



**Original Article**

**Further observations on Enhancing Soybean Performance with Green-Synthesised Silver Nanoparticles from Neem Leaves: Effects on Physicochemical Properties, and Nutritional Composition**

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**ABSTRACT**

Plant nanotechnology provides sustainable solutions for improving crop productivity and quality. This study evaluated the effects of neem (*Azadirachta indica*) derived biosynthesised silver nanoparticles (AgNPs) on agromorphological traits, physicochemical properties, and nutritional characteristics of three soybean (*Glycine max*) genotypes from Nigeria (SOY-MNA-001, SOY-MKW-001, and SOY-MNA-002). Silver nanoparticles were synthesised using neem leaf extract and characterised by UV-Vis spectroscopy, dynamic light scattering, and scanning electron microscopy. Soybean seeds were primed with AgNPs at 0, 25, 50, 75, and 100 ppm and evaluated under field conditions using a factorial experimental design. AgNP application significantly supported growth, yield, seed quality, and oil characteristics in a dose- and genotype dependent manner. Moderate concentrations (25–50 ppm) generally promotes vegetative growth, branching, pod number, seed yield, protein, lipid, and mineral contents, while maintaining acceptable moisture and fibre levels. Improvements in oil quality were observed at optimal doses, including reduced acid and peroxide values and enhanced iodine and saponification values in responsive genotypes. Conversely, higher concentrations (75–100 ppm) induced growth suppression, nutritional trade offs, and oxidative instability in some genotypes. In general, neem derived AgNPs functioned as effective bio-stimulants at optimized concentrations, indicating their potential for sustainable soybean productivity and quality enhancement in Nigeria. Neem-derived AgNPs should be evaluated in combination with conventional fertilizers and organic amendments to develop integrated, cost-effective, and environmentally sustainable soybean production systems. Future research should focus on elucidating the physiological, biochemical, and molecular mechanisms underlying AgNP-mediated improvements in growth, yield, and seed quality.

**Keywords:** Green-synthesised silver nanoparticles, Neem, Soybean, Nutritional composition,

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## INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) is known to be among the most significant economic leguminous crops all over the world {1}, regarded as a rich source of high-quality plant protein, minerals, lipids, vitamins, and essential amino acids, which serve as industrial raw materials for food manufacturing industries. Cultivation of soya bean has improved over the past 20 years as a result of its contributions to food production, animal feed formulation, and agricultural food processing. {2} Upon its improvement in cultivation, the yield of this plant is still very low, perhaps as a result of diseases, loss of soil nutrient, and non-improved agronomic practices. These challenges address the need for more sustainable, profitable that will improve the growth of soya bean, increase agronomic performance, and enhance its nutritional and physicochemical characteristics

Modern development in nanobiotechnology provided new ideas to address these agricultural challenges. Among many engineered nano materials, silver nanoparticles (AgNPs) have received considerable attention as a result of its unique physicochemical properties that arise from their nanoscale size, antimicrobial exceptional catalytic, redox properties and high surface area to volume ratio{3}. AgNPs is considered to be a modifying agent in plants metabolism and physiological processes, such as photosynthesis, nutrient uptake, enzyme activation, stress tolerance and seed germination. The synergy between the plant tissues and AgNPs help to increase roots elongation improve biosynthesis of chlorophyll, regulate phytohormone pathways, and increase resistance

activities of fungal and bacterial diseases. Although, the biological activities of AgNPs confide majorly on duration, particle size, exposure synthesis route, dose, and genotypic nature of the plant {4}.

The procedures of nanoparticle production play a vital role in controlling its biocompatibility and agricultural significance.{5}. Even though physical and chemical synthesis paths control industrial processes, these techniques often involve toxic reagents, environmentally harmful by products and high energy demands. In comparison, green synthesis has emerged as a safer, ecologically friendly, and economically viable techniques of nanoparticle synthesis using biological molecules from plants, fungi, and microorganisms as reducing and stabilizing agents. Biosynthesis is particularly important due to the presence of many phytochemicals such as, saponins, phenolics, flavonoids, terpenoids, alkaloids, and tannins that facilitate rapid nanoparticle formation and enhance surface stability {6}

Neem (*Azadirachta indica*) leaf extract is known to be one of the strongest botanical substrates for green synthesis of AgNPs due to its rich phytochemical diversity and effective bio-reducing potential.{7}. Neem contains quercetin, azadirachtin, nimbin, nimbolide, and various triterpenoids, which serve both as a reducing and capping agents, yielding nanoparticles with strong antioxidant, antimicrobial, and growth-modulating properties. According to Edo *et al* 2025, Plant based AgNPs from neem leaves have proved to be strong in increasing strength in seed vigour, improving plant metabolic activities and suppressing soil-borne diseases. Their natural origin, however, improve environmental compatibility and

ameliorate the toxicological concerns associated with chemically synthesized nanoparticles. {7}

Despite the expansion of application of biosynthesized AgNPs, there still limited information on their effects on indigenous Nigerian soybean varieties, particularly with regard to their physicochemical traits and proximate composition. Soybean genotypes vary significantly in genetic component, biochemical composition, growth behavior and stress response mechanisms. Studying the behavior of biosynthesized AgNPs with these genetically diverse genotypes is germane for designing targeted nanotechnological interventions that contribute to crop improvement programmed {8}. Moreover, while some studies have investigated the influence of AgNPs on general growth characteristics, systematic investigations that combine seed quality attributes such as protein, oil quality, carbohydrate, fibre, ash, and, moisture content are still largely lacking {9}.

Furthermore, physicochemical properties such as bulk density, water absorption capacity, seed pH, thermal stability and emulsification index are critical indicators of soybean's processing suitability and nutritional value. It is possible that green-synthesized AgNPs may promote or modulate these traits and provides a novel area of scientific findings with direct implications for food science, agro-processing industries, and crop quality improvement initiatives in Nigeria.

It is well known that biosynthesized silver nanoparticles from neem leaf extracts offer a promising, sustainable pathway for improving soybean performance. Yet, empirical evidence on their dose-response relationships, differential effects across

genetic accessions, and influence on nutritional quality still lacking. This gap necessitates a comprehensive study that not only evaluation of the agronomic and morphological effects of neem derived AgNPs on soybean but also explores their impact on seed physicochemical parameters and proximate composition.

Therefore, this study investigates the effects of biosynthesized silver nanoparticles from neem (*Azadirachta indica*) leaf extracts on physicochemical properties, and proximate composition of selected Nigerian soybean accessions. The findings are expected to contribute valuable insights into the potential of green-synthesized nanomaterials as bio-stimulants for crop improvement, provide scientific evidence to support sustainable agricultural practices, and strengthen Nigeria's capacity for nanotechnology driven food security interventions.

## MATERIALS AND METHODS

### Description of Study Area

The experiment was conducted at the Plant Biology laboratory and centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna. Niger State, is situated within the geographical coordinates of latitude 9°37' N and longitude 6°33' E in North Central, Nigeria. With about 1.2 million people living there, it has a land area of 88 km<sup>2</sup>. The mean annual temperature, rainfall and relative humidity, in Minna are 30.20 °C, 1334.00 mm, and 61.0 %, respectively, indicative of a tropical climate. The region's climate may be divided into two separate seasons, the rainy season (May to October), and the dry season (November to April). Typically, the area's vegetation is guinea savannah with a predominance of grass and a few scattered trees.

### Seed Collection

Seeds of three different genotypes were collected from local farmers from three different areas in Niger State. The first was collected from Bosso, Minna, Niger State (SOY-MNA-001), the second was collected from farmers in Mokwa, Niger State (SOY-MKW-001) and the third was collected from Chanchaga, Minna, Niger State (SOY-MNA-002), and stored in well labeled paper envelopes and stored in the refrigerator until further use.

### Source of Silver Nanoparticles

Fresh and healthy neem (*Azadirachta indica*) leaves were collected from the biological garden of Federal University of Technology Minna. The leaves were first properly cleaned under running tap water, then again with deionized water to remove all dusts and undesirable particles. After that, the leaves were cut into small pieces and allowed to air dry at room temperature {10}.

### Preparation of Leaf Extract

After transferring 10 g of the dried leaves to a 250 ml beaker with 100 ml deionized water added to it, the mixture was agitated for 20 minutes at 80 °C using a magnetic stirrer. For subsequent studies, the extract was placed in Erlenmeyer flasks and cooled at 4 °C after being filtered twice with Whatman filter paper. Sterile conditions were maintained for the entire experiment to ensure efficacy and correctness of the findings {10}.

### Biosynthesis of Silver Nanoparticles

In a 250 ml Erlenmeyer flask, 1 ml was used out of 1M silver nitrate ( $\text{AgNO}_3$ ) prepared, and 10 % leaf extract was added for the reduction of  $\text{Ag}^+$  ions. The entire mixture was maintained at 30 °C on a magnetic stirrer. Along with routine sampling and

scanning by UV-Visible (UV-Vis) spectrophotometer, time and colour change were also recorded. Appropriate controls were kept in place throughout the experiment. The colour shift from light to yellowish colloidal brown indicated the complete reduction of  $\text{Ag}^+$  ions. The colloidal solution was then kept aside for a whole day in order to observe saturation and total bio-reduction using UV-Vis spectrophotometric scanning. After which the solution was properly covered and stored in the refrigerator after which spectrophotometric method was used to confirmed the synthesis of silver nanoparticles {10}.

### Determination of Surface Morphology of the Biosynthesized Nanoparticles

The nanoparticle size was determined by preparing nanoparticle suspension or solution while making sure that the particles were evenly distributed and did not aggregate. The solution was filtered through a filter whose pore size was less than the size of the expected nanoparticles in order to get rid of large particles and aggregates. The dynamic light scattering (DLS) device was aligned, calibrated, and set up in a vibration-free environment in accordance with the manufacturer's instructions. To minimize errors, a refractive index that was almost identical to the solvent's was used. Following the placement of the nanoparticle suspension in an appropriate cuvette or sample cell, the sample cell was put into the device, and measurements were made. A detector collected the dispersed photons at 25 °C after the instrument's laser beam entered the sample cell. To guarantee statistical accuracy, many runs of data were obtained at the proper acquisition periods. The data was analyzed using the instrument's software or specialized DLS analysis software. The results, which included

metrics like mean particle size and polydispersity index, were displayed as a size distribution plot (either intensity- or volume-weighted) (National Research Centre for Working Environment {11}).

Following the process of nanoparticle synthesis, the forms of the particles were established. Millipore filters with a pore size of 0.2  $\mu\text{m}$  were used to filter the nanoparticle solution in order to remove any impurities that might interfere with the images captured by the scanning electron microscope (SEM). A pipette was used to extract about 25  $\mu\text{l}$  of the filtered material, which was then put onto a copper stub that was specifically constructed and shaped like a small cylinder with a diameter of about 1 mm. The stub had double-sided carbon material applied to one side and the stub was firmly fastened to a holder after the sample was loaded onto the carbon material. Before collecting the samples for additional examination, the holder which could hold up to four samples at once went through two 10-minute sessions. The pictures of the nanoparticles were taken using the SEM. These pictures had embedded information on the specifics of applied voltage, magnification, and image content size {12}.

### Seed Treatment

In de-ionized water, the biosynthesized silver nanoparticles were dissolved at different concentrations (0, 25, 50, 75, and 100 ppm). After cleaning, the soybean seeds were primed by soaking in a solution containing different concentrations of the silver nanoparticles for about two hours. After which, the seeds were air dried before planting {13}.

### Determination of Proximate Composition

The crude Fibre, Protein content, Fat content, The ash, Moisture contents are determined by method of AOAC (2011)

### Determination of carbohydrate content

Following the {14} approach, the differences between the percentage of crude fibre, ash, fat, moisture, and protein content were subtracted from 100 to calculate the amount of carbohydrates present. Percentage carbohydrate (%) =  $100 - (\% \text{ Crude fiber} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Moisture} + \% \text{ Crude protein})$ .

### Physicochemical Analysis

The Oil extraction , refractive index , specific gravity , Acid value contents, free fatty acid, iodine value, peroxide value and saponification value were determined by AOAC (2011:2016)

## RESULTS AND DISCUSSION

### Characterization of green synthesized Silver nanoparticle (AgNP)

The present study examined the effects of biosynthesized silver nanoparticles (AgNPs) from *Azadirachta indica* (neem) leaf extracts on development of three Nigerian soybean genotypes (SOY-MNA-001, SOY-MKWA-001, and SOY-MNA-002). The UV-Vis spectrum of the synthesized AgNPs showed a strong absorption peak at 415 nm, attributed to the surface plasmon

resonance (SPR) from collective oscillations of free electrons in resonance with the light wave {17}. This confirms the bioreduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  during synthesis. SPR peaks below or above 400 nm typically indicate smaller or larger nanoparticles, respectively {18}. Dynamic light scattering (DLS) analysis revealed a mean hydrodynamic diameter of 112.7 nm and a polydispersity index (PDI) of 0.393 (Table 1). Scanning electron microscopy (SEM) confirmed that the particles were predominantly spherical, though some irregular shapes were observed (Fig. 1) {17}; {18}. These DLS and SEM results align with {19} and recent peer-reviewed studies, such as those reporting PDI values around 0.35–0.39, hydrodynamic diameters of 40–113 nm, and spherical morphology with sizes of 20–50 nm in green-synthesized AgNPs {17}; {20}.

### Proximate Composition

The proximate composition of soybean seeds from these genotypes were treated with different concentrations of neem-derived silver nanoparticles (AgNPs) which showed significant and dose dependent differences across all nutritional parameters evaluated (Table 2.) These differences showed that AgNPs exert both stimulatory and inhibitory effects on seed nutritional quality, depending on nanoparticle concentration and genetic composition of the soybean genotype. Moisture content across all treatments and accessions remained below the maximum limits recommended by CAIFS (8.5–10.0%), suggesting that AgNP application did not compromise seed storability. Moderate AgNP concentrations (25–50 ppm) generally reduced moisture content, particularly in SOY-MNA-001 and SOY-MKW-001, which may improve shelf life and reduce post-

harvest microbial contamination. However, high moisture contents observed at higher concentrations (75 ppm) in SOY-MKW-001 suggests that excessive nanoparticle exposure may interfere with water regulation mechanisms during seed development. Similar moisture-modulating effects of nanoparticles have been reported in legumes treated with metal-based nanomaterials {21}. Ash content, an indicator of total mineral accumulation, fell within the CAIFS recommended range (4.0–7.0%) for all genotypes. Moderate AgNP treatments slightly increased ash content in SOY-MKW-001 and SOY-MNA-002, indicating improved mineral uptake and translocation. This improvement may be as a result of nanoparticle induced stimulation of root absorption efficiency and membrane permeability. But, reductions at higher concentrations implies that excessive AgNPs may disrupt mineral homeostasis, corroborating earlier findings that nanoparticle benefits are concentration dependent {22}. Crude fat content showed significant accession specific responses. In SOY-MKW-001, fat content peaked at 25 ppm, while SOY-MNA-002 recorded its highest lipid concentration at 75 ppm. Conversely, lower doses reduced fat accumulation in SOY-MNA-001 before increasing at 100 ppm. These patterns demonstrated that AgNPs promotes lipid biosynthesis pathways, likely by modulating enzymatic activity associated with fatty acid synthesis. Similar dose dependent effects on oil content have been reported in soybean and other oilseed crops exposed to silver nanoparticles {23}. Most observed values were within or close to FAO-recommended ranges for soybean oil content (18–22%) Protein content responded positively to AgNP treatments at moderate concentrations, particularly

in SOY-MKW-001 and SOY-MNA-002, where protein levels exceeded CAIFS recommended values (35–40%). This increment may be linked to improved nitrogen assimilation and increased activity of nitrogen metabolizing enzymes induced by nanoparticles. However, protein content reduced at higher concentrations in SOY-MNA-001, suggesting potential metabolic stress or toxicity. Previous studies have shown that silver nanoparticles can promote protein synthesis at low doses but reduce it when applied excessively [24]; [25]. Crude fiber content remained largely within acceptable nutritional limits (4.0–6.0%) across all treatments. Slight reductions at moderate AgNP concentrations, especially in SOY-MKW-001, may be nutritionally advantageous, as lower fiber levels improve digestibility and nutrient availability. In the other hand, increased fiber content at higher concentrations in SOY-MNA-002 demonstrated improved synthesis of structural carbohydrates, possibly as a stress response. These results highlight the role of AgNPs in modulating carbohydrate partitioning during seed maturation. Findings from carbohydrate content revealed an inverse relationship with protein and fat in several treatments, showing a redistribution of seed reserves. An increase in carbohydrate levels at higher nanoparticle concentrations in SOY-MNA-001 may reflect reduced protein synthesis under stress conditions. In comparison, SOY-MNA-002 exhibited an increase in carbohydrate at 50 ppm, exceeding FAO recommended values (30–35%). Such shifts in carbon nitrogen balance have been widely reported in nanoparticle treated crops and are attributed to altered metabolic fluxes during seed filling [26]. All these demonstrated that neem-derived silver nanoparticles significantly

promotes the nutritional composition of soybean seeds in a dose dependent and genotype specific manner. Moderate concentrations (25–50 ppm) were generally optimal, enhancing protein, fat, and mineral contents without affecting moisture or fiber levels. In comparison, higher concentrations (75–100 ppm) often induced nutritional tradeoffs, perhaps due to nanoparticle induced oxidative or metabolic stress.

### Physicochemical Properties

The physicochemical properties of soybean oil extracted from three genotypes treated with neem-derived silver nanoparticles revealed significant differences across treatments and genotypes (Table 3). These parameters are critical indicators of oil quality, stability, degree of unsaturation, and industrial suitability. The findings demonstrated that AgNPs showed dose dependent and genotype specific effects on oil physicochemical traits, meriting their influence on lipid metabolism during seed development. The refractive index of oils from all genotypes ranged between 1.469 and 1.471, which are within the acceptable value [27]. So also, specific gravity values (0.911–0.913) were all compared across treatments and did not exceed acceptable limits. The little differences observed showed that AgNP treatments did not significantly alter the physical properties of the oils. This stability demonstrated that neem-derived AgNPs, even at higher concentrations, do not adversely affect oil purity or molecular structure, reports have it that nanoparticle application primarily promotes biochemical rather than physical oil attributes [28]. Free fatty acid (FFA) and acid value and contents are indicators of oil hydrolysis and deterioration. In SOY-MNA-001, acid value

and FFA increased at 25 ppm, suggesting influenced lipid hydrolysis at low nanoparticle concentration. However, values declined at 50 ppm, showed improved oil stability at mild AgNP doses. In SOY-MKW-001, acid value and FFA were lowest at 50–75 ppm, reflecting good oil quality and reduced degradation. Conversely, SOY-MNA-002 revealed increased acid and FFA values at 25–50 ppm, followed by reductions at higher concentrations. These trends revealed that moderate AgNP application (50–75 ppm) may suppress lipase activity and oxidative breakdown, while excessive or suboptimal doses may increase hydrolytic reactions. Similar concentration dependent effects of silver nanoparticles on lipid stability have been documented in oilseed crops {23}. Iodine value reflects the degree of unsaturation of fatty acids and is directly linked to nutritional quality and oxidative susceptibility. In SOY-MNA-001, iodine values reduced progressively with enhanced AgNP concentration, suggesting a reduction in unsaturated fatty acids at higher doses. In comparison, SOY-MKW-001 showed its highest iodine value at 25 ppm, followed by a significant reduction at 75 ppm, showed altered fatty acid composition. SOY-MNA-002 revealed increasing iodine values at 50–75 ppm, exceeding NAFDAC recommended ranges (124–139 I<sub>2</sub>/100 g), suggesting enhanced unsaturation and nutritional value. These genotype specific responses implies that AgNPs promotes desaturase enzyme activity differently across genotypes, there by modifying fatty acid profiles {29}. Peroxide value is a measure of primary lipid oxidation and oil rancidity. In SOY-MNA-001, peroxide values increased steadily at higher nanoparticle concentrations (75 ppm), suggesting oxidative stress and reduced oil stability. In contrast, SOY-MKW-001 revealed

significantly lower peroxide values at 50–75 ppm, suggesting improved oxidative stability under mild AgNP application. SOY-MNA-002 maintained relatively low peroxide values across all treatments, remaining within {27} acceptable limits (0–10 meq O<sub>2</sub>/kg). The results suggest that neem derived AgNPs may promotes antioxidant defense mechanisms at optimal concentrations but induce oxidative damage at excessive levels, these corroborated with earlier reports on nanoparticle induced redox imbalance {30}.

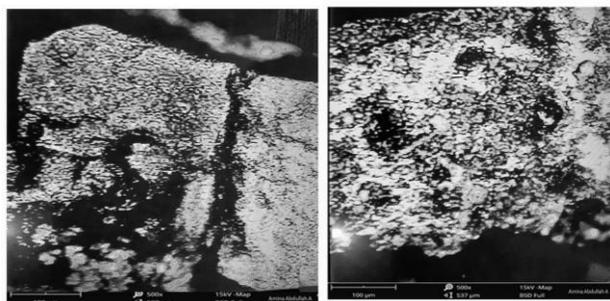
Saponification value provides insight into the average molecular weight of fatty acids and the industrial suitability of oils. In SOY-MKW-001 and SOY-MNA-002, saponification values increased significantly at 50–75 ppm, exceeding CAIFS recommended ranges (189–195 mg KOH/g), indicating a higher proportion of short-chain fatty acids and improved soap-making potential. SOY-MNA-001 revealed relatively stable values across treatments, suggesting limited nanoparticle influence on fatty acid chain length in this accession. Enhanced saponification values under moderate AgNP treatments suggest stimulated fatty acid biosynthesis and altered lipid composition, consistent with nanoparticle-mediated metabolic regulation {31}.

Overall, neem-derived silver nanoparticles significantly promote the physicochemical quality of soybean oil in a concentration and genotype dependent manner. Moderate concentrations (50–75 ppm) generally improved oil quality by reducing acid and peroxide values while promoting saponification and iodine values in responsive accessions. despite higher concentrations ( $\geq 75$  ppm) induced oxidative instability in some genotypes,

highlighting the importance of dosage optimization

**Table 1: Characteristic Properties of the Silver Nanoparticles Extracted from Neem (*Azadirachta indica*) Leaves**

Peaks	Size (d.nm)	% intensity	St Dev (d.nm)
Peak1	68.67	59.5	15.72
Peak2	371.7	21.4	58.11
Peak3	191.6	19.2	27.36
<b>Z-Average (d.nm): 112.7 , Pdi: 0.393, Intercep t: 0.699</b>			



**Figure 1: Characterisation of the Silver Nanoparticles Extracted from Neem (*Azadirachta indica*) Leaves**

**Table 2: Proximate Compositions of SOY-MNA-001, SOY-MKW-001 and SOY-MNA-002 Treated with Silver Nanoparticles**

Treatments	Nano dose (ppm)	Moisture Content (%)	Ash Content (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)	Carbohydrate (%)
<b>SOY-MNA-001</b>							
Control	0	3.01±0.00 <sup>b</sup>	6.13±0.11 <sup>ab</sup>	16.20±0.10 <sup>d</sup>	44.04±0.20 <sup>e</sup>	4.04±0.07 <sup>ab</sup>	26.59±0.12 <sup>a</sup>
G1C1	25	5.48±0.02 <sup>e</sup>	6.33±0.21 <sup>b</sup>	13.54±0.07 <sup>a</sup>	40.59±0.03 <sup>c</sup>	4.18±0.14 <sup>b</sup>	29.90±0.28 <sup>c</sup>
G1C2	50	2.46±0.01 <sup>a</sup>	6.29±0.05 <sup>b</sup>	15.19±0.00 <sup>c</sup>	42.99±0.05 <sup>d</sup>	4.15±0.01 <sup>b</sup>	28.91±0.02 <sup>b</sup>
G1C3	75	4.98±0.02 <sup>d</sup>	5.64±0.09 <sup>a</sup>	14.15±0.00 <sup>b</sup>	39.89±0.03 <sup>b</sup>	3.72±0.06 <sup>a</sup>	31.63±0.15 <sup>d</sup>
G1C4	100	4.48±0.00 <sup>c</sup>	5.86±0.24 <sup>ab</sup>	16.91±0.00 <sup>e</sup>	37.46±0.01 <sup>a</sup>	3.87±0.16 <sup>ab</sup>	31.43±0.39 <sup>d</sup>
<b>SOY-MKW-001</b>							
Control	0	4.95±0.00 <sup>c</sup>	6.09±0.39 <sup>ab</sup>	14.71±0.67 <sup>b</sup>	31.00±0.05 <sup>a</sup>	4.02±0.26 <sup>ab</sup>	38.56±0.69 <sup>c</sup>
G2C1	25	2.82±0.00 <sup>b</sup>	6.37±0.23 <sup>b</sup>	20.98±0.00 <sup>d</sup>	39.45±0.03 <sup>b</sup>	4.20±0.15 <sup>b</sup>	26.19±0.35 <sup>a</sup>

G2C2	50	4.95±0.00 <sup>c</sup>	5.58±0.09 <sup>a</sup>	17.78±0.00 <sup>c</sup>	42.51±0.03 <sup>d</sup>	3.68±0.06 <sup>a</sup>	25.49±0.13 <sup>a</sup>
G2C3	75	9.85±0.00 <sup>d</sup>	5.54±0.13 <sup>a</sup>	13.07±0.03 <sup>a</sup>	42.23±0.01 <sup>c</sup>	3.66±0.09 <sup>a</sup>	25.66±0.25 <sup>a</sup>
G2C4	100	2.19±0.40 <sup>a</sup>	6.15±0.15 <sup>ab</sup>	14.83±0.00 <sup>b</sup>	42.51±0.03 <sup>d</sup>	4.06±0.10 <sup>ab</sup>	30.26±0.62 <sup>b</sup>
<b>SOY-MNA-002</b>							
Control	0	4.48±0.00 <sup>c</sup>	6.68±0.12 <sup>bc</sup>	18.75±0.00 <sup>b</sup>	42.75±0.04 <sup>d</sup>	4.41±0.08 <sup>bc</sup>	22.93±0.17 <sup>a</sup>
G3C1	25	4.48±0.01 <sup>c</sup>	5.85±0.03 <sup>a</sup>	19.33±0.00 <sup>c</sup>	43.58±0.01 <sup>e</sup>	3.86±0.02 <sup>a</sup>	22.89±0.06 <sup>a</sup>
G3C2	50	4.50±0.00 <sup>c</sup>	5.85±0.34 <sup>a</sup>	12.76±0.29 <sup>a</sup>	36.56±0.03 <sup>a</sup>	3.86±0.22 <sup>a</sup>	36.47±0.88 <sup>c</sup>
G3C3	75	3.98±0.00 <sup>b</sup>	6.86±0.06 <sup>c</sup>	22.86±0.04 <sup>e</sup>	37.72±0.04 <sup>b</sup>	4.53±0.04 <sup>c</sup>	24.05±0.09 <sup>a</sup>
G3C4	100	3.06±0.04 <sup>a</sup>	6.19±0.16 <sup>ab</sup>	20.67±0.00 <sup>d</sup>	38.75±0.13 <sup>c</sup>	4.09±0.11 <sup>ab</sup>	27.25±0.43 <sup>b</sup>
<b>CAIFS, FAO STD</b>		<b>CAIFS, 2019 (8.5- 10.0 %)</b>	<b>CAIFS, 2019 (4.0- 7.0 %)</b>	<b>FAO, 2019 (18.0-22.0 %)</b>	<b>CAIFS, 2019 (35.0- 40.0 %)</b>	<b>CAIFS, 2019 (4.0-6.0 %)</b>	<b>FAO, 2019 30.0-35.0 %</b>

Values are expressed as means±standard error, and DMRT testing indicates that values that have the same superscript in the same column do not differ significantly at  $p>0.05$ . CAIFS: Codex Alimentarius International Food Standards, FAO: Food and Agriculture Organisation

**Table 3: Physicochemical Parameters of SOY-MNA-001, SOY-MKW-001 and SOY-MNA-002 Treated with Silver Nanoparticles**

Treatment	Dose (ppm)	Refractive Index	Specific Gravity	Acid Value (mg KOH/g)	Free Fatty Acid (%)	Iodine Value (I2/100g)	Peroxide Value (meq O2/kg)	Saponification Value (mg KOH/g)
<b>SOY-MNA-001</b>								
Control	0	1.471±0.001a	0.913±0.001a	2.259±0.006a	1.130±0.003a	11.781±0.036e	9.950±0.202a	209.955±0.243b
G1C1	25	1.470±0.001a	0.913±0.001a	7.194±0.260d	3.597±0.130d	11.447±0.011d	20.700±0.058d	210.376±0.162b
G1C2	50	1.469±0.001a	0.911±0.001a	2.475±0.001a	1.238±0.001a	8.852±0.018b	19.300±0.173c	202.732±0.041a
G1C3	75	1.469±0.001a	0.911±0.001a	3.826±0.002c	1.913±0.001c	8.556±0.007a	39.300±0.173e	214.723±0.081c
G1C4	100	1.469±0.001a	0.912±0.001a	3.370±0.001b	1.685±0.001b	9.160±0.022c	16.800±0.115b	215.845±0.243d
<b>SOY-MNA-001</b>								
Control	0	1.467±0.001a	0.913±0.001a	3.485±0.065d	1.742±0.032d	13.885±0.014d	37.650±0.087d	213.742±0.162a
G2C1	25	1.471±0.001a	0.911±0.001a	3.147±0.000c	1.574±0.000c	14.604±0.007e	11.100±0.058c	227.767±0.162c
G2C2	50	1.470±0.001a	0.912±0.001a	1.911±0.065a	0.956±0.033a	11.529±0.109c	10.900±0.058c	246.280±0.810e
G2C3	75	1.470±0.001a	0.912±0.001a	1.827±0.016a	0.913±0.008a	7.579±0.011a	9.500±0.058a	236.462±0.000d
G2C4	100	1.471±0.001a	0.911±0.001a	2.259±0.006b	1.130±0.003b	9.608±0.018b	9.900±0.058b	218.791±0.162b
<b>SOY-MNA-002</b>								
Control	0	1.471±0.001a	0.913±0.001a	2.035±0.001a	1.018±0.003a	6.615±0.036a	6.150±0.087a	211.217±3.239a
G3C1	25	1.469±0.001a	0.913±0.001a	6.351±0.033c	3.175±0.016c	6.804±0.073b	6.400±0.000b	231.553±0.405c
G3C2	50	1.470±0.001a	0.913±0.001a	6.857±0.065d	3.428±0.032d	10.824±0.007d	7.850±0.087e	226.645±0.810b
G3C3	75	1.469±0.001a	0.911±0.001a	2.361±0.065b	1.180±0.032b	12.210±0.007e	7.610±0.006d	243.565±0.815d
G3C4	100	1.469±0.001a	0.912±0.001a	2.304±0.032b	1.152±0.016b	9.948±0.004c	7.290±0.006c	235.901±0.324c
<b>CAIFS, NAFDAC STD</b>		<b>CAIFS (2022) 1.466-1.470</b>	<b>CAIFS (2022) 0.919-0.925</b>	<b>CAIFS (2022) 1.00 - 4.00</b>	<b>CAIFS (2019) 0.6</b>	<b>NAFDAC (2019) 124 - 139</b>	<b>CAIFS (2022) 0 - 10</b>	<b>CAIFS (2022) 189 - 195</b>

Values are expressed as means ± standard error, and DMRT testing indicates that values that have the same superscript in the same column do not differ significantly at  $p>0.05$ .

## CONCLUSION

Biosynthesised silver nanoparticles derived from neem leaf extracts significantly influenced soybean growth, yield, nutritional composition, and oil physicochemical quality in a varying concentration and genotype dependent

manner. Moderate AgNP doses (25–50 ppm) consistently enhanced vegetative development, reproductive performance, seed nutritional value, and oil stability across most accessions, whereas higher concentrations often induced physiological and biochemical stress. The variations observed among soybean

genotypes underscore the importance of genotype-specific optimization in nanoparticle application. These results demonstrated that neem-based AgNPs can serve as eco-friendly bio stimulants for soybean improvement when applied at appropriate doses, offering a promising strategy for sustainable agriculture and food quality improvement in sub-Saharan Africa.

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