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The Effect of Silver Nanoparticles Stabilized with Tannic Acid for Nano-Priming on *Zea mays* L. Seeds Germination

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Abstract

Silver nanoparticles (AgNPs) have various benefits for application in the agricultural sector, such as nano-seed priming to enhance seedling growth and development. In this research, the effectiveness of AgNPs sizes and concentration to enhance *Zea mays* seeds germination has been investigated. The AgNPs were synthesized using various concentrations of tannic acid (0.025, 0.25, and 5 mM) to produce AgNPs with different sizes to know their optimum size and concentration. The synthesized AgNPs were characterized using a UV-Vis spectrophotometer to determine the absorption spectrum of AgNPs within 400 to 500 nm. Besides that, a transmission electron microscope (TEM) was used to determine the size and shape of the AgNPs, and an atomic absorption spectrophotometer was used to determine the concentration. The results show AgNPs with sizes of 13.39±2.40, 27.25±4.09, and 46.7±10.75 nm, respectively. Subsequently, AgNPs with concentrations of ~8, ~16, and ~24 mg l^{-1} were exposed to *Z. mays* seeds for 24 hours, then germinated for 14 days. The results revealed that AgNPs with a size of ~27 nm and a concentration of ~24 mg l^{-1} showed the highest germination rate and growth despite the control and other treatments. This indicates that the AgNPs with those properties have the potential as a seed nano-priming agent.

Keywords: germination; nano-priming; silver nanoparticles; tannic acid; *Zea mays*

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INTRODUCTION

One of the primary applications of nanotechnology in agriculture is the development of nanoparticle-based solutions, such as nano-fertilizers (León-silva et al., 2018; Lasso-Robledo et al., 2022), nano-pesticides (Shang et al., 2019; Pereira et al., 2021), and nano-priming as agrochemical sources (Acharya et al., 2017; Sharma et al., 2021; Hassanisaadi et al., 2022). These nanoparticles can penetrate plant tissues more efficiently than conventional materials because of their small size (< 100 nm) and high surface area-to-volume ratio, which allows for controlled release and targeted delivery. Nanoparticles can enhance nutrient absorption, improve resistance to environmental stresses, and facilitate plant defense mechanisms against pathogens. Nanotechnology's ability to manipulate materials at the molecular level (1 to 100 nm) offers unique opportunities for precision farming and sustainable agricultural practices (Shang et al., 2019; Maluin et al., 2021).

Among the many types of nanoparticles, metal-based nanomaterials are extensively used in various sectors, with silver nanoparticles

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(AgNPs) being one of the most commonly applied. AgNPs exhibit unique properties, particularly their localized surface plasmon resonance (LSPR), which makes them ideal for use as antimicrobial agents, as well as in biological and chemical sensors, biomarkers, and biomedical applications (Roy et al., 2019; Odeniyi et al., 2020; Velsankar et al., 2020). These nanoparticles are utilized in various industries, including healthcare, food production, industrial applications, household products, medical device coatings, optical sensors, cosmetics, drug delivery systems, and agriculture (Dhand et al., 2015; Loza and Epple, 2019). Their antimicrobial properties, along with their ability to enhance plant growth, make AgNPs especially valuable. AgNPs can interact with plant cells, influencing important physiological processes such as seed germination, photosynthesis, and stress responses.

Several methods for synthesizing AgNPs have been developed, encompassing physical (Khatoon et al., 2017), chemical, and biological approaches (Shalaby et al., 2015; Siddiqi et al., 2018; Moradi et al., 2020). Each method comes with its own set of advantages and drawbacks. Among the various techniques, the green synthesis method with chemical and biological approaches, stands out as a widely used method for AgNPs synthesis (Khandel et al., 2018). This is mainly attributed to their simplicity, the use of basic equipment, and eco-friendly nature. Green synthesis involves three key components in the nanoparticle synthesis process: the metal precursor, reducing agent, and capping agent (Siddiqi et al., 2019; Handayani et al., 2021).

One of the green synthesis methods used is the utilization of organic compounds, such as sodium citrate for nanoparticle synthesis (Ranoszek-Soliwoda et al., 2019). The utilization of organic compounds in nanoparticle synthesis has advantages such as ease of production, low cost, and high yield (Ravichandran et al., 2019). Sodium citrate is a compound that can be used for silver nanoparticle synthesis. Citrate is a common choice for producing AgNPs in a colloidal solution, the result tends to produce diverse shapes and sizes of nanoparticles (Millour et al., 2020). Sodium citrate functions as a reducing agent and stabilizer in the synthesis of AgNPs. During the synthesis process, sodium citrate reduces silver ions (Ag^{+}) to silver atoms (Ag^{0}), leading to the formation of AgNPs. Additionally, sodium citrate acts as a capping agent, stabilizing the nanoparticles by preventing their aggregation. The citrate ions adsorb onto the surface of the nanoparticles, providing a negative charge that leads to electrostatic repulsion between particles, thus maintaining their stability in solution. Utilizing the green synthesis method with chemical reduction employing sodium citrate not only demonstrates its primary advantage but also its capability for subsequent nanoparticle functionalization (Cherian et al., 2020; Loza et al., 2020).

Meanwhile, tannic acid is a non-toxic and biodegradable polyphenolic substance. In this research, tannic acid has multiple roles as a reductor, stabilizer, and capping agent (Ranoszek-Soliwoda et al., 2019; Matras et al., 2022). The capping agent is crucial as a stabilizer that can inhibit excessive growth of nanoparticles and prevent aggregation in colloid synthesis. The ligands of the capping agent stabilize the surface where the nanoparticles will later interact with the precursor. AgNPs are known as metals that have toxic properties. The release of AgNPs into aquatic ecosystem will cause disturbance and toxicity to organisms living in those habitats (Fahimirad et al., 2019; Khan et al., 2019). Therefore, the release of AgNPs into the ecosystem raises awareness about environmental safety and toxicity effect. However, the toxicity of AgNPs depends on their size and concentration.

In addition to having toxic effects that can disrupt plant growth and development, the exposure of plants to AgNPs can inhibit plant growth by reducing seed germination and seedling growth. AgNPs can accumulate in plant organs such as roots or leaves, triggering defense mechanisms at the cellular and tissue levels, which can alter metabolic pathways (Song and He, 2021). Exposure to AgNPs can affect the formation of reactive oxygen species (ROS), superoxide dismutase (SOD) activity, H_2O_2 levels, chlorophyll, proline, phenolic content, ascorbic acid, and glutathione, as well as the rate of photosynthesis and transpiration (Gupta et al., 2018; Krishnasamy et al., 2024; Pintos et al., 2024). This accumulation can lead to oxidative stress in plants, ultimately disrupting the physiological and biochemical balance, thereby reducing plant productivity and quality. However, it is important to determine the optimal concentration and size of AgNPs that do not exhibit toxic effects, ensuring the benefits of AgNPs without compromising plant health (Nile et al., 2022; Shelar et al., 2023). However, it is still unclear to what extent the toxicity is related to the released Ag^{+} because AgNPs

are susceptible to various environmental transformations (Pu et al., 2019).

AgNPs that are released into the environment or taken up by organisms will undergo modifications to their physicochemical properties and affect their transport, pathways, and toxic properties. In addition to being toxic, the current research suggests that, owing to the physicochemical properties of AgNPs and the reactions they can trigger in plants, these substances could be classified within the category of compounds beneficial for crops plant, commonly referred as bio-stimulants (Tortella et al., 2023). The effectiveness of these materials, when administered in typically modest quantities, is notable. Therefore, this nanomaterial has the potential to be developed as nano-priming. The seed priming technique will enhance seed germination, seed growth, and crop yields while also conferring resistance to pathogen. Nano-priming stands out as a significantly more efficient approach when compared to all other existing seed priming methods (Acharya et al., 2017). This is caused by nanoparticles having a large surface area-to-volume ratio, allowing seeds to absorb nutrients and water more effectively.

This research aims to investigate the effect of AgNPs synthesized with different size and concentration using the green synthesis method with sodium citrate and tannic acid on the germination and growth of maize (*Zea mays*) seeds. Maize is a crop that also has a relatively fast germination phase. Variations in the size and concentration of AgNPs are also tested in this study, and it has not been previously determined whether the synthesized AgNPs will have a toxic effect or act as nano-seed priming at appropriate sizes and concentrations. The synthesized results will be characterized using UV-Vis spectrophotometry to observe the wavelength and absorbance, transmission electron microscope (TEM), X-ray diffraction (XRD), and atomic absorption spectrophotometry (AAS).

MATERIALS AND METHOD

Synthesis and characterization of AgNPs

The synthesis process commenced by preparing a 100 ml solution containing 50 ml of 5 mM trisodium citrate ($Na_3C_6H_5O_7$) [Merck] and tannic acid ($C_{76}H_{52}O_{46}$) [Sigma-Alrdrich] at varying concentrations, starting from 0.025 (for synthesizing ~10 nm), 0.25 (~27 nm), and 5 mM (~50 nm) of AgNPs, respectively, based on the desired nanoparticle size. This solution was then transferred into a two-necked flask equipped with a condenser and a thermometer. The solution inside the flask was heated for 20 minutes using a heating mantle until the temperature reached 97±2 °C. After that, 1 ml of 25 mM $AgNO_3$ [Merck] was added into the solution. Subsequently, the solution was cooled and characterized using a UV-Vis spectrophotometer [Genesys S10, Thermoscientific] both before and after undergoing centrifugation for 20 minutes at a speed of 12,000 rpm. Afterward, the supernatant was poured off, and double-distilled water was added to the pellet. The solution was then subjected to another round of centrifugation at a speed of 12,000 rpm for 10 minutes. The resulting AgNPs were further analyzed using a TEM [FEI Tecnai G2 SuperTwin TEM/STEM] to determine the shape and size of the nanoparticles, and XRD to identify different phases present in the AgNPs, such as metallic silver or any silver compounds that might be formed during synthesis or treatment processes. Additionally, an AAS was utilized to ascertain the concentration of AgNPs. The AAS results are presented in the form of a standard curve to determine the concentrations of AgNPs. The equations derived from the AAS results are: $y = 0.1216x - 0.0016$, $R^2 = 1$ (~13 nm); $y = 0.141x + 0.0143$, $R^2 = 0.9911$ (~27 nm); and $y = 0.1459x + 0.0092$, $R^2 = 0.9932$ (~50 nm).

Seed germination test

In this research, AgNPs of sizes 15, 27, and 50 nm were synthesized, and their concentrations were set at approximately 8, 16, and 24 mg l^{-1}. As a result, dilution of the AgNPs solution was necessary. Double distilled water was used as a control. Before dilution, all synthesis repetitions in each size variation were combined and the UV-Vis spectrum was ascertained. The corn kernels used were *Z. mays* var. Baruna [Garuda seed]. The seeds used in the testing were pre-sterilized. Initially, the seeds were cleaned and sorted by soaking them in water, and only the submerged seeds with good viability were selected for further use. Subsequently, the chosen seeds were rinsed under running water for 10 minutes. Afterward, the seeds were sterilized by soaking them in sodium hypochlorite [Bayclin] 5.25% (v/v) for 15 minutes, followed by rinsing with distilled water. Next, the seeds were immersed in AgNPs solutions with different size and concentration, each corresponding to

its specific size (15, 27, and 50 nm) with concentration 8, 16, and 24 mg l^{-1}, for 24 hours. After this, the seeds were placed in plastic containers lined with filter paper. Each container held 15 seeds, and each treatment included three replicates. The humidity of the filter paper was maintained periodically throughout the experiment by adding distilled water. The germination process was monitored for a 14-day duration, with the seeds stored in a dark environment at a constant temperature of 28±2 °C. Seeds that exhibited successful germination were identified by the presence of radicles reaching a length of 1 to 2 mm.

Germination percentage

Germination percentage (%) is the ability of seeds to germinate normally under favorable circumstances after a specified time. Germination was observed in normally germinated seeds and calculations are carried out on the 14th day using the formula ISTA (1972) as Equation 1 (Kader, 2005; Biba et al., 2021).

$$GP(\%) = \frac{\text{Total normal sprout}}{\text{Total seeds}} \times 100\% \quad (1)$$

The germination rate was calculated using Equation 2 according to Lesilolo et al. (2012).

$$GR = \frac{N1T1+N2T2+N3T3\ldots+NxTx}{\text{Number of seeds that germinate}} \quad (2)$$

Where, GP = Germination percentage, GR = Germination rate, N = The number of seeds germinated in units of time (days), Nx = Total number of germinated seeds from the initial test to the final test, Tx = The amount of time between the initial test and the final test at a given interval.

The germination rate index was calculated using Equation 3 according to Lesilolo et al. (2012).

$$GRI = \frac{G1}{D1}+\frac{G2}{D2}+\frac{G3}{D3}+\ldots+\frac{Gn}{Dn} \quad (3)$$

Where, GRI = Germination rate index, G = The number of seeds germinated on a given day, D = The time corresponding to the amount, n = Number of days in the last day.

Seed growth parameters

In addition to germination parameters, the length of the shoot, root, as well as the fresh wight, dry weights, and water content (%) of the seedlings were also measured. The shoot length was determined from the longest leaf tip, and the root length was measured from the tip of the primary root. The fresh weight was directly measured using digital scales. The dry weight of the plant was determined by drying the samples in the oven at 70 °C until the weight constant, followed by weighing it using digital scales (Gupta et al., 2018). On the 14th day of germination, the GP (%), seedling length (include shoot and root), and biomass were used to calculate seed vigor index (SVI) according to Equation 4 and Equation 5 (Yagız and Çalışkan, 2024).

Seed vigor index I
(SVI I) = Gemination (%) × seedling length rate (mm) (4)

Seed vigor index II
(SVI II) = Gemination (%) × seedling weight (g) (5)

H_2O_2 analysis

For the measurement of H_2O_2 content, a sample of fresh leaf tissue weighing 0.1 g treatment^{-1} group, with each treatment having three replications, was required. The H_2O_2 level was determined spectrophotometrically according to Gupta et al. (2018). Firstly, 0.1 g of fresh leaf tissue was homogenized using a homogenizer with the addition of 5 ml of cold 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was then centrifuged at 12,000 g at 4 °C in a refrigerated centrifuge for 15 minutes, and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI). This mixture was incubated in the dark for 1 hour, and the absorbance was measured at 390 nm using a spectrophotometer. The amount of H_2O_2 was reported as µmol g FW^{-1}. The H_2O_2 content was calculated according to Equation 6, where V = volume of leaf extract (ml) and FW = fresh weight of leaf sample per unit volume of extract (g ml^{-1}).

$$H_2O_2\left(\frac{\mu mol}{g}\right) = \frac{Abs_{390}+0.004}{3.727 \text{ x V x FW}} \quad (6)$$

Data analysis

The results were statistically analyzed using a Two-Way ANOVA to determine the effects of AgNPs size, AgNPs concentration, and the interaction between size and concentration on seed germination, seed growth, and H_2O_2 ($p < 0.05$). A post hoc analysis was subsequently performed using the Tukey test with IBM SPSS Statistics 24.

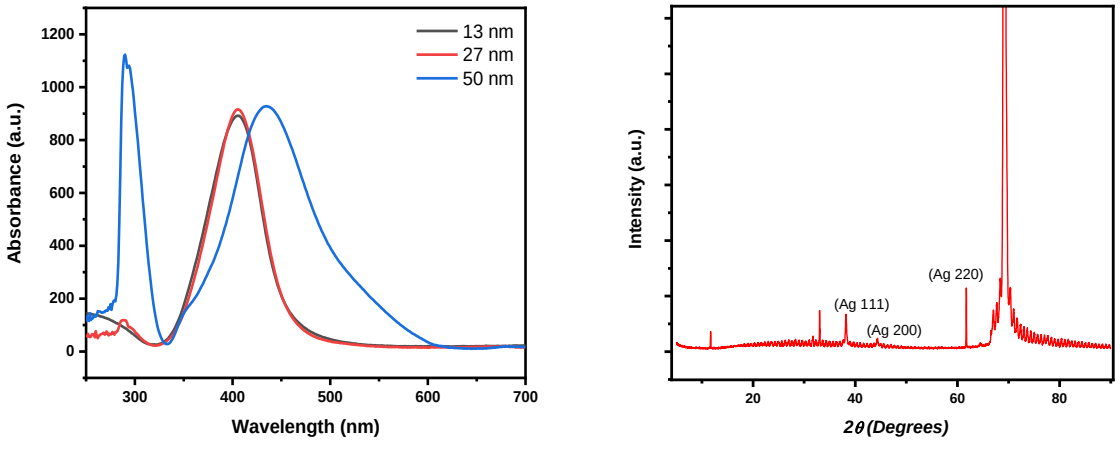
RESULTS AND DISCUSSION

AgNPs synthesis

Figure 1a represents the UV-Vis spectrum and the XRD results of the AgNPs solution. The spectrum shows the presence of absorption peaks in the range of 200 to 300 nm. These peaks correspond to the tannic acid and sodium citrate spectrum, which still have chromophore groups (unsaturated covalent groups) (Alqadi et al., 2014). This indicates that the synthesized AgNPs solution still contains reducing and capping agents. Meanwhile, the formation of AgNPs showed from the absorption peaks in the range of 400 to 450 nm. These peaks indicate a tendency for a wavelength shift with increasing tannic acid concentration. The combination of sodium citrate (CA) and tannic acid (TA) can produce CA-TA complexes that control reaction conditions to yield AgNPs with specific shapes and sizes (Ranoszek-Soliwoda et al., 2019). Shifts in the λ_{max} peak and peak width can indicate an increase in the size of AgNPs. The formation of AgNPs was also confirmed using the XRD. The results obtained show the spectrum of pure silver (Figure 1b). XRD can identify the presence of different phases or compounds within the sample. In addition to metallic silver, AgNPs may contain other phases or compounds resulting from surface oxidation or chemical reactions during synthesis.

The TEM image results also demonstrate that the synthesis yields AgNPs with spherical shapes and three different sizes when using three different tannic acid concentrations. At tannic acid concentrations of 0.025 mM, the size is 13.39±2.40 nm; at 0.25 mM, the size is 27.25±4.09 nm; and at 5 mM, the size is 46.37±10.75 nm (Figure 2a, 2b, and 2c). Meanwhile, the size distribution of AgNPs in the solution can be seen in Figure 2d, 2e, and 2f. These results indicate that tannic acid acts as a stabilizer to maintain and control the size of AgNPs. AgNPs with various sizes are subsequently used to soak *Z. mays* seeds to evaluate their toxicity or nano-priming activity.

Tannic acid, also known as tannin or gallotannin, is a type of polyphenolic compound found in various plant tissues, such as fruits, leaves, bark, and wood. It consists of a central glucose (sugar) core that is esterified with multiple galloyl groups. These galloyl groups are derived from gallic acid and are attached to the glucose core through ester bonds. Therefore, tannic acid can play a role as a weak reducing agent known to undergo hydrolysis under either acid or alkaline conditions to form glucose and gallic acid (Ranoszek-Soliwoda et al., 2019; Matras et al., 2022). Glucose is known as a weak reducing agent also highly effective as a stabilizer in alkaline conditions. Meanwhile, sodium citrate can function both as a reducing agent and a stabilizing agent. It can stabilize nanoparticles electrostatically by utilizing the free electrons in the carbonyl group and act as a coordinating agent with metal atoms that have available orbitals. The capping agent is crucial as a stabilizer that can inhibit excessive growth of nanoparticles and prevent aggregation in the colloidal nanoparticle synthesis (Figure 3). Ligands from the capping agent stabilize the surface of AgNPs. This capping agent also plays an important role in altering its biological activity and environmental perspective. The steric effects of the adsorbed capping agent on the nanoparticle's surface are responsible for



a. b.

Figure 1. The UV-Vis spectrum (a) and the XRD (b) result of the AgNPs colloid from each synthesis result

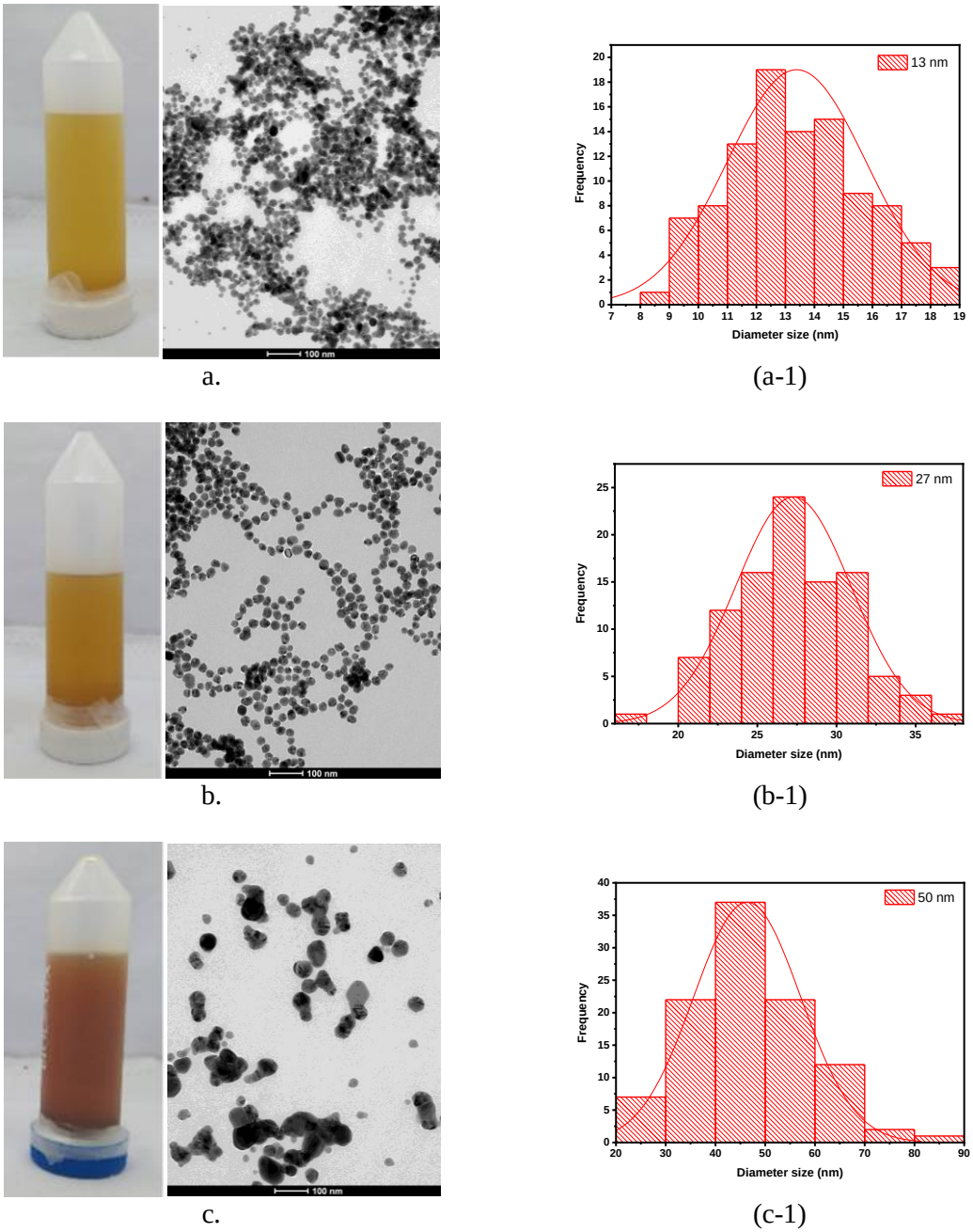


Figure 2. The colloid solution and TEM images of AgNPs with sizes (a) 15 nm, (b) 27 nm, and (c) 50 nm. The size distribution of AgNPs from each different concentration of tannic acid (a-1) 0.025 mM (13.39±2.40 nm), (b-1) 0.25 mM (27.25±4.09 nm), (c-1) 5 mM (46.37±10.75 nm)

changes in the physicochemical properties and biological activity of the nanoparticles (Orlowski et al., 2018; Liu et al., 2021).

In previous research, Bastus et al. (2014) synthesized AgNPs coated with sodium citrate with different concentrations. The result also showed nanoparticles in sizes ranging from 10 to 200 nm through the reduction of silver nitrate reacted with sodium citrate and tannic acid. This disclosed that sodium citrate and tannic acid promoted the enlargement of AgNPs dimensions through nitrate reduction (Bastús et al., 2014). Meanwhile, Zhang et al. (2016) and Liu et al. (2021) used tannic acid as a reducing agent and a capping agent in the production of spherical AgNPs with sizes ranging from 8 to 22 nm. Therefore, this synthesis method using sodium citrate and tannic acid can be used to determine precise control of the shape and dimensions of the AgNPs.

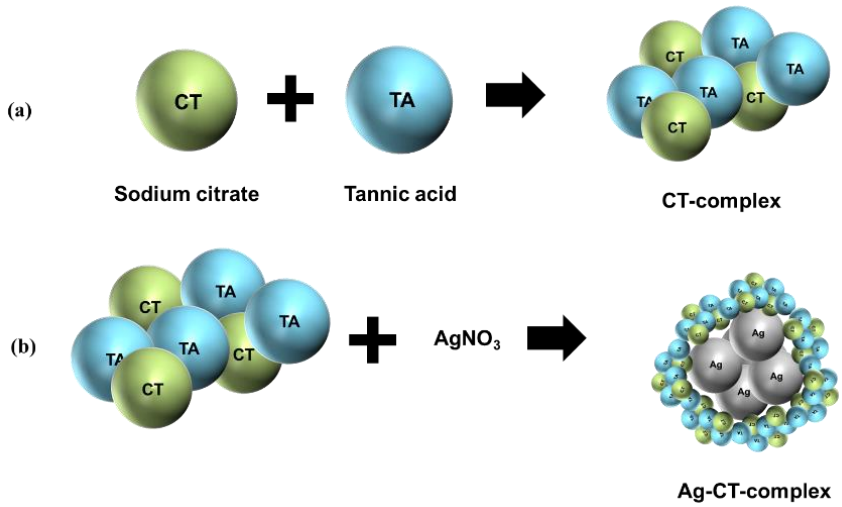


Figure 3. The synthesis mechanism illustration of AgNPs using tannic acid (TA) and sodium citrate (CT). (a) Formation of complexes of sodium citrate and tannic acid, (b) The complexes of sodium citrate and tannic acid act as a reducer and stabilizer in the process of AgNPs synthesis

The effect of AgNPs on seed germination

Germination parameters are characterized by the values of GP, GR, and GRI (Figure 4). The results showed no significant differences in seed GP and GR, while GRI indicated a significant difference in the effect of AgNPs size and the interaction between size and concentration of AgNPs. However, the concentration of AgNPs has not had a significant effect on GRI. Some treatments showed lowest results of GRI at AgNPs 50 nm with concentration ~24 mg l^{-1}, while AgNPs 27 nm at 24 mg l^{-1} showed the highest rate. This indicates that others variations in size and concentration of AgNPs did not have a significant impact on seed GP or GR. In other words, the size and concentration of nanoparticles do not significantly affect the number of seeds that germinate or the speed of germination. GP reflects the seed's germination potential in each treatment and can be an indicator of seed quality and its ability to germinate according to the treatment given (Figure 4). The GRI is significantly influenced by the size of AgNPs and the interaction between nanoparticle size and concentration. This means that the combination of size and concentration of nanoparticles has a significant effect on the GRI. It suggests that certain combinations of size and concentration may be more effective in influencing the germination speed, even though they do not have a significant impact on the overall GP or GR. This implies that variations in nanoparticle concentration alone, without considering size, are not strong enough to influence the GRI.

Meanwhile, the GR was determined by counting the number of days it took for the radicle to emerge from the seed, which was 14 days, although radicles typically began to appear on days 2 to 3. The average number of days for seed germination was calculated to assess the response of each treatment to the seeds throughout the observation period. Figure 4 shows AgNPs of 50 nm at ~24 mg l^{-1} have a GR lower than the control. This explains that the following AgNPs size and concentration tend to have slower GR. In the case of AgNPs with a size of 13 nm, the higher the concentration, the GR tends to be higher. The same holds for the 27 nm in all concentration variations, while it's not the case for the 50 nm variation. The results indicate a tendency for the GR to decrease as AgNPs concentration increases. AgNPs with a size of 27 nm at a concentration of ~24 mg l^{-1} tend to have the lowest GR and GRI. Based on the result, these nanoparticles show potential for improving plant performance by enhancing germination at those sizes, concentrations, and differences in phytotoxicity based on synthesis methods, influencing the overall environmental safety of AgNPs (Yilmaz et al., 2021).

The effect of AgNPs on seedling growth

The growth parameters were assessed by measuring the length of roots and shoots (Figure 5). The data for shoot length is normally distributed and meets the assumption of homogeneity of variance (both *p*-values > 0.05). Meanwhile, the Two-Way ANOVA results

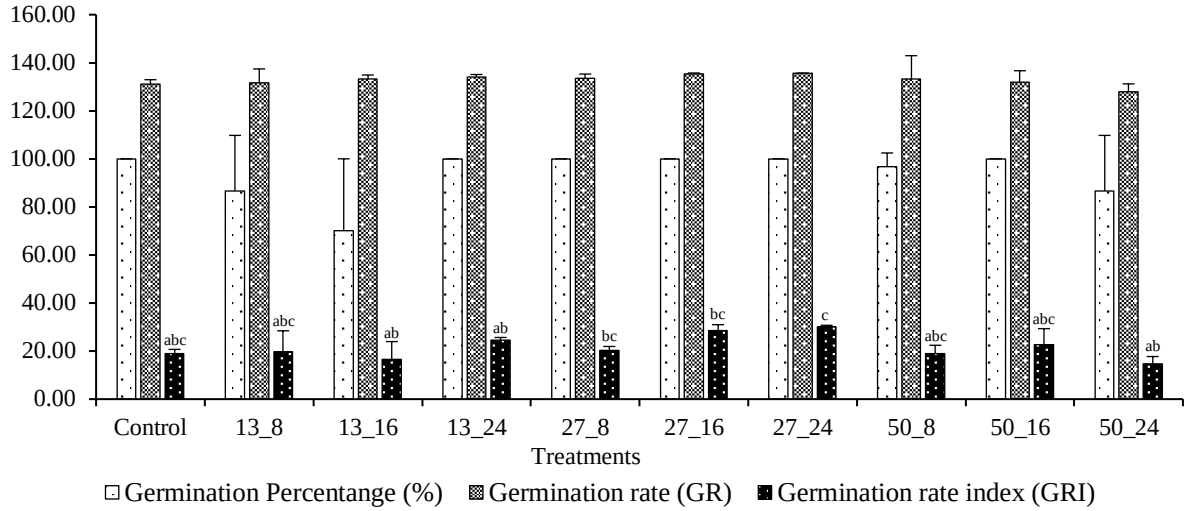


Figure 4. The GP (%), GR, and GRI after 14 days of germination of *Z. mays* treated with AgNPs sizes 13, 27, and 50 nm and concentrations 8, 16, and 24 mg l^{-1}

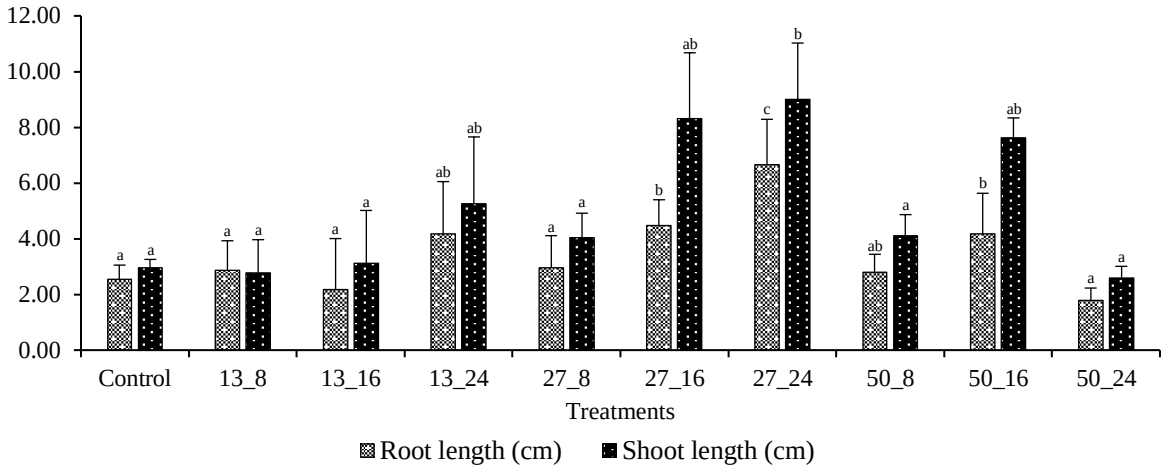


Figure 5. Shoots and roots length of *Z. mays* seedling treated with AgNPs sizes 13, 27, and 50 nm and concentrations 8, 16, and 24 mg l^{-1}

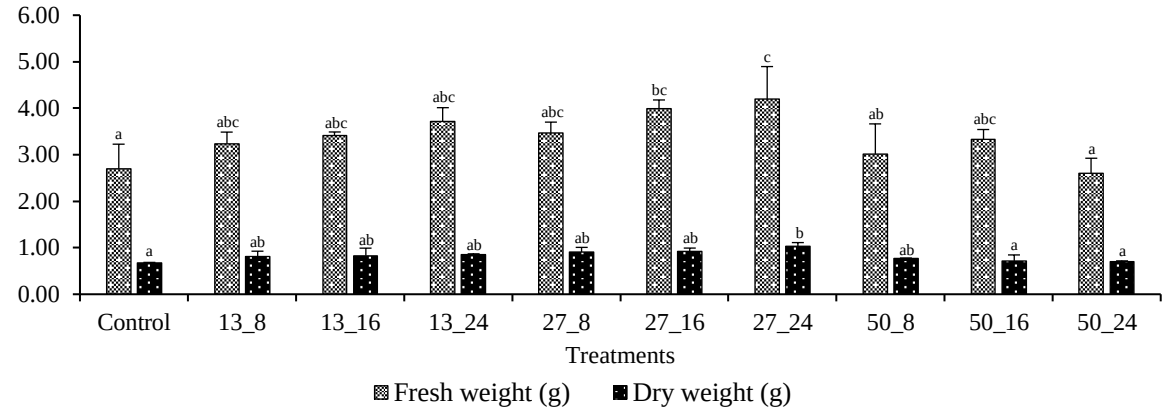
indicate a statistically significant effect of both nanoparticle size and concentration on shoot length ($p < 0.05$). This means that changes in both the size and concentration of nanoparticles significantly affect shoot length. The post hoc Tukey test further reveals significant differences between specific groups in terms of both nanoparticle size and concentration. This suggests that different sizes of nanoparticles and their varying concentrations contribute distinctively to shoot length. Additionally, the interaction between nanoparticle size and concentration has a significant effect, indicating that the combined influence of size and concentration is also important in determining the length of the shoot.

As for the shoot length, the analysis shows that both nanoparticle size and the interaction between size and concentration have a statistically significant effect on shoot length ($p < 0.05$). This means that the size of the nanoparticles and their interaction with concentration play a crucial role in influencing shoot length. However, the concentration of nanoparticles alone does not have a significant effect on root length ($p > 0.05$). This indicates that while the size of the nanoparticles and their combination with concentration influence root growth, changes in concentration by itself are not enough to significantly impact root length. For shoot length, both nanoparticle size and concentration have a significant impact, with interaction effects playing an important role. The post hoc analysis using the Tukey test further highlights specific differences between groups, indicating distinct influences of different sizes and concentrations. The 27 nm size and ~24 mg l^{-1} concentration resulted in the most significant shoot and root

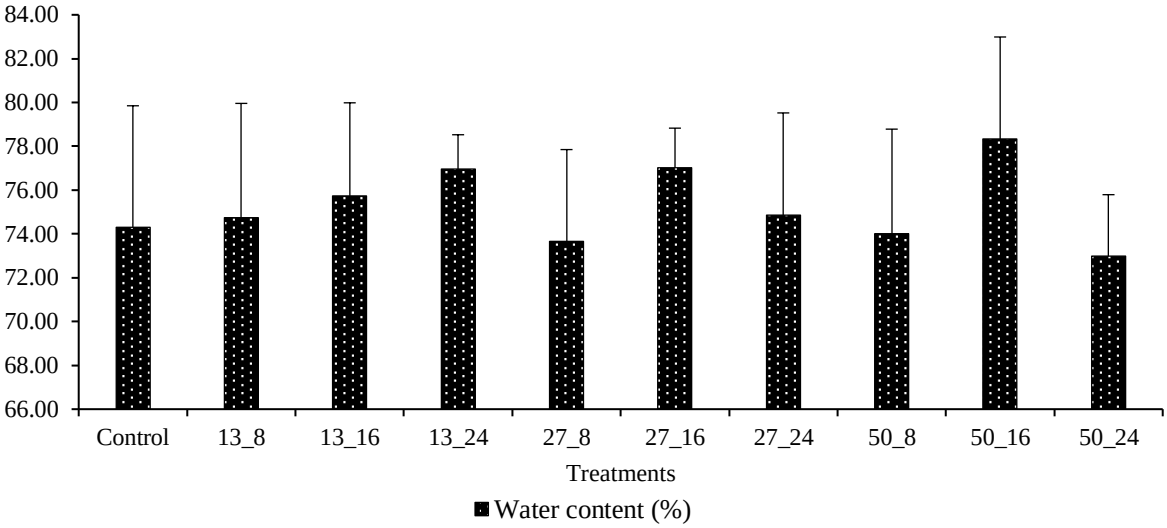
length than control and other treatments. Meanwhile, for root length, only the AgNPs size and the interaction between size and concentration show significant effects, while concentration alone does not have a significant effect. This indicates that root length is more sensitive to the physical size of nanoparticles and the combined interaction with concentration rather than the concentration alone. These results suggest that both shoot and root growth are differentially affected by AgNPs size and concentration, with shoot length being influenced by both factors independently, whereas root length depends more on the size and interaction effects.

Research by Nair and Chung (2014) showed rice germination over 7 days with exposure to 20 nm AgNPs at concentrations of 0, 0.2, 0.5, and 1 mg l^{-1}. The results indicated that shoot length tended to decrease with increasing concentration, although not significantly compared to the control. Meanwhile, it was also observed that root length in rice decreased with AgNP treatment (Nair and Chung, 2014). Research by Hasan et al. (2021) studied lettuce germination treated with 12 nm AgNPs at various concentrations (25, 50, and 100 ppm) and Ag^+ ions ($AgNO_3$) over 25 days. The results showed that AgNP treatments at higher concentrations (> 50 ppm) and Ag^+ ions ($AgNO_3$) had adverse effects, leading to a significant decrease in shoot and root length in lettuce. However, at certain concentrations, lettuce treated with 25 and 50 ppm AgNPs had significantly longer shoots compared to the control. In terms of root length, lettuce treated with 25, 50, and 100 ppm AgNPs had shorter roots, although the results were not significantly different between AgNP exposures (Hasan et al., 2021).

Figure 6a presents the values of fresh and dry weight. The results showed that AgNPs size



a.



b.

Figure 6. Fresh weight and dry weight (a), and water content in % (b) of *Z. mays* seedling treated with AgNPs sizes 13, 27, and 50 nm and concentrations 8, 16, and 24 mg l^{-1}

has a significant effect on fresh weight ($p < 0.05$). The results indicate the highest fresh weight in treatments with sizes 27 nm at a concentration of ~24 mg l^{-1} (4.19±0.7 g). Meanwhile, the lowest concentration was at AgNPs 50 nm at concentration ~24 mg l^{-1} (2.6±0.32 g). This means that variations in the AgNPs size significantly influence the fresh weight of the plants. Larger or smaller nanoparticles may contribute differently to the plant's ability to absorb water or nutrients accumulation, thereby affecting fresh weight. In this case, concentration and the interaction between AgNPs size and concentration do not have a significant effect on fresh weight ($p > 0.05$). This suggests that changes in concentration or the combination of size and concentration are not strong enough to impact the fresh weight of the plants. The size of the AgNPs is the primary factor of fresh weight, which is a parameter that can indicate the plant's metabolic activity (Parveen and Rao, 2015; Hasnain et al., 2019).

Similar to fresh weight, AgNPs size also has a significant effect on dry weight ($p < 0.05$). The treatment with AgNPs of 50 nm at ~24 mg l^{-1} shows the lowest dry weight (0.69±0.02 g). The treatment with a size of 27 nm at a concentration of ~24 mg l^{-1} has the highest dry weight values compared to the control and other treatments (1.033±0.07 g). This indicates that the size of the nanoparticles influences the overall biomass or dry matter produced by the plants. The size-dependent effect may be related to how the nanoparticles interact with plant tissues or metabolic processes, impacting dry biomass accumulation. Again, neither concentration nor the interaction between size and concentration show significant effects on dry weight ($p > 0.05$). Meanwhile, in the case of AgNPs of 50 nm, the values are not significantly different from the control (Figure 6b). Plant dry weight can indicate the plant's nutritional status and biomass, serving as an indicator of whether growth and development processes are occurring properly. This reinforces the idea that AgNPs size is the primary factor influencing dry weight, while concentration, whether alone or in combination with size, does not significantly alter the outcome. Greater biomass suggests normal metabolic processes, but lower results may indicate hindrances in the plant's metabolism (Noshad et al., 2019).

Figure 6b shows the percentage of water content in *Z. mays* seedlings treated with various sizes and concentrations of AgNPs. The result shows no significant effect of AgNPs size, concentration, or the interaction between them on water content ($p > 0.05$). This suggests that the water retention or hydration level of the plants is not significantly influenced by the size or concentration of AgNPs. The plants' ability to maintain water content remains relatively stable regardless of the AgNPs treatments. The results obtained do not indicate significant differences between the control and treatments. There are no significant differences in water content, suggesting that the treatments do not impact the plant's water retention capacity. The water content in seedlings can be expressed as a percentage of the total weight, reflecting how much the seedling's tissue is filled with water. This can provide information about plant moisture and its physiological conditions. Measuring water content in seedlings is typically done as part of plant growth analysis and scientific research can reduce plant water losses due to transpiration under drought-stress conditions. In this study, the AgNPs size significantly affects plant biomass, the water content is unaffected, and nanoparticle concentration alone does not play a significant role in influencing these growth parameters.

Figure 7 shows that AgNPs size, concentration, and the interaction between size and concentration have a significant impact on SVI I ($p < 0.05$). This means that both the size and concentration of AgNPs independently affect the vigor of the seeds, and their combination further amplifies the effect. Treatment with the highest SVI I was the seed soaked in AgNP 27 nm, with a concentration of ~24 mg l^{-1} (1,567.00), and the lowest at AgNP 50 nm, with a concentration of ~24 mg l^{-1} (381.3). Meanwhile, variations in AgNPs size significantly affect the seed vigor, potentially due to the different mechanisms of various sizes of AgNPs interacting with the seeds. The different concentrations of AgNPs also significantly influence SVI I, implying that the number of AgNPs present affects seed vitality. Besides that, the significant interaction effect indicates that the combination of nanoparticle size and concentration produces a combined influence on seed vigor that is distinct from their individual effects. A higher SVI indicates better seed quality and performance, which can lead to more uniform and vigorous plant stands in the field (Shah et al., 2021).

In SVI II (Figure 7), both AgNPs size and the interaction between size and concentration have a significant effect ($p < 0.05$). The findings reveal that treatment with AgNPs size 50 nm at

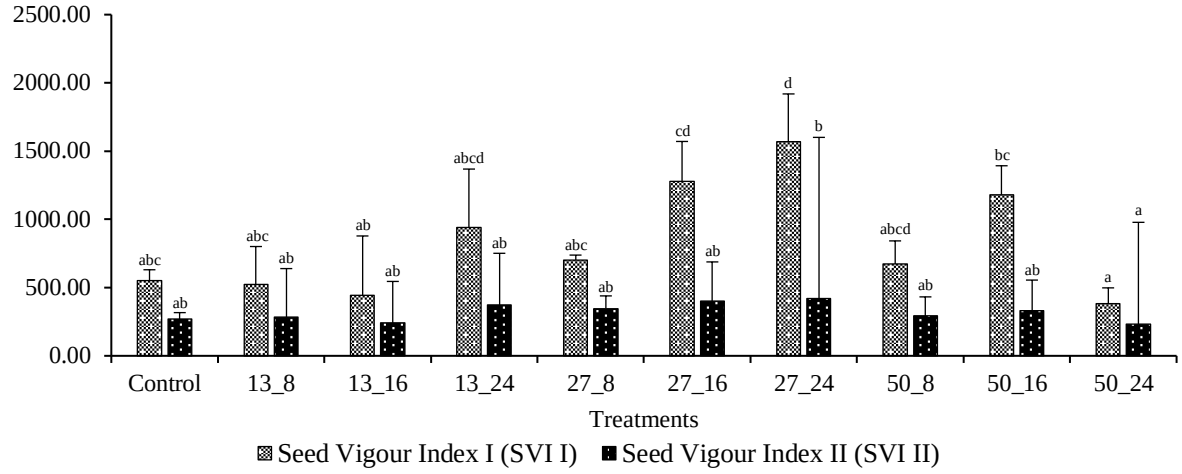


Figure 7. Effect of *Z. mays* nano-priming using AgNPs sizes 13, 27, and 50 nm and concentrations 8, 16, and 24 mg l^{-1} on SVI I and SVI II

24 mg l^{-1} concentration had the lowest SVI II (230.00), and AgNPs size 27 nm at 24 mg l^{-1} concentration had the highest SVI II (419.33). Unlike SVI I, the concentration of AgNPs alone does not have a significant effect on SVI II ($p > 0.05$). This implies that while size and the interaction between size and concentration matter, the concentration of AgNPs by itself does not have a strong influence on SVI II. This suggests that the physical size of the AgNPs is a crucial factor in determining seed vigor, and the interaction between AgNPs size and concentration also plays a role. As in SVI I, the size of the AgNPs has a significant influence on SVI II, likely affecting the growth potential or strength of the seedlings. The interaction between size and concentration is also significant, indicating that specific combinations of AgNPs size and concentration have a distinct impact on the seeds' vigor. This can ultimately result in better crop establishment, improved yield potential, and overall crop productivity (Bairwa et al., 2023). This result also showed nano priming treatments using AgNPs synthesis using tannic acid can improve the GRI and uniformity of seed germination in *Z. mays*. Nanomaterials may facilitate water uptake by the seeds and promote the activation of metabolic processes involved in germination. At appropriate sizes and concentrations, AgNPs may enhance seed germination, root elongation, and overall growth by promoting nutrient uptake and physiological processes. However, at higher concentrations, AgNPs can inhibit seed germination and seedling growth due to their toxicity (Hassanisaadi et al., 2022).

Figure 8 presents that there are no significant differences in H_2O_2 content in *Z. mays* seedlings treated with three different sizes and concentrations of AgNPs ($p > 0.05$). The control group shows a very low H_2O_2 content, which is expected as no AgNPs treatment was applied. This suggests that neither the size of the nanoparticles nor their concentration had a notable effect on the levels of H_2O_2 in the seedlings. The treatment with 27 nm AgNPs at 24 mg l^{-1} stands out, showing a large increase in H_2O_2 content compared to other treatments. However, the large error bar suggests high variability in this group, which means that while the average H_2O_2 content is high, the variation within this treatment group is also substantial. This could indicate inconsistent effects of this AgNPs size and concentration on oxidative stress in the seedlings. H_2O_2 is often a marker for oxidative stress in plants, and these results indicate that the application of AgNPs, regardless of size or concentration, did not induce significant oxidative stress in the seedlings. This might suggest that *Z. mays* seedlings are either tolerant to the AgNPs treatments at the applied levels, or that the concentrations and sizes used in the experiment do not significantly impact the oxidative processes within the seedlings (Gupta et al., 2018; Nile et al., 2022). The absence of significant changes in H_2O_2 content implies that the AgNPs treatments did not lead to increased oxidative stress in *Z. mays* seedlings. This could indicate that the nanoparticles, at the tested sizes and concentrations, do not disrupt cellular processes related to H_2O_2 production.

Since H_2O_2 content alone may not provide a complete understanding of the physiological response of *Z. mays* seedlings to AgNPs, additional physiological parameters should be analyzed to gain deeper insights into plant stress

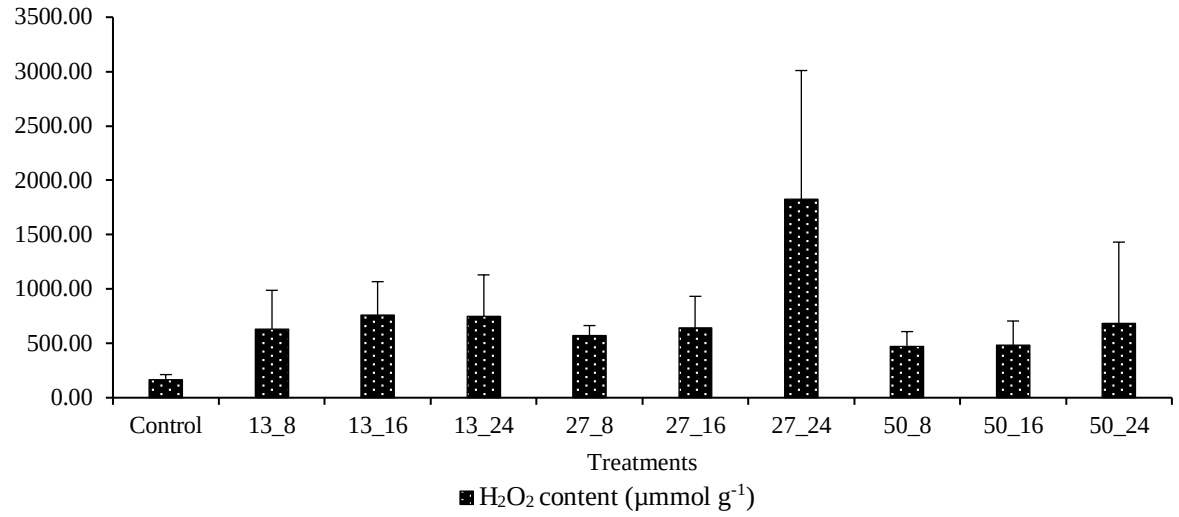


Figure 8. The H_2O_2 content of *Z. mays* seedlings treated with three different sizes and concentrations of AgNPs solution

responses toward their interaction with AgNPs. These include protein content, which measures total protein content that can help to assess the overall metabolic state of the plant and whether the application of AgNPs affects protein synthesis or degradation. In addition, the activities of catalase (CAT), SOD, and phenylalanine ammonia-lyase (PAL) should be analyzed to understand the metabolite mechanism that plays crucial roles in plant defense mechanisms within cells (Shah et al., 2021; Garza-Alonso et al., 2023). Silver ions released by AgNPs drive excessive ROS production, damaging mitochondrial functions. The percentage of successful seed germination was influenced by nano-priming depended on the concentration and AgNPs size (Sonawane et al., 2021).

Silver ions released from the AgNPs lead to cellular enzyme inactivation, membrane structure disruption, impaired electron transfer processes, reduced redox potential levels, decreased mitochondrial membrane potential (MMP), and increased intracellular ROS accumulation (Yan and Chen, 2019). AgNPs have also been reported to increase intracellular ROS accumulation by disrupting electron transfer processes, increasing the $NADP^+$/NADPH ratio, and interfering with mitochondrial functions when the dose is inappropriate. AgNPs bind strongly to plant cell membranes, where they can generate ROS and cause peroxidation of the outer membrane lipids (Kandhol et al., 2022; Nile et al., 2022). Altered lipid content from the cell membrane can result in increased cell permeability, leading to uncontrolled NP transport from the extracellular environment into the cytoplasm, where further cell damage may occur if the doses and physicochemical properties of the nanoparticles are not suitable. However, AgNPs with sizes ranging from 6 to 27 nm (10 and 20 mg l^{-1}) are known to increase the expression of the aquaporin gene in *Oryza sativa*. Also, AgNPs with sizes 10 to 30 nm and a concentration of 50 mg l^{-1} increased seed and seedling vigor on wheat seeds (*Triticum aestivum*). Therefore, if the dose is appropriate, AgNPs will not inhibit plant growth but act as a biostimulant (Shelar et al., 2023; Tolisano and Del Buono, 2023), in this case, as seed nano-priming.

Nanoparticles can be classified as nano-seed priming when present within specific concentration ranges, typically at low levels, they can enhance plant growth and act as biostimulant (Guzmán-Báez et al., 2021; Tolisano and Del Buono, 2023). Plant biostimulants derived from nanoparticles can positively impact seed germination, root development, nutrient absorption, stress tolerance, and overall crop productivity (Guzmán-Báez et al., 2021; Tortella et al., 2023). The use of nanoparticles as biostimulants is an emerging area of research aimed at optimizing agricultural practices and promoting sustainable farming. As for nano-seed priming, this mechanism involves the application of nanoparticles to seeds before planting, which can influence various physiological and biochemical processes within the seeds. Nano-seed priming can improve seed vigor, nutrient uptake, water absorption, and overall plant performance (Shelar et al., 2023; Tortella et al.,

2023). It is used to optimize seedling establishment and enhance crop yields, particularly under challenging environmental conditions. This technique leverages the unique properties of nanoparticles depending on their size and concentration to benefit seed and plant development.

CONCLUSIONS

The findings suggest that AgNPs sized at 27 nm and at a concentration of 24 mg l^{-1} demonstrate potential as nano-seed priming agents to facilitate the germination and growth of *Z. mays* seeds based on the trend of biometric data. Meanwhile, AgNPs sized at 50 nm with a concentration of 24 mg l^{-1} exhibit inhibitory effects on seedling growth. This study highlights the importance of considering both the size and concentration of AgNPs, as they can elicit diverse responses in *Z. mays* germination. Notably, toxicity, manifested as growth inhibition, becomes apparent at certain concentrations of AgNPs, which can disrupt seed physiology, hindering proper germination and development. Therefore, further investigation is warranted to ascertain the optimal application dosage and physicochemical properties of AgNPs, as well as their interaction with different environmental conditions. Furthermore, exploring the effects of AgNPs synthesized using sodium citrate and tannic acid on various plant species, particularly crop plants, is crucial for evaluating their efficacy as nano-seed priming agents and determining the appropriate dosage. Nano priming offers promising opportunities for enhancing the performance and stress tolerance of *Z. mays* seeds, potentially leading to improved crop productivity and sustainability in agriculture. However, further research is needed to optimize nano priming protocols, evaluate their long-term effects on crop growth and environmental safety, and assess their practical feasibility for large-scale application for sustainable agriculture.

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