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Silver Nanoparticle Synthesis and Characterization for the Treatment of Ganoderma Fungus

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ABSTRACT

Almost every producing country's Gross Domestic Product (GDP) has benefited from the large-scale growth of the oil palm industry in recent years. However, there are a few risks to the palm trees that can have an impact on the GDP of our nation, one of which is the disease known as Ganoderma Basal Stem Rot (BSR). The lignin trunk of palm trees is broken down or degraded by a specific fungus called Ganoderma Boninense, Therefore, silver nanoparticles (AgNPs) have been created for this purpose due to their potential for treating fungus. The AgNPs were synthesized into a spherical shape by using Silver Nitrate ($AgNO_3$), Sodium Borohyrdide (NaBH4), and Polyvinylpyrrolidone (PVP) due to its straightforward and adaptable setup. Regarding to the AgNPs characterization, the optical properties showed the greatest absorption peak of transverse suface plasmon resonance (t-SPR) for AgNPs is 2.670 intensity at wavelength of 420nm. The structural properties of AgNPs exhibit an intensity peak as indexed (1 1 1), at 2(Theta) = 38.10°. The surface density for morphological properties is 0.1211 ± 0.000333. The study shows that 25.17% of fungal growth around Negative Control area, 2.09% around AgNPs Control area, and 0% of fungal growth around Positive Control area during the disc diffusion experiment after 7 days.

Keywords: AgNPs; Characterization; Ganoderma; Synthesis; Control

INTRODUCTION

Since nanotechnology can generate a wide variety of materials at the nanoscale level, it has been a recognized research area in this era of advanced technology since the last century. Nanotechnology produces materials of various types at the nanoscale level. Nanoparticles (NPs) are a wide class of materials that include particulate substances, which have at least one dimension less than 100 nm [1]. There are also various types of nanoparticles, such as iron nanoparticles, silver nanoparticles, and zinc nanoparticles. Silver nanoparticles (AgNPs) are the most stable metal nanoparticles and are among the most well-known materials in the field of nanotechnology due to their distinctive optical property, which depends on size, shape, and local refractive index. AgNPs have a wide range of applications, such as a drug carrier, catalyst, biosensor, sensing material, and, most significantly, the treatment of Ganoderma fungus, which is the subject of this project.

Basal stem rot, a common disease that affects oil palm plant and other related plants, which is caused by the Ganoderma fungus as shown in Figure 1. It can attack both hybrid and local varieties of oil palm within plantations leading to a decrease in crop yield [2]. Visible dark brown to black rot at the base of the stem, wilted leaves, and reddish-brown discoloration of internal tissues indicate this disease. The fungus's velvety, dark, and white spores on the Ganoderma are visible under a microscope. This disease can lead to wilting, defoliation, and reduced economic yield. This work focuses on the synthesis and characterization of AgNPs, to treat Ganoderma fungus using AgNPs' antibacterial characteristics.

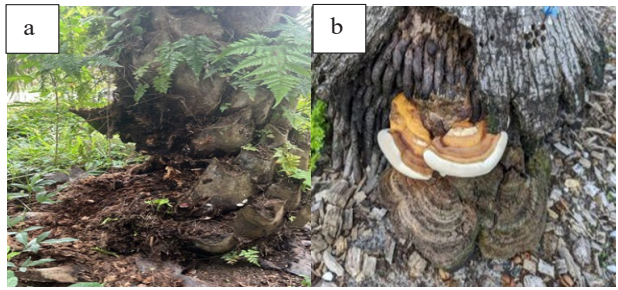

Figure 1: (a) Basal Stem Rot of oil palm, (b) Ganoderma fungi

Previous research has demonstrated the potential of nanoparticles in treating fungal infections. The use of gold nanoparticles (AuNPs) to limit the growth of Ganoderma boninense was studied by Fowotade *et al.* [3] and demonstrated strong antifungal action. This research emphasizes the increasing curiosity and positive outcomes in utilizing metal nanoparticles as antifungal agents. Nevertheless, there is a lack of studies that specifically concentrate on the use of AgNPs to combat Ganoderma Fungi, especially in oil palm fields. This research seeks to address this void by investigating the ability of AgNPs to prevent and potentially eliminate Ganoderma fungus, presenting a new method for controlling this destructive disease in agricultural settings.

METHODOLOGY

Synthesis of AgNPs

For the synthesis process of AgNPs, three different types of chemical solutions are used, which are Silver Nitrate ($AgNO_3$), Sodium Borohydride ($NaBH_4$), and Polyvinylpyrrolidone (PVP). Table 1 shows the recipe to synthesize the AgNPs solution. Firstly, the 0.001M $AgNO_3$ solution was prepared by dissolving 0.017g of $AgNO_3$ powder into 100 mL distilled water. Next, prepare 0.002M of $NaBH_4$ solution by dissolving 0.0189g of NaBH4 powder into 250 ml distilled water. PVP solutions are prepared by dissolving 0.1g of PVP into 33 ml of distilled water. After these four solutions are already prepared, then the next procedure can be started. Firstly, 30ml of 0.002M Sodium Borohydride ($NaBH_4$) was added to an Erlenmeyer flask. Then, a magnetic stir bar was added to the flask and placed in an ice bath on a stir plate. Let the NaBH4 solution be on ice so that the rate of decomposition will be reduced. Next, 2 ml of 0.001M Silver Nitrate ($AgNO_3$) was dripped into the stirring NaBH4 solution at approximately 1 drop per second. The stirring process was done by using the magnetic stirrer. The stirring process will be stopped once all the $AgNO_3$ solution has been completely added. After that, a 4ml drop of 0.027M Polyvinylpyrrolidone (PVP) was added to the other test tube mixed with the previous solution. The PVP solution can prevent aggregation in the solution, and it makes the mixed PVP solution yellowish-brown in color.

Table 1: Recipe of AgNPs solution

Material	Molarity (M)	Volume (mL)
Silver Nitrate, $AgNO_3$	0.001	2
Sodium Borohydride, NaBH4	0.002	30
Polyvinylpyrrolidone, PVP	0.027	4

Characterization of synthesized AgNPs

The characterization process will be conducted using three methods to determine the structural, optical, and morphological properties of the synthesized AgNPs. In this project, three characterization methods are used: X-Ray powder diffraction (XRD) for structural properties, Ultraviolet-Visible (UV-Vis) spectroscopy for optical properties, and field emission scanning electron microscopy (FESEM) for morphological properties.

Optical properties of AgNPs

The optical properties of a material are analyzed using an UV-1800 Spectrophotometer, focusing on near-infrared, visible, and near-ultraviolet light absorption. UV-Vis spectroscopy measures the absorption spectrum and wavelength of nanoparticles. The sample was prepared by dripping 1mL of AgNPs solution into six cuvettes, 3 of them are filled with Dionized water as base. UV-Vis spectroscopy is highly accurate in collecting absorption spectra and size information, with 420nm peaks being a characteristic feature of AgNPs [4].

Structural Properties of AgNPs

The structural properties of AgNPs were analyzed using X-ray diffraction (XRD) using an X'Pert Powder diffractometer from PANalytical. XRD is a non-destructive method used to examine the atomic or molecular structure of materials, including AgNPs. XRD analysis was used Cu Kα radiation (λ = 1.54 A). The wavelength of the X-rays used is the same as the distance between atoms in a crystalline lattice.

Morphological properties of AgNPs

The morphological properties of materials were analyzed using the Field Emission Scanning Electron Microscopy (FESEM) model JSM-7600F from JEOL, an advanced technology for capturing nanostructure images with high resolution. To perform field emission in FE-SEM, FEG will apply low voltages to an electron source, which is typically a single tungsten filament with a pointed, sharp tip that concentrates low- and high-energy electrons at a low electrical potential with increased spatial resolution [5]. The spherical shape was expected, as previous research showed spherical AGNPs and their specific size. To make the FESEM sample, Fluorine-doped Tin Oxide (FTO) was prepared and AgNPs solution was dropped onto it and left it until dry.

Preparation of extracted fungi

The Ganoderma fungi have been obtained freshly from the oil palm tree plantation located in Muar, Johor. Figure 2 shows the Ganoderma Fungi before were extracted and the fungi after extraction. The fungus will be extracted directly by scraping the white and black surface of the Ganoderma with a clean spatula and placed in a clean petri dish. Extracted Ganoderma fungi can be stored for a reasonable period if kept under conditions to maintain their potency and effectiveness [6].

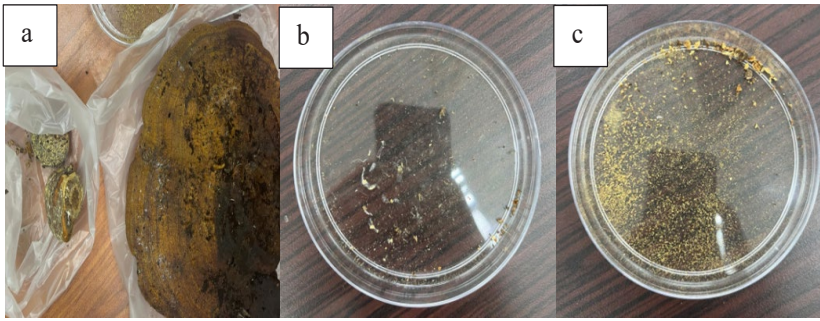

Figure 2: Ganoderma Fungi; (a) before extracted and (b and c) Ganoderma fungi after extracted

Preparation of Agar medium

Testing for antifungal susceptibility to the Ganoderma fungus is done on agar. Agar is used as a medium for antifungal susceptibility testing to assess the effectiveness of antifungal chemical samples against Ganoderma fungus [7]. Weighing the agar powder and DI water, diluting it with 1.5g for 75ml, heating it to 315°C for around 10-15 minutes, and then filling three Petri plates are the steps involved in the preparation. Regarding the purpose of ensuring the validity, generalizability, and reliability of the experimental data, three Petri dishes are used as replicas.

Disc Diffusion Method

The disc diffusion method involves sterilized filter paper discs, sterilized forceps, three controls (Negative Control, Positive Control, AgNPs sample), and an agar medium. Ganoderma fungi are placed at the dish center, with three controls placed 2 cm away as shown in Figure 3. Three controls were presented in this method: AgNPs sample created from Phase I and II; Amphotericin B functioning as the Positive Control; and DI water acting as the Negative Control. Amphotericin B is a well-known antifungal compound that acts as a fungicide that effectively against a wide range of fungi [8]. Disks are inserted into the agar medium using sterile forceps, ensuring complete contact. The experiment is observed under a microscope from day 1 to day 7, dividing disks into four zones for each disk.

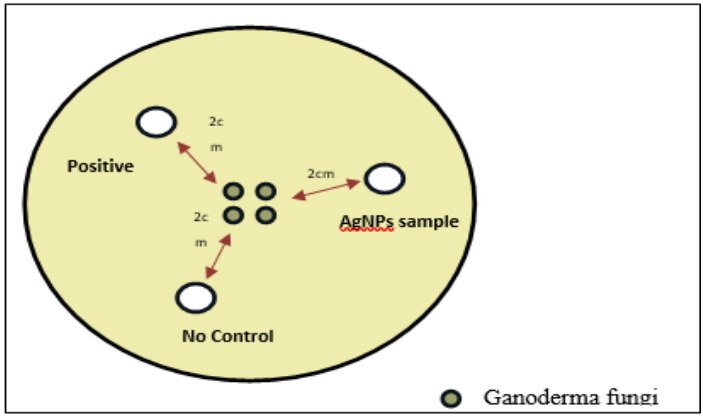

Figure 3: Disc Diffusion Setup

ImageJ Analysis

ImageJ is a Java-based image processing program created by the National Institutes of Health (NIH) for scientific research. It enables quantitively image analysis, measuring pixel values, regions, and distances, and supports complex processes like particle analysis [9]. The analysis involves setting a scale of 0.25mm, counting fungi by total area, standard deviation, and deviation error, and calculating the average surface density in centimeters, cm unit using the Eq. (1).

$$\text{Average surface density} = \frac{Total\ area\ of\ fungi}{Image's\ Pixel} \text{ x } 100\% \quad (1)$$

RESULTS AND DISCUSSION

Synthesis of AgNPs

Synthesis of silver nanoparticles is successfully done by using three materials: Silver Nitrate, Sodium Borohydride, and PVP. The result showed the change of the pale-yellow color of the mixed solution into a yellowish-brown color as the temperature and incubation period increased, which indicates the formation of nanoparticles as shown in Figure 4. This color shift is caused by the peculiar optical characteristics of AgNPs that react with light in a particular manner. The color change is influenced by the concentration and ratio of the reducing material in the reaction mixture, affecting the final color of the synthesized AgNPs [10].

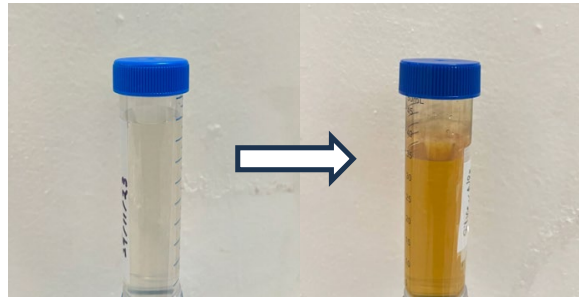

Figure 4: Change in color of mixed solution

Characterization of Optical Properties

The absorption spectrum of nanoparticles obtained from UV-visible absorption spectroscopy is presented in Figure 5, which shows a peak at 420nm and 2.670 of intensity corresponding to the surface plasmon resonance.

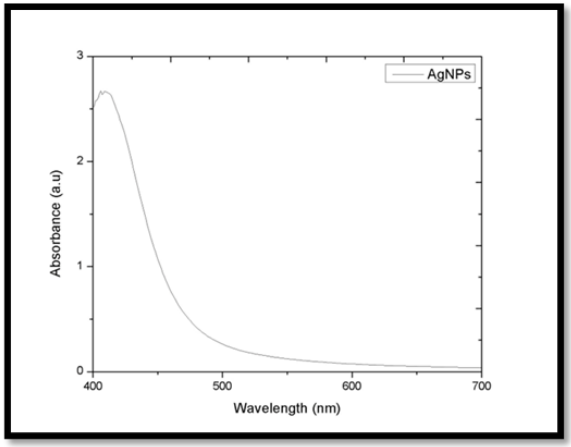

Figure 5: UV-Vis spectrum of synthesized silver nanoparticles

Due to the isotropic shape of nanoparticles obtained, it typically appears as a single and distinct peak. Therefore, the result only displays one peak. According to Gürsoy [11], AgNPs are characterized by peaks at 420 nm. Furthermore, the graph was started at 400nm and can't be adjusted because the access to the machine as an undergraduate is restricted to what is already configured.

Characterization of Structural Properties

The X-ray diffraction pattern of the synthesized silver nanoparticles is shown in Figure 6. The (111) plane of Ag is attributed to the diffraction peak at $2\theta = 38.10^{o}$. The size of coherently diffracting domains is measured in XRD, and it is occasionally consistent with the size of crystalline grains. The Ag peak (111) intensity is proven the Ag structure [12]. The (111) plane's peak is more intense than that of the other planes. The broadening of these peaks is mainly due to the effect of nanoparticles.

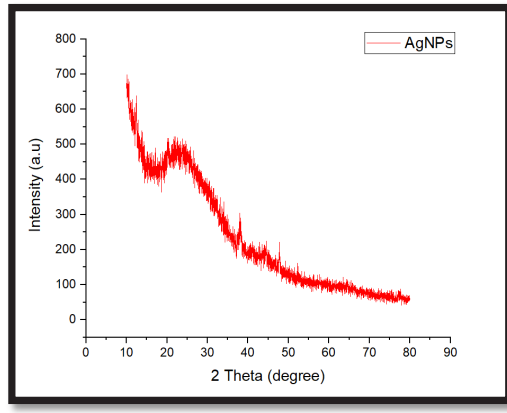

Figure 6: X-ray Diffraction peaks of silver nanoparticle

Characterization of Morphological Properties

Field Emission Scanning Electron Microscopy confirmed the morphology of the synthesized silver nanoparticles. The result of FESEM analysis is presented in Figure 7 which shows the synthesized silver nanoparticles are spherically shaped and size ranges from 100nm to 110nm in diameter. The analysis was done using ImageJ software and it also showed the nanoparticles are slightly aggregated. However, the spherical shape was obtained from the FESEM and it matches previous research that revealed spherical AgNPs of various sizes which suitable for antifungal properties [13].

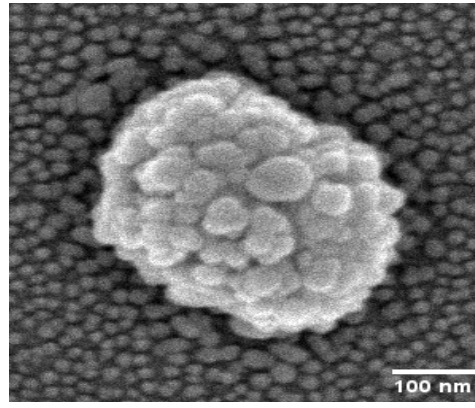

Figure 7: FESEM photograph of AgNPs

Disc Diffusion Method

The experiment involved setting up controls in three Petri dishes containing Ganoderma Fungi and Agar medium, including a Negative Control group and Positive Control group, alongside the AgNPs Control group from day 1 until day 7. Over a week, fungal growth was observed under a microscope. Seven days of observation was a great period for this assessment because Ganoderma fungi have optimum growth within 48 hours as stated by previous researchers [14]. The Negative Control area had the highest fungi count, while the Positive Control area had the lowest. The total fungi count in the AgNPs area was low, indicating good antifungal functions. Figure 7, Figure 8, and Figure 9 show the count of fungi growing near each affected control area on three replications. Meanwhile, Figure 10 shows the percentage of average surface density of fungi growth on three replications by using Image J and Eq. (1). The observation of the experiment. The experimental observations were taken for 7 days.

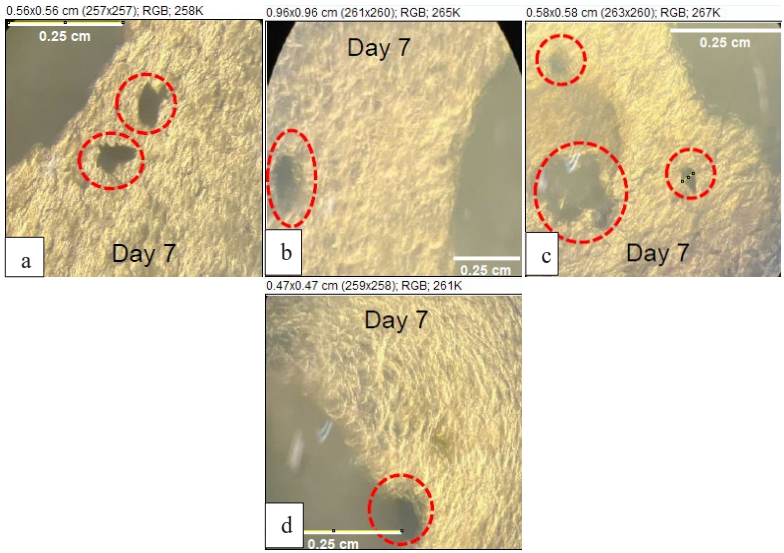

Figure 7: Observation on Replication 1 at day 7. Fungi count in: (a, b, and c) Negative Control Area and (d) AgNPs Area

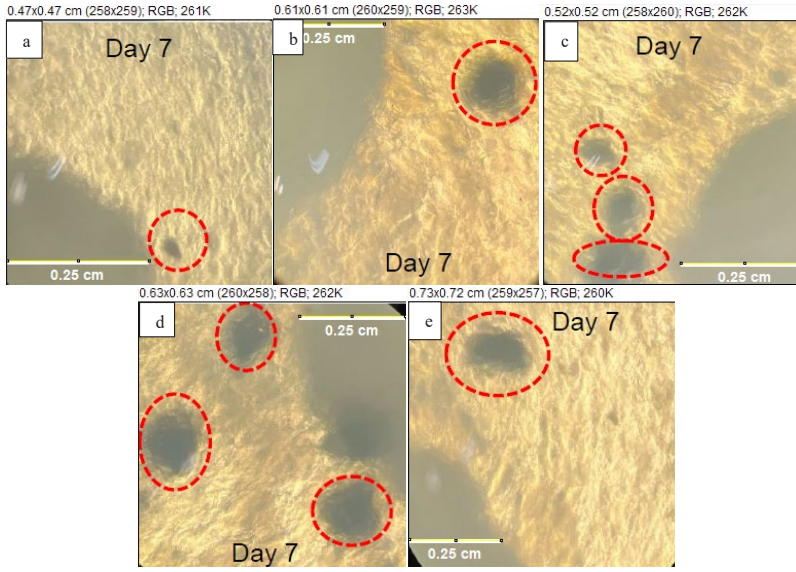

Figure 8: Observation on Replication 2 at day 7. Fungi count in: (a, b, c, and d) Negative Control Area and (e) AgNPs Area

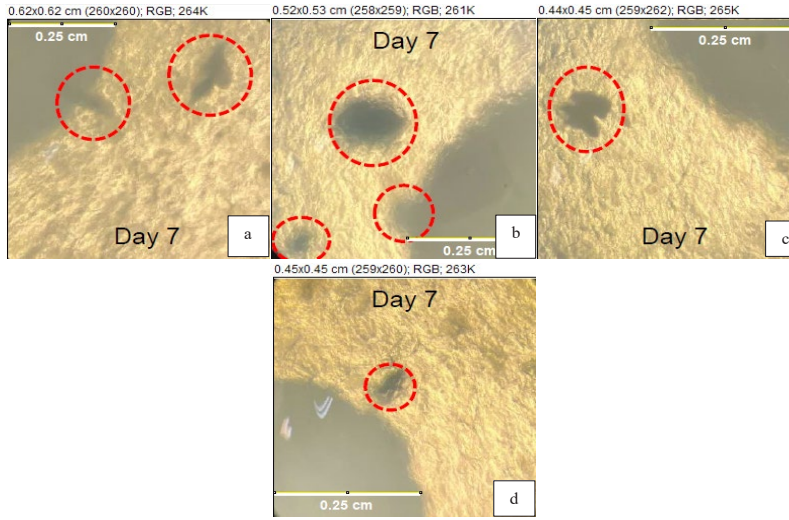

Figure 9: Observation on Replication 3 at day 7. Fungi count in: (a, b and c) Negative Control Area and (d) AgNPs Area

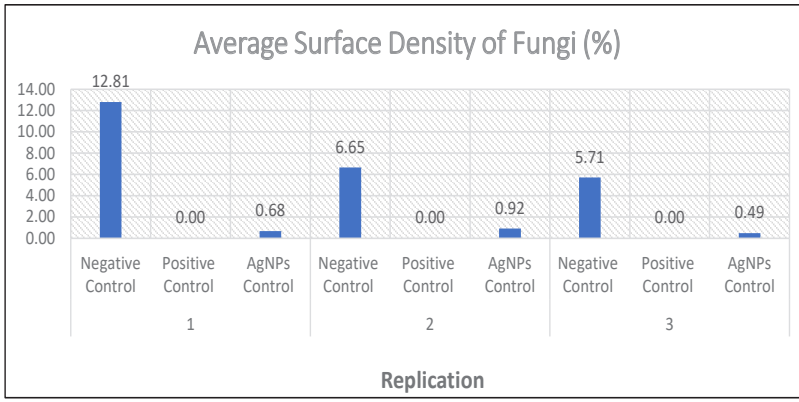

Figure 10: Percentage of average surface density of fungi growth on three replications.

According to Figure 7, Figure 8, Figure 9, and Figure 10, the analysis revealed 12.82% of fungal growth around the Negative Control area for Replication 1 by using the ImageJ software analysis method. Meanwhile, 2.09% of fungal growth around the AgNPs Control area, and 0% of fungal growth around the Postive Control area. The percentage of fungi growth around the Negative Control area in Replication 1 has the highest average surface density because of the huge sizes of fungus. In Replication 2, there is 6.65% of fungal growth around the Negative Control area and 0.92% of fungal growth around AgNPs Control area. In replication 3, 5.71% of fungal growth was around the Positive Control area meanwhile 0.49% was around AgNPs Control area, and 0% was around the Positive Control area. However, the analysis showed the highest fungal growth around the Negative Control area and slight growth around AgNPs area. However, it can be claimed that AgNPs successfully inhibit fungal growth. In this method, the spherical form of AgNPs is essential for the treatment of fungus because it increases the antibacterial activity of the particles. Because of its high surface area to volume ratio, this form may interface with fungal cell membranes more effectively. AgNPs' spherical shape allows them to break through the fungal cell wall and interfere with cellular functions [15]. This disruption can cause cell death and prevent fungi from growing and multiplying. It also releases silver ions more effectively due to their spherical shape, which can cause oxidative stress and damage to fungal cells. This process adds much more to AgNPs' antifungal qualities.

CONCLUSION

The project aimed to synthesize silver nanoparticles using Silver Nitrate and Sodium Borohydride, with polyvinylpyrrolidone added for a spherical shape. Characterization of the nanoparticles was achieved using FESEM, XRD, and UV-Vis. The disc diffusion method was used for silver nanoparticles sample to treat Ganoderma fungus, evaluating its antifungal properties against No-Control (DI water) and Positive Control (Amphotericin B) eventually showing a favorable outcome after 7 days. It showed the efficiency and effectiveness of AgNPs in combating the Ganoderma Fungi. The results of this study could be improved upon in order to provide a better oil palm plantation disease in the future. To enhance in production of good samples of AgNPs, some future work will be implemented by increasing the observation time of disc diffusion method to obtain the optimum fungi growth for excellent results. The experiment also will be repeated by enchancing the recipe of synthesizing AgNPs with other chemical materials and the handling during the experiment also will be improved to obtain better results.

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