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# Study on the production of silver nanoparticles from plant extracts for application in stimulating maize seed germination

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ABSTRACT

Silver nanoparticles are among the widely applied nanomaterials due to their strong antibacterial properties and effective ability to eliminate fungi and bacteria. In agriculture, silver nanoparticles are being studied as a potential alternative to chemical pesticides, contributing to enhanced productivity and biosafety. The green synthesis method with the aims to save cost and minimize chemical during silver nanoparticles production protecting the environment. Among various plant extracts, Terminalia catappa leaf extract was selected as a suitable reducing agent for the synthesis of silver nanoparticles. Silver nanoparticles were successfully synthesized from terminalia catappa leaf extract, and their structural morphology was evaluated by modern techniques such as SEM, XRD, and FTIR, yielding a spherical silver nanoparticles shape with an average diameter ranging from 20 to 100 nm. The study also investigated the impact of synthesis conditions on the germination of maize seeds, with the optimal conditions 0.005M $AgNO_3$ concentration, synthesis temperature of 40°C, and reaction time of 30 minutes that resulted in the highest germination rate and seed vigor index. Maize seeds treated with a diluted silver nanoparticle solution at concentrations of 4-6 times showed the ability to stimulate germination, providing a high germination rate and vigor index, with uniform development of the seeds.

## Introduction

Nanotechnology is one of the most essential fields in modern life and has attracted significant attention from scientists due to its applications. Nanomaterials are generally defined as materials with at least one external dimension in the range of 1 to 100 nanometers (nm). In agriculture, nanotechnology plays an important role in enhancing production, such as through silver nanoparticle-based sensors for crop protection and disease detection, as well as nano-chemicals used in fertilizers and pesticides to improve plant growth. In the medical field, nanomedicine is an emerging discipline inspired by biological phenomena. Notably, nanomaterials laid the foundation for the development of SARS-CoV-2 (COVID-19) vaccines. In addition, nanotechnology is being utilized to exploit the excellent electrical conductivity and other advantageous properties of nanomaterials to transform the electronics industry, making quantum computing and atomic



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electronics more feasible. Therefore, nanotechnology is regarded as a groundbreaking platform for sustainable development.

Currently, various methods are available for the synthesis of nanoparticles, as chemical, physical, and biological approaches. Each method has its own merits and demerits; however, physical and chemical methods have significant drawbacks such as high cost and environmental impacts. In contrast, green synthesis offers several outstanding advantages over conventional physical and chemical techniques, this method is energy- and time-efficient, requires simple equipment, reduces the generation of by-products, gets rid of unnecessary processing steps, and is in accordance with ecosystems. It is also environmentally friendly, safe for human health, and feasible for large-scale industrial production [1]. Green synthesis utilizes naturally occurring compounds found in plants, microorganisms, and other biological sources as reducing agents in the nanoparticle formation process. Therefore, green synthesis is regarded as a promising approach that ensures safety, cost-effectiveness, sustainability, and environmental friendliness.

Silver nanomaterials have been known since ancient times, when silver containers were used to store water or silver coins were placed in liquids for their antibacterial properties [2]. From 1980 to 2010, there were approximately 7,500 patents and inventions related to silver nanoparticles worldwide [3]. A 2011 study reported that over 300 consumer products incorporated silver nanoparticles. Among the various applications of silver nanoparticles, their primary use is attributed to their strong antibacterial activity, followed by their optical properties. The ionic form of silver ($Ag^+$) has also been used to coat clay-based water filters for antimicrobial purposes [4]. The most common method for synthesizing silver nanoparticles is the chemical reduction method. This involves the transfer of electrons to $Ag^+$ ions, reducing them to metallic $Ag^0$ atoms. These $Ag^0$ atoms then aggregate to form larger silver nanoparticles [5]. Typically, this method uses silver ion precursors such as $Ag_2SO_4$, $AgNO_3$, or $AgClO_4$, along with reducing agents like citrate salts, sodium borohydride, ascorbic acid, glucose, formaldehyde, ethylene glycol, or plant extracts. Chemical reduction offers advantages such as simplicity, low cost, and high synthesis efficiency. However, it often involves the use of toxic and environmentally hazardous chemicals such as $NaBH_4$ and hydrazine [4]. To address these concerns, recent research has focused on replacing such harmful reagents with more eco-friendly alternatives, such as plant extracts [6].

The Indian almond tree (Terminalia catappa) is one of the most common plant species in Vietnam. The fresh leaf extract of this tree contains various bioactive compounds such as flavonoids, phenolic acids, alkaloids, saponins, carbohydrates, amino acids, proteins, and terpenoids. These compounds act as reducing and stabilizing agents in the synthesis of silver nanoparticles. Therefore, the use of fresh Terminalia catappa leaf extract represents a green synthesis approach, satisfying several principles of green chemistry, such as utilizing readily available natural sources and being environmentally friendly.

At present, there are still very few studies focusing on the synthesis of silver nanoparticles using fresh Terminalia catappa leaf extract. In this study, silver nanoparticles were synthesized according to a green synthesis method using the leaf extract of terminalia catappa and then tested for their potential application in promoting germination in maize seeds. Advanced analytical techniques as SEM, XRD and FTIR were used to assess the morphology and structural properties of the nanoparticles. Additionally, the study aimed to optimize the synthesis conditions to achieve the highest germination rate, root length, and shoot length of maize seeds under laboratory conditions.

## Experimental

### *Materials*

Fresh Terminalia catappa leaves were collected in Hanoi, Vietnam, ensuring only undamaged leaves free from insect infestation were selected. The leaves were thoroughly washed with distilled water to remove contaminants and then dried in an oven at a constant temperature until a consistent weight was achieved. The dried leaves were subsequently ground into a fine powder using a suitable grinding method. The chemicals used in this study included silver nitrate ($AgNO_3$, 99.95% purity, China), ethanol ($C_2H_5OH$, 96% purity, China), and distilled water.

### *Solution preparation*

0.01 M $AgNO_3$ Solution: Accurately weigh 0.432 g of $AgNO_3$ and dissolve it in distilled water. Transfer the solution to a 250 mL volumetric flask and make up to the mark with distilled water.

Terminalia catappa leaf extract: Accurately weigh 12 g of finely ground Terminalia catappa leaf powder and add it to an Erlenmeyer flask containing 240 mL of 70% ethanol. The mixture is then sonicated for 30 minutes using an ultrasonic bath. After sonication, the mixture is

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shaken and heated at 60°C with a stirring speed of 180 rpm for 30 minutes. The resulting solution is filtered using a vacuum filtration system to obtain the leaf extract.

*Synthesis of nano silverparticles*

60mL of Terminalia catappa leaf extract solution was slowly added dropwise to 30 mL of $AgNO_3$ solution at the investigated concentration, with a dropping rate of approximately 1 drop per second. The reaction was carried out in an Erlenmeyer flask under stirring at a speed of 700 rpm. After the complete addition of the leaf extract solution, the mixture was stirred for an additional 15 minutes. A portion of the synthesized silver nanoparticle solution was diluted 5 times (at a ratio of 1:4, where each sample consisted of 10mL of silver nanoparticle solution + 40mL of distilled water) for germination stimulation evaluation on maize seeds. Another portion was centrifuged at 13,000 rpm for 10 minutes and washed multiple times with ethanol and distilled water. The obtained samples were vacuum-dried at approximately 60°C for 12 hours. The dried product was then used for XRD, FTIR, and SEM measurements.

## Results and discussions

*The evaluation of silver nanoparticles's morphology and structure*

The morphological analysis of the silver nanomaterial using SEM showed the results presented in Figure 1a. The synthesized silver nanoparticles exhibited a near-spherical shape with an average size ranging from 20-100nm, the presence of speckled white patterns could be attributed to aggregates of organic compounds from the Terminalia catappa leaf extract adhering to the surface.

The structure of the silver nanomaterial, studied by X-ray diffraction (XRD), is shown in Figure 1b. The diffraction peak at 2θ ≈ 46–47° is able to correspond to the (200) crystal plane of the face-centered cubic (FCC) structure of silver nanoparticles [7]. The XRD pattern shows characteristic diffraction peaks appearing at 2θ≈32.8 ° and 55° [8], indicating the clear presence of $Ag_2O$. Due to the oxidation of silver nanoparticles into $Ag_2O$ when the residual organic film from the extract was washed away and the nanoparticles were exposed to air.

The Terminalia catappa leaf extract contains various organic compounds such as polyphenols, flavonoids, and tannins, which can create amorphous or small crystalline phases during the synthesis process. These compounds may produce a diffraction peak at 2θ ≈ 28°.

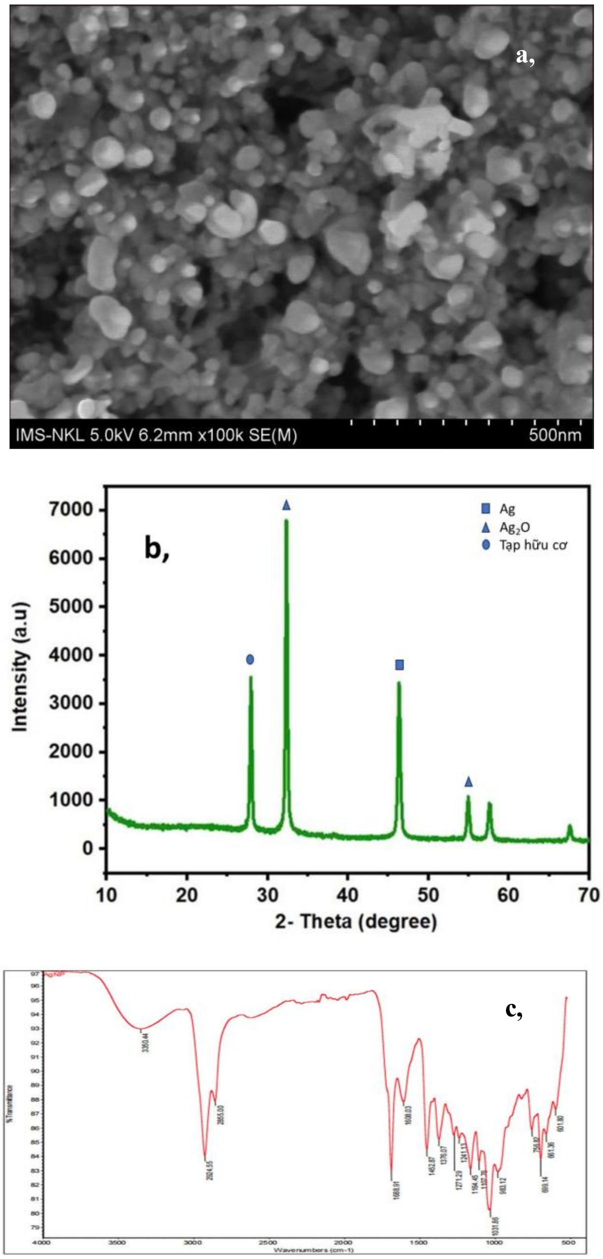


Figure 1: The silver nanoparticles synthesized using Terminalia catappa leaf extract at a volume ratio of $AgNO_3$ to leaf extract of 1:2 were characterized using the following analytical methods: (a) SEM; (b) XRD; (c) FTIR

FT-IR spectroscopy is a crucial tool for evaluating the involvement of chemical components in the Terminalia catappa leaf extract in the elimination of $Ag^+$ to $Ag^0$. The FT-IR spectrum of the AgNPs (Figure 1c) shows a large absorption peak at 3350.44 $cm^{-1}$, characteristic of the stretching vibration of the O-H bond [9]. The peaks at 2924.55 $cm^{-1}$ and 2855.0 $cm^{-1}$ reflect the vibration of C-H bonds in $-CH_2$ groups [10], while the peak at 1689.91 $cm^{-1}$ corresponds to the C=O bond [11], and the peak at 1608.03 $cm^{-1}$ confirms the presence of the C=C bond [12].

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Furthermore, the absorption peak at 1452.87 $cm^{-1}$ is characteristic of the C=C stretching vibration in the aromatic ring [13], while the peak at 1376.07 $cm^{-1}$ represents the bending vibration of the C-O bond [14]. Notably, the absorption peak at 699.14 $cm^{-1}$ is a characteristic indicator of the stretching vibration of the Ag-O bond [15], suggesting an interaction between the silver nanoparticles and the functional groups of the organic compounds. This result in line with previous studies while flavonoids and polyphenols were identified as the main components acting as stabilizing agents, helping to control the formation and stability of silver nanoparticles during the synthesis process.

*Optimization of Conditions for Silver Nanoparticle Synthesis*

Silver is one of the essential micronutrients that assists plants growth and protects the life source of plant species. Determining the synthesis conditions under which silver nanoparticles can promote seed germination and development under normal conditions is crucial. In this research, silver nanoparticles synthesized from plant extracts are applied to stimulate germination in maize seeds.

The optimal conditions for silver nanoparticle synthesis were evaluated by monitoring the seed germination rate and then determining the seed vigor index using the following formula.

Determination of Germination Rate: The average germination rate was determined according to a modified Scott method [16] by randomly selecting 20 seeds from each surveyed sample. The number of germinated seeds meeting the requirements at 48 hours (calculated from the start of sowing) was counted and calculated using the formula:

$$GP(\%) = \frac{A}{A+B} \times 100 \quad (1)$$

Whereas, GP: Average germination percentage; A: Number of seeds that germinated successfully; B: Number of remaining seeds that did not germinate properly.

Determination of Vigour Index: The seed vigour index (VI) was determined according to the formula proposed by Abdul-Baki [17]:

$$VI = GP \times [D_{root} + D_{shoot}] \quad (2)$$

Whereas, VI: Vigour Index, GP: Average Germination Percentage, $D_{root}$: Root Length (cm), $D_{shoot}$: Shoot Length (cm).

Data processing: Data were collected and statistically processed using Microsoft Excel. Each experiment was repeated 3 times. The samples were processed similarly.

*The impact of initial $AgNO_3$ concentration on germination stimulating ability of maize seeds*

According to the graphs in Figure 2, it can be seen that the germination rate and vigor index of the 0.0075M sample were the lowest among the five surveyed samples (accounting for a rate of 73.33±3.33% and a vigor index of 118.50±7.56mm). The graphs show an increasing trend from the 0.001M sample to the 0.005M sample, followed by a decreasing trend at 0.0075M, with a slight but insignificant increase at 0.01M. The germination rate and vigor index of the five surveyed samples peaked at the 0.005M sample, with values of 88.33±1.67% and 224.47±7.01mm, respectively. Maize seeds soaked in the 0.005M sample exhibited the most uniform development.

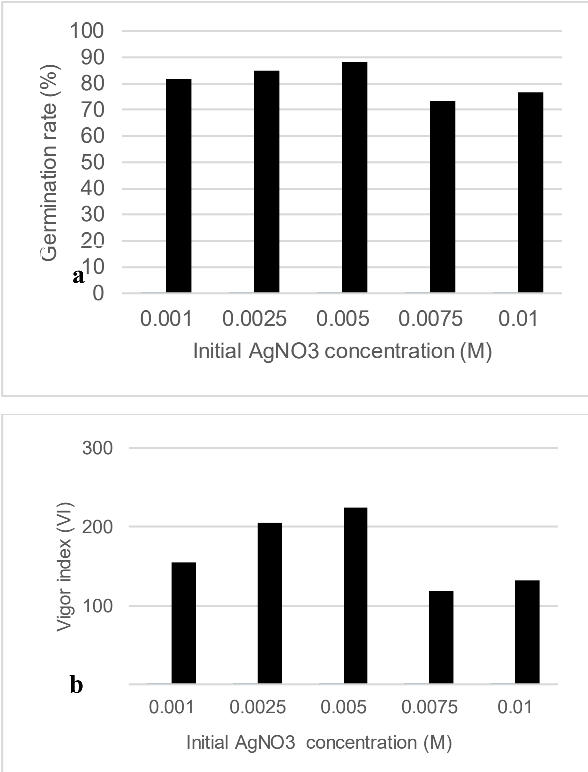


Figure 2: The effect of initial $AgNO_3$ concentration on the germination rate (a) and the vigor index (b) of maize seeds

*The effect of silver nanoparticle synthesis temperature on the germination stimulating ability of maize seeds.*

According to the graphs in Figure 3, it can be shown that the germination rates for samples ranging from 30°C to 60°C were over 80%. The germination rate and vigor index of the 70°C sample were the lowest (with a germination rate of 76.67±1.67% and a vigor index of 136.53±4.54mm). The graphs show an increasing trend from the 30°C sample to the 40°C sample and a decreasing trend in the 50°C to 70°C samples. The

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germination rate and vigor index among the five surveyed samples were optimal at the 40 °C sample, with values of 91.67±1.67 % and 250.63±6.91 mm, respectively. Seeds soaked in this sample showed the most uniform development and the highest radicle length among the five surveyed samples.

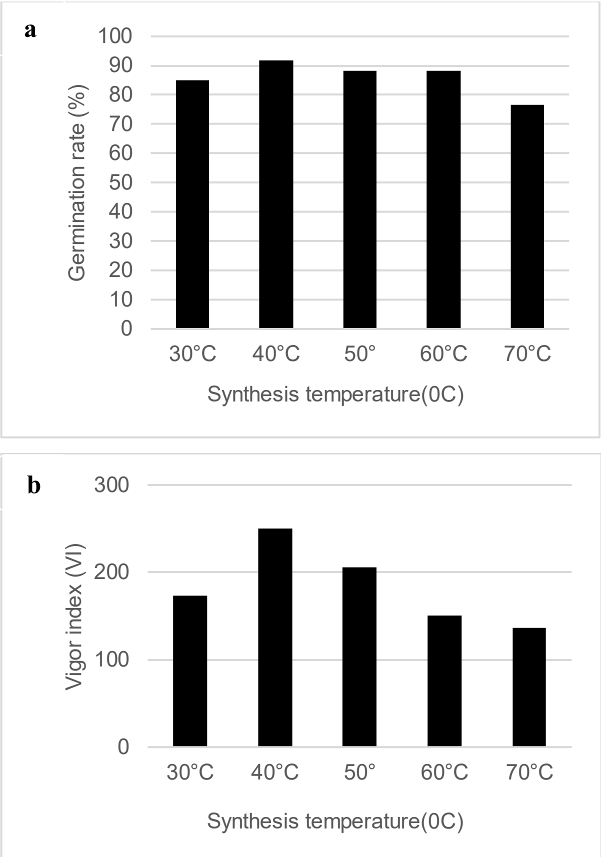


Figure 3: The effect of silver nanoparticle synthesis temperature on the germination rate (a) and the vigor index (b) of maize seeds

*The effect of silver nanoparticle synthesis time on the germination stimulating ability of maize seeds*

Regarding to the graphs in Figure 4, it can be observed that the germination rates for samples ranging from 20 minutes to 50 minutes were above 80%. The germination rate and vigor index of the 10-minute sample were the lowest among the five surveyed samples (with a germination rate of 65.00±2.90% and a vigor index of 84.88±5.44mm). The graphs show an upward trend from the 10-minute sample to the 30-minute sample and a decreasing trend in the remaining two samples. Maize seeds soaked in the 30-minute sample achieved a germination rate of 96.66±1.67%, resulting in the highest vigor index of 331.02±8.52mm, with uniform seed development and the best radicle length development.

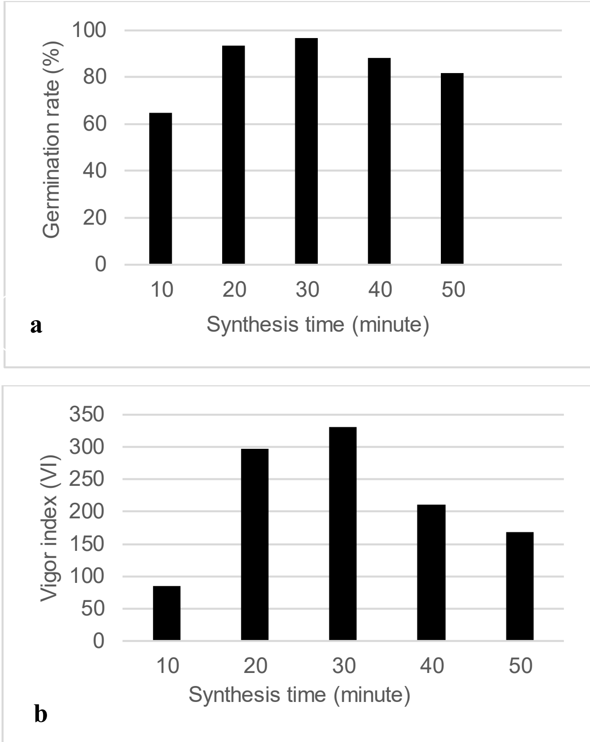


Figure 4: The effect of silver nanoparticle synthesis time on the germination rate (a) and the vigor index (b) of maize seeds

*Survey of the control samples for stimulating germination in maize seeds.*

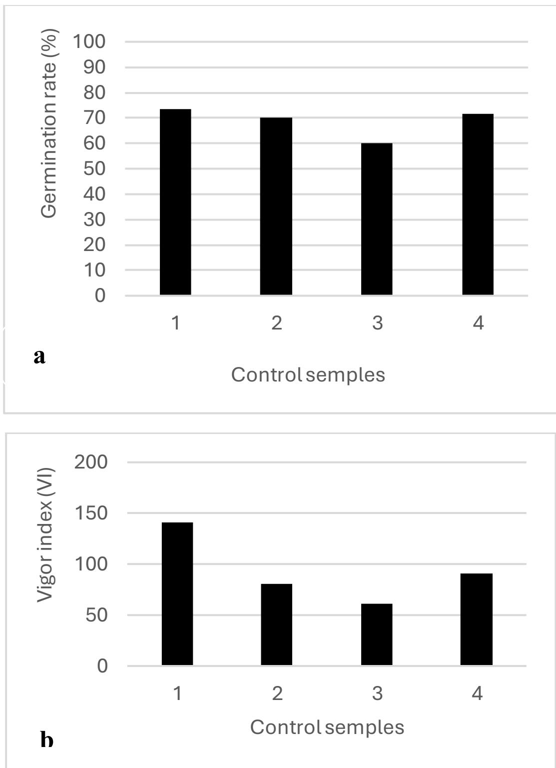


Figure 5: The effect of control samples on the germination rate (a) and the vigor index (b) of maize seeds

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After the optimal parameters and concentration for stimulating maize seed germination were established, the influence of various control samples was investigated to provide a comparative baseline. This evaluation involved four distinct control groups: (1) a 0.005 M $AgNO_3$ solution, (2) Terminalia catappa leaf extract, (3) 70% alcohol, and (4) distilled water. The comparative effects of these treatments on the germination rate and vigor index are illustrated in Figure 5.

Based on the graphs in Figure 5, it can be presented that the germination rate and vigor index of the 70-degree alcohol control sample were the lowest among the four surveyed samples (with a germination rate of 60.00±2.89% and a vigor index of 60.93±4.30). Among the four control samples, the highest germination rate was observed in the $Ag^+$ (0.005M) solution sample, at 73.33±1.67%, resulting in the highest vigor index of 141.34±4.25 among the four surveyed samples. However, the highest values among the four control samples were still significantly lower than those of the optimized nano-silver sample, which was prepared under optimized conditions of initial $AgNO_3$ concentration, temperature, time, and silver nanoparticle concentration. Maize seeds treated with the control samples all exhibited uneven development, with poorly developed average root and shoot lengths.

## Conclusion

In this study, silver nanomaterials were successfully synthesized using a green synthesis method from Terminalia catappa leaf extract. The morphology and structure of the synthesized silver nanomaterials were evaluated using modern analytical methods such as SEM, XRD, and FTIR. The formed silver nanomaterials were spherical in shape with an average diameter of 20-100nm. Optimal parameters for silver nanoparticle synthesis that best stimulate maize seed germination were identified. Silver nanoparticles synthesized at an $AgNO_3$ concentration of 0.005M, a synthesis temperature of $40^0$C, and a synthesis time of 30 minutes demonstrated the ability to stimulate germination, resulting in a high maize seed germination rate and the highest seed vigor index.


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