showcased a drastic color change from yellowish orange to dark brown which was visible by the naked eye. This dark brown extract was transferred into falcon tubes and centrifugation was initiated thrice at 10,000 rpm for 15 min. The supernatant was discarded, and the pellet was washed thrice with distilled water. As a result, greyish nanoparticles were observed at the bottom of the falcon tubes. The obtained nanoparticles were resuspended using 1ml distilled water. The entire process described above is illustrated in Figure 2

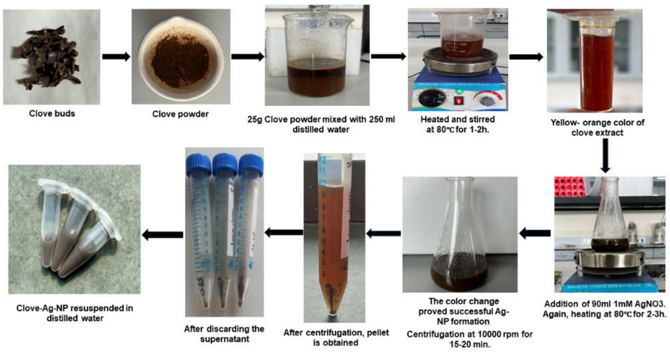

Figure 2: Green synthesis of the clove extract mediated Ag-NPs.

2.5. Characterization of the attained AgNPs

The samples were loaded into a UV-Vis spectrophotometer and the analysis was performed in the range of 200-800nm to investigate the successful formation of AgNPs. To evaluate the surface morphology,10µl supernatant of silver nanoparticles was taken on a glass stub (1cm. X 1cm.) and vacuum dried. The sample was then subjected to scanning electron microscopy using FEI Quanta 200 (FEI, Hillsboro, OR, USA).

2.6. Antifungal response on the mycelial growth of Rhizoctonia solani

To assess the response of Rhizoctonia solani upon exposure to the synthesized silver nanoparticles (AgNPs), the experimentations were carried out in triplicate with Potato Dextrose Agar (PDA) in the Petri plates under aseptic conditions by differing the concentration of the AgNPs. A standard sample without treatment of AgNPs was considered as control and different concentrations of the AgNPs were taken following the Poison plate method. The control plate was prepared by pouring 20 ml of PDA that was allowed to be set for some time, followed by the addition of a mycelial plug (5mm) at the centre that was obtained from the edge of the 7-day-old colonies of the received mother plate. A similar process was carried out for the plates having various concentrations of AgNPs. The plates were then subjected to incubation and were checked after a brief time period of 24,48 and 72 hrs.

Figure 3 displays a schematic diagram of the experimental design for the evaluation of the in vitro anti-fungal activity

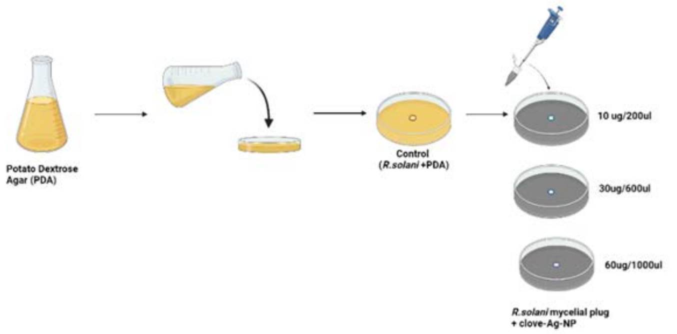

Figure 3: Experimental design carried out for the anti-fungal evaluation. Created with BioRender.com.

3. Results and Discussion

This section produces the various results of the optical properties demonstrated by the synthesized Ag-NPs followed by the assessment of the antifungal behavior of the AgNPs.

3.1. Visual inspection and absorbance analysis by UV-Vis Spectroscopy

The synthesis process was carried out with the aqueous extract and silver nitrate in the presence of heat. The addition of silver nitrate to the solution led to a color change from orangish yellow to dark brown Figure 4a which strongly inferred the formation of the clove-AgNPs. The formation of AgNPs was further confirmed by analyzing the synthesized nanoparticles as well as the clove extract with a UV-Vis spectrophotometer as demonstrated in Figure 4b.

These bio-transformed products were characterized by UV VIS spectroscopy performed at 24h, 48h, and 72h time points in order to study the change in light absorption of the solution with an increase in color intensity; a graph of which is shown in Figure 4c. It was generally recognized that the brown coloration in water was due to the surface plasmon resonance (SPR) effect and reduction of AgNO3 [25]. The strong surface plasmon resonance centered at ca.400 – 450nm increases with time. The absorption peak was about 405 nm, this is a typical range for Ag nanoparticles supported by literature.

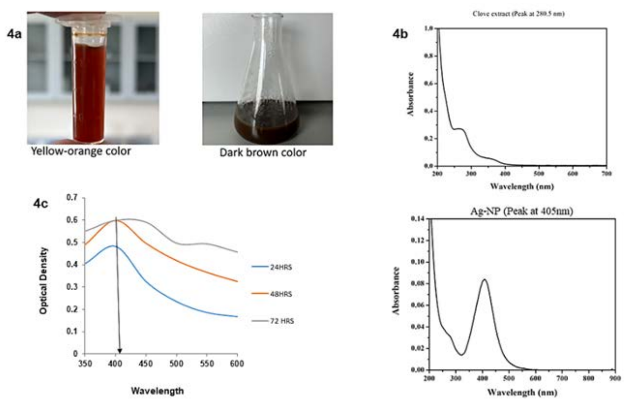

Figure 4: Characterization of the bio-fabricated clove-Ag-NPs. (4a) Preliminary identification of the nanoparticles through colour change. (4b) UV-visible spectrum for clove extract as well as clove-Ag-NP extract. (4c) Graph measured at the required time intervals indicating the absorption peak.

3.2. The morphological study by Scanning Electron Microscopy

Figure 5 shows a micrograph recorded from clove extract-based AgNPs. The structural features revealed that individual silver nanoparticles were spherical in shape. The vast majority of them were single crystalline in nature. The SEM studies confirmed the formation of the nanoparticles in the range of 20-100 nm thereby providing a clear indication of the formation of the silver nanoparticles.

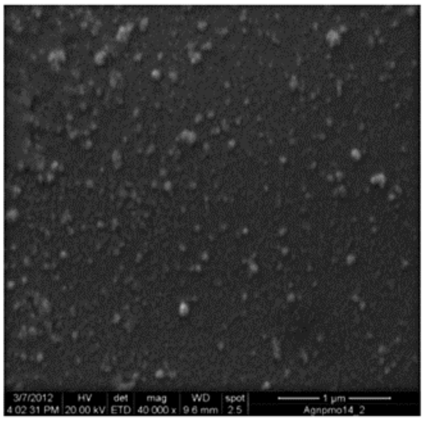

Figure 5: Scanning Electron Microscopy of the silver nanoparticles.

3.3. Analysis of the in vitro antifungal activity of the Clove-AgNPs

Figure 6 illustrates the antifungal activity of the Clove-AgNPs against the phytopathogenic fungi Rhizoctonia solani cultivated in agar mixed with varying concentrations of (10ug/200ul,30ug/600ul, 60ug/1000ul) AgNPs at observed time intervals of 24,48 and 72 hrs. This figure mainly depicts how using the poison food technique the silver nanoparticles caused a delay in the growth of fungi. A decrease in the growth of R. solani was recorded for a concentration of 30ug/600ul and 60ug/1000ul indicating that the percentage inhibition of the radial growth of R. solani increased as a function of concentration and exposure time.

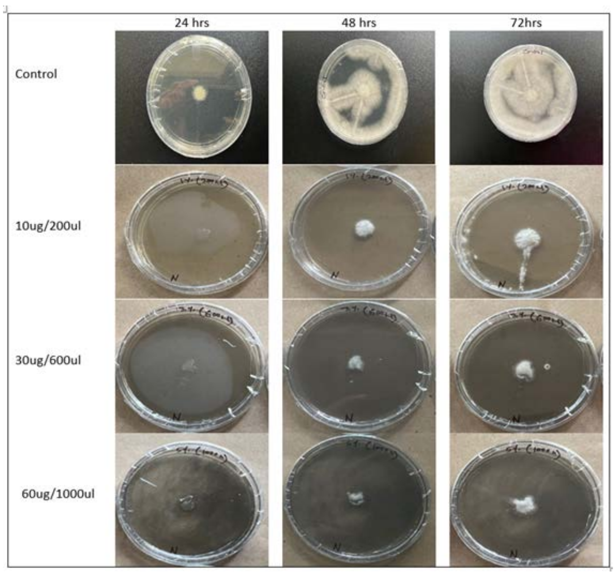

Figure 6: Pictographic record of the fungal mycelium growth of Rhizoctonia solani

Figure 7 shows the mycelial growth measurement in each plate recorded as a function of time and AgNP concentration in the form of a histogram.

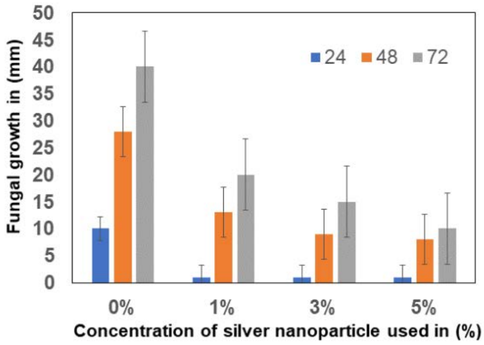

Figure 7: Histogram depicting fungal growth with respect to the various concentration of nanoparticles in use.

In support of our findings, the antifungal activities of several bio-based inorganic metal/ metal oxides have been under investigation previously. The studies performed by various researchers have been in support of the green synthesis of nanoparticles and their use as antifungal agents to protect the plant from fungal pathogens in a sustainable manner. In various other studies where, in vitro, the antifungal capability was researched against Botrytis cinerea, Alternaria solani, and many more using AgNPs. It was determined that the inhibitory action was increased by accelerating the concentration [26-28]. The vigorous inhibitory activity of the nanoparticles shown in the present study could be due to the small size and the capability to permeate the cell altering the fungal cell activity as reported by AshaRani et al., [29]. Numerous studies have also been performed to explain the antifungal mechanisms of the AgNPs and studies performed by various researchers suggest that the process involves oxidative stress which leads to the reticence of the spore germination initiating cell death.

4. Conclusion

The present study revealed the eco-friendly, cost-effective, and fast method of the synthesis of AgNPs using the aqueous extract obtained from Syzygium aromaticum that was frequently used in traditional medicine. The various characterization techniques were confirmative of the successful formation of the spherical and crystalline silver nanoparticles which further provided evidence of the role of the phytochemicals as the reducing and circumscribing agents in the conflation of AgNPs.

Likewise, upon biological evaluation, the AgNPs were found to have good anti-fungal properties against Rhizoctonia solani, a phytopathogen that causes extensive damage to rice plants all over the world.

Hence, this study vouches that the AgNPs obtained via green synthesis hold tremendous potential to tackle fungal diseases. This study performed could be of benefit in the sustainable agriculture fields.

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Declaration of Interest

The authors declare no conflict of interest

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