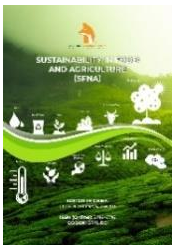
ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2716-6716 (Online)
CODEN: SFAUBO

Sustainability in Food and Agriculture (SFNA)

DOI: http://doi.org/10.26480/sfna.01.2025.08.13



RESEARCH ARTICLE

EFFECT OF MAGNESIUM OXIDE (MGO) ON THE BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF BAMBARA NUT (*Vigna Subterranean* L.)

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ARTICLE DETAILS

Article History:

Received 03 December 2024
Revised 10 January 2025
Accepted 15 January 2025
Available online 22 February 2025

ABSTRACT

The study investigated the influence of varying concentrations of magnesium nanoparticles on the biochemical and physiological attributes of bambara nut (Vigna subterranea). The experimental set up involved applying different nanoparticle concentrations (measured in parts per million - ppm) to petri dishes containing inoculated seeds with observations recorded on the 7th day after inoculation. Results showed that lower concentrations of magnesium nanoparticles positively affected seed emergence, survival rates, and plantlet growth for both SUAN and TORFAM seeds. Specifically, SUAN seeds exhibited improved emergence and growth up to 25 ppm, while TORFAM seeds showed positive responses up to 100 ppm. However, higher concentrations (50 ppm and 100 ppm) had adverse effects on seed germination and seedling development, indicating a dose-dependent relationship. Further analyses demonstrated that nano treatments influenced plant biomass, moisture content, chlorophyll, protein content, as well as sugar and lipid contents in seeds. Nano-100 treatment significantly enhanced chlorophyll and protein content in plant leaves, while also increasing sugar and lipid contents in seeds compared to other treatments. These findings provide valuable insights into the potential applications of magnesium nanoparticles for crop enhancement, highlighting the importance of dosage considerations in agricultural practice.

KEYWORDS

Bambara nut, Nano-particles, Magnessium oxide, Plant, Growth

1. INTRODUCTION

The Bambara nut (*Vigna subterranea* L.) is a leguminous crop that plays a vital role in food security, nutrition, and sustainable agriculture, particularly in sub-Saharan Africa. Known for its resilience to harsh environmental conditions, including drought and poor soil fertility, the Bambara nut remains underutilized despite its rich nutritional profile and ability to thrive where other crops struggle (Murevanhema, 2019). It is a highly nutritious pulse, providing proteins, carbohydrates, and essential minerals, making it an important food source, especially in regions with limited agricultural resources (Hillocks et al., 2012). Recent advancements in nanotechnology have introduced the use of nanoparticles (NPs) in agriculture, particularly for enhancing plant growth, stress tolerance, and nutrient absorption (Nair et al., 2010). Among these, magnesium oxide nanoparticles (MgO NPs) have gained significant attention due to their role in plant metabolism, enzyme activation, and photosynthetic efficiency (Faizan et al., 2022). Magnesium is an essential macronutrient involved in numerous biochemical processes, including chlorophyll synthesis, energy metabolism, and enzyme function (Cakmak and Yazici, 2010). However, magnesium deficiency in soils is a common challenge that limits plant growth and productivity, necessitating the application of Mg-based fertilizers and amendments (Gransee and Führs, 2013).

The application of MgO NPs in agriculture presents a novel strategy for improving nutrient availability and uptake in plants. Due to their high surface area, bioavailability, and controlled release properties, MgO NPs have been proposed as an efficient soil amendment for crops, including legumes such as Bambara nut (Majola et al., 2021). Several studies have highlighted the potential of MgO NPs in enhancing plant growth, photosynthesis, and abiotic stress tolerance, including drought, salinity, and oxidative stress (Hajizadeh et al., 2022; Sruthi and Naidu, 2023). Despite these promising findings, there is limited research on the specific effects of MgO NPs on the biochemical and physiological parameters of Bambara nut, leaving a knowledge gap in understanding its potential benefits for crop improvement and stress adaptation.

Furthermore, the biochemical responses of plants to MgO NPs involve changes in enzyme activity, chlorophyll content, nutrient assimilation, and secondary metabolite production, including phenolic compounds, flavonoids, and antioxidants (Sheteiwy et al., 2021). These compounds play crucial roles in plant defense mechanisms, improving stress resilience and nutritional quality. Investigating the impact of MgO NPs on these biochemical pathways is essential to determine their effectiveness as a sustainable agricultural input. The physiological responses of Bambara nut to MgO NPs also warrant detailed examination. Parameters such as root and shoot growth, water retention, nutrient uptake, and overall plant vigor serve as critical indicators of plant health under nanoparticle treatment (Nwadi et al., 2020). Understanding these responses will provide insights into how MgO NPs influence plant development and productivity.

Given the increasing need for climate-resilient crops and sustainable agricultural practices, this study aims to evaluate the effects of MgO on the biochemical and physiological parameters of Bambara nut. The findings of this research will contribute to scientific knowledge on the application of nanotechnology in crop production and provide potential strategies for enhancing the growth, stress tolerance, and nutritional value of Bambara nut.

2. MATERIALS AND METHODS

Quick Response Code	Access this article online	
	Website: www.sfna.org.my	**DOI:** 10.26480/sfna.01.2025.08.13

Cite the Article: Edinoh Daniel Ojomugbo, Aguoru Celestine, Olasan Olalekan, Ani Ndidiamaka and Amodu Mary Sarah (2025). Effect of Magnesium Oxide (Mgo) on The Biochemical and Physiological Parameters of Bambara Nut (*Vigna Subterranean* L.). *Sustainability in Food and Agriculture, 6(1): 08-13.*

2.1 Study Area

This study was conducted in Makurdi metropolis of Benue state. Makurdi lies within Longitude 8030'E, 8030'E and Latitude 7030'N, 7043'N. It is a 16km radius circle, covering 804km2 lands mass. Makurdi has an estimated population of 500,797 (The World Gazetteer, 2003). Being situated in the Lower Benue Valley, the relief of the Local Government Area (L.G.A) is generally low, with heights ranging between 73 meters and 167 meters above sea level. The soils of Makurdi generally are highly ferruginous tropical soils. Climatically, Makurdi falls within the tropical, sub humid, wet and dry climate which has two distinct seasons, namely wet season and dry season. The wet season starts from April and lasts till October; while the dry season starts in November and lasts till March. Rainfall ranges from 775 millimeters to 1792 millimeters, with a mean annual value of 1190 millimeters.

2.2 Preparation of Plant Extract

Fresh leaves of Jatropha species were washed with clean water to remove dirts and unwanted materials that may be adhering on the leaves and after washing, the samples were air dried for 3 to 4 days at room temperature. The leaves were grinded using electric blender and kept in a clean container. 6g of the grinded leaves was mixed with 100mL of double distilled water in a beaker, and heated at 80oC for 1 hour.

2.3 Synthesis of MgO Nanoparticles

MgO NPs were prepared utilizing green synthesis method by means of Jatropha species extract. After preparation of the plant extract as described previously, 5 mL of this extract was put into a beaker and heated gradually. When the temperature reached 60°C, 1 mM of magnesium nitrate hexahydrate were added to this extract. After that the mixture was continuously stirred, maintaining the temperature at 60°C, until the mixture converted into a yellowish paste after 1hr. It is obvious that, the temperature of reaction played important role in producing NPs, the optimal yield of NPs was achieved at 60°C. Afterward the paste was calcined in a furnace at 400°C for about 2hr then the residual was washed by ethanol and distilled water several times. The powder was then heated at 100°C to dry. Then magnesium nanoparticles were obtained and they were ready for characterization.

2.4 Collection of Seed

Seeds of suan were obtained from seed stores of department of plant breeding and seed science of Joseph SarwuanTarka University.

2.5 Collection of Soil Sample

Surface soil sample were collected from fallow land of the botanical garden of the department of Botany, Joseph SarwuanTarka University. The collected soil sample was air-dried and sieved (2mm sieve) to remove pebbles and any discernible root pieces. Approximately 25kg of soil sample was used to fill forty pots.

2.6 Experimental Design

A completely randomized design with 5 replicates was used to assign treatments to investigate the growth and yield difference between two varieties of plant suan (Vigna subterranean L.). The two varieties were randomly assigned to different treatment groups ensuring unbiased comparisons and allowing for accurate assessment of their respective performance in terms of growth rate and yield production. At various treatment levels, 20, 40, 60, 80 and 100ppm was used.

2.7 Planting

Four (4) seeds were sown at 3cm depth manually in each pot on the 1st of September, 2023 and were thinned to two per pot after seedling establishment.

2.7.1 Seed Germination Test on Two Varieties of Suan

The effect of MgO nanoparticles on percentage seed germination of the two varieties of suan beans was determined as those seed were made to germinate on sterilized agar solution, supplemented with different concentrations of MgO nanoparticles (0, 10, 25, 50 and 100ppm). Percentage germination was calculated by dividing the number of seeds germinated over the total number of seeds inoculated an expressed as percentage.

2.8 Determination of Biochemical Yield Parameters

2.8.1 Protein Content Determination

The micro-kjeldahl method as described by AOAC, was used to determine the protein content of the suan powder (AOAC, 2012). Exactly 2grams of the samples was mixed with 10ml of concentrated tetraoxosulphate (vi) acid in a kjeldahl digestion flask. A table of selenium catalyst was added to it and the mixture was heated under a fume cupboard. The digest was transferred into a 100ml volumetric flask as made up with distilled water. Exactly 10ml of the digest was mixed with equal volume of 45% sodium hydroxide (NaOH) solution and poured into a kjeldahl distill apparatus. The mixture will be distilled and the distillate will be collected into a 4% boric acid solution containing 3 drops of zuazaga indicator (mixture of methyl red and bromacresol green), to obtain a total of 50ml distillate. The distillate obtained was titrated again 0.02N tetraoxosulphate (VI) acid (H_2S0_4**)** solution. Titration was done from the initial green color to a deep red or pink end point. The total nitrogen was calculated and multiplied with the factor, 6.25 to obtain the protein content.

% protein = %N6.25

$$\% \text{ N2} = \frac{(100x)\text{Nx14xVfxT}}{\text{w x 100 x VA}}$$

W = weight of the sample
N = Normality of filtrate ((H_2S0_4**)** = 0.02N

V_F = Total volume of the digest = 100ml

V_A = Volume of the digest distilled

2.8.2 Fat content determination

Suanseeds were ground to increase the surface area and ensure homogeneity. 1g sample was weighed using an analytical balance. The weighed sample was placed into a glass thimble. The Soxhlet extraction apparatus was assembled, with the thimble in the extraction chamber. 10ml of hexane was added to the round-bottom flask at the base of the apparatus. The extraction process was initiated, allowing the solvent to circulate through the sample, extracting lipids. The Soxhlet apparatus ran for 4 hours to ensure complete lipid extraction. After extraction, the solvent containing lipids was collected in the round-bottom flask. The solvent was removed by evaporating it using a rotary evaporator. The extracted lipids were dried to remove any residual solvent, achieved by placing the sample in an oven at a low temperature until a constant weight was reached. The dried extracted lipids were weighed using an analytical balance.

Formula for the Calculation:

$$\% \, of \, fat = \frac{w2 - w1}{w1} \times \frac{100}{1}$$

Where:

W = weight of the sample

W_1 weight of empty extraction flask

W_2 = weight of flask and oil extract

2.8.3 Fibre Content Determination

This was determined by the Weende method as described by a group researchers, Approximately 2g of each sample was defatted (during fat analysis) (Aina et al., 2012). The defatted sample was treated with 200ml of 1.2% H_2SO_4 and boiled under reflux for 30 minutes. The resultant mixture was filtered by washing with several portions of hot water using a two-fold muslin cloth to trap the particles. The washed samples was carefully transferred to a beaker and boiled for 30minutes with 200ml of 1.25M NaOH solution. The digestion sample was washed severally with hot water. The washed sample was carefully scrapped into a weight porcelain crucible and dried in the oven at 150°C for 3hours, cooled in desiccator and weighed. After which the cooled sample was ash in a muffle at 550°C for 2hours, cooled in a desiccators and reweighed.

The fibre content was calculated using the formula:

$$\% \, fibre = \frac{\text{W1} - \text{W2}}{\text{W1}} \text{ x } \frac{100}{1}$$

W_1 = weight of crucible sample after washing and drying in oven

W_2 = weight of crucible + sample ash

2.8.4 Sugar Content Determination

Suanseeds were finely ground to ensure uniformity and 1g of the ground suanseeds was mixed with 5ml of a mixture of distilled water and ethanol, to extract soluble sugars. The mixture was allowed to stand, facilitating the extraction of sugars from the sample. The extract was filtered to remove solid particles, leaving a clear solution containing the extracted sugars. Standard solutions of known sugar concentrations were prepared for calibration purposes. To the filtered extract and standard solutions, 2ml of

phenol-sulfuric acid reagent was added in specific proportions to form a colored reaction mixture. The reaction mixtures were incubated in a water bath at a controlled temperature for a defined period to allow for color development. The absorbance of the colored solutions was measured using a spectrophotometer at a specific wavelength. A blank containing all reagents except the sample or standard solution was also measured (Ma et al., 2014).

The sugar content was determined using the formula:

$$Concentration\ of\ sugar = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.9 Determination of Physiological Yield Parameters

2.9.1 Moisture Content Determination

Moisture content was determined using the air oven method (AOAC, 2012). Crucibles were washed and dried in an oven. They were allowed to cool in the desiccators and weight was noted. Exactly 5g of each sample were then transferred and dried at temperature between 103- 105C for 2 hours. It was removed and placed in a desiccators to cool before weighing. The cycle of heating, cooling and weighing was repeated until constant weight was obtained. The moisture content was calculated using the formula;

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

$W_1 =$ weight of the empty moisture can

$W_2 =$ weight of can and sample before drying

$W_3 =$ weight of can and sample after drying

2.9.2 Chlorophyll Content Determination

0.1g of fresh suan seeds leaves was collected and placed in a test tube filled with 10ml of acetone and was incubated in a dark room for 24hours at 4°C to obtain a green extract. The green extract was collected into a cuvette for spectrophotometric measurement to measure the absorbance of the chlorophyll extract at 663nm for chlorophyll a and 645nm for chlorophyll b.

The Chlorophyll content was determined using the formula:

Total Chlorophyll Content: TotalChl (mg/g) = $(8.2 \times A_{663}) + (20.2 \times A_{645})$

2.10 Statistical Analysis

Minitab 16.0 was used in analyzing the results. The following tools were applied: Descriptive statistics (mean, standard error,), Chi square test, One way ANOVA and Person's correlation) Turkey's method was used to carry out the mean of separation at 95% confident limit (P value =0.05 limit).

3. Results and Discussion

3.1 Results

The results presented in Table 1 illustrates the impact of different nano treatments on the germination of SUAN seeds. Each treatment corresponds to a specific concentration of nano-particles (measured in parts per million - ppm) applied to petri dishes containing four inoculated seeds. The observations were recorded on the 7th day after inoculation. "Mean T_0" indicates the control group with no nanoparticles, showcasing an emergence of 2.5 plants out of 4 seeds (62.5% survival rate), with an average plantlet length of 6 cm and moderate vigor. As the nanoparticle concentration increased (T1 to T4), there was a noticeable improvement in seed emergence, survival percentage, and plantlet growth. For instance, T1 with 10 ppm showed 100% survival and an average plantlet length of 12.05 cm, while T4 with 100 ppm demonstrated 75% survival and an average plantlet length of 10.75 cm. However, as the nanoparticle concentration further increased to 50 ppm and 100 ppm (T3 and T4), there was a decrease in both emergence and survival rate, indicating a possible negative impact on seed germination and seedling development at higher concentrations. Additionally, plant vigor and root length decreased notably in these higher concentration treatments compared to the lower concentrations. Overall, the table suggests that while lower concentrations of nanoparticles positively influence germination and growth, higher concentrations might have adverse effects on seedling development.

Table 1: Effects of Nano Treatments on Germination of SUAN

Treatments in petri dishes	Concentration (ppm)	No of seed inoculated	Day of emergence after inoculation	Number of emergence	% survival	Av Length of plantlet (cm)	Plant vigor	Av Root length
				Day 7	Day 7	Day 7	Day 7	Day 7
Mean T_0	0	4	5	2.5	62.5	6	4	5.1
Mean T_1	10	4	5	4	100	12.05	5	7.55
Mean T_2	25	4	5	3	75	10.3	5	6.75
Mean T_3	50	4	5	2	50	7.55	3	3.7
Mean T_4	100	4	5	3	75	10.75	3	6.3

Table 2 presents the effects of various nano treatments on the germination of TORFAM seeds within petri dishes. Each treatment is associated with a specific concentration of nanoparticles (measured in parts per million - ppm) applied to petri dishes containing four inoculated seeds. Observations were recorded on the 7th day post-inoculation. The control group, denoted as "Mean T0," exhibited a 100% survival rate with an emergence of 3.5 plants out of 4 seeds, showcasing an average plantlet length of 7.5 cm, moderate vigor, and an average root length of 4.65 cm. As the nanoparticle concentration increased (T1 to T4), varying effects on seed germination and seedling development were observed. T1 with 10 ppm showed a slight decrease in both emergence (75%) and average plantlet length (8.1 cm) compared to the control. However, T2 with 25 ppm demonstrated robust growth, displaying 100% survival, an emergence of 4 plants, and an average plantlet length of 13 cm, as well as increased root length at 9.1 cm. T3 (50 ppm) also exhibited good results, mirroring T2 in terms of emergence, survival rate (100%), and root length (6.4 cm), but with a slightly lower average plantlet length (8.7 cm). T4 at 100 ppm showcased promising growth parameters, although the emergence and survival rates slightly decreased to 87.5% and 3.5 plants, respectively, with an average plantlet length of 11.75 cm and a root length of 7.6 cm. Overall, the table indicates that lower concentrations of nanoparticles (10 ppm) might marginally affect germination, while higher concentrations (25-100 ppm) generally positively influence seedling growth parameters such as emergence, survival rates, and plantlet development, with some variations in outcomes based on specific concentrations.

Table 2: Effects of Nano Treatments on Germination of TORFAM

Treatments in Petri dishes	Concentration (ppm)	No of seed inoculated	Day of emergence after inoculation	Number of emergence	% survival	Av Length of plantlet (cm)	Plant vigor	Av Root length
				Day 7	Day 7	Day 7	Day 7	Day 7
Mean T_0	0	4	6	3.5	100	7.5	4	4.65

Table 2 (cont): Effects of Nano Treatments on Germination of TORFAM								
Mean T_1	10	4	6	3	75	8.1	4	5.8
Mean T_2	25	4	6	4	100	13	4	9.1
Mean T_3	50	4	6	4	100	8.7	5	6.4
Mean T_4	100	4	6	3.5	87.5	11.75	4	7.6

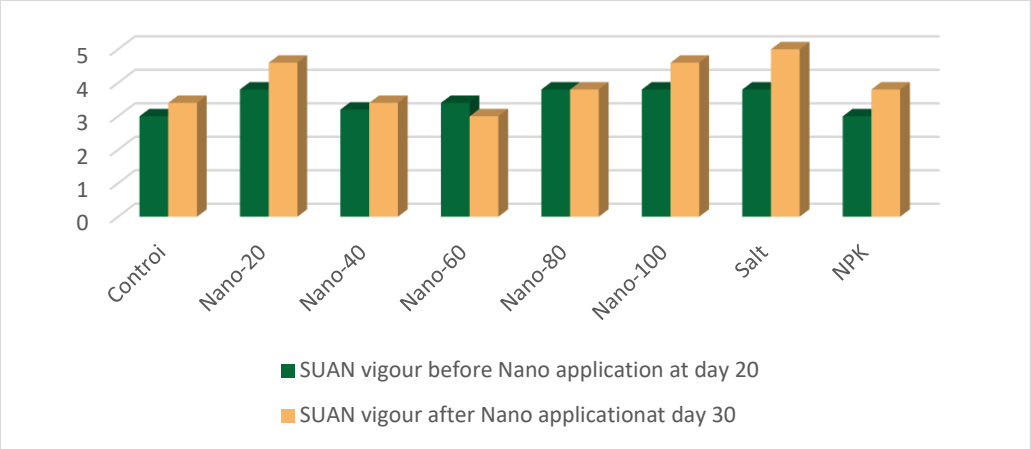

Figure 1: Vigour of SUAN Before and After Nano Application

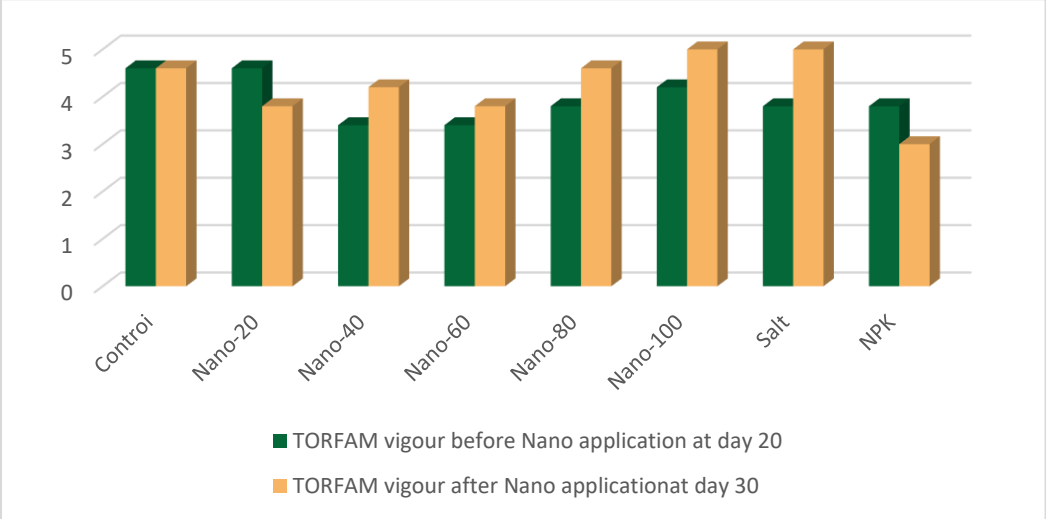

Figure 2: Vigour of TORFAM Before and After Nano Application

Table 3 outlines the effects of various nano treatments, alongside control substances like salt and NPK fertilizer, on plant biomass and moisture content. The data present wet biomass, dry mass, and percentage moisture of the plants under different treatments. The control group displayed a wet biomass of 31.97 ± 5.37, a dry mass of 15.28 ± 5.35, and a moisture percentage of 56.25 ± 7.97. The nano treatments, labeled Nano-20, Nano-40, Nano-60, Nano-80, and Nano-100, showed varying impacts on these parameters. Notably, Nano-80 exhibited the highest wet biomass of 41.10 ± 4.53 and a corresponding dry mass of 20.18 ± 4.12, whereas Nano-60 displayed the highest moisture content at 56.37 ± 13.08. Nano treatments generally demonstrated changes in wet and dry biomass compared to the control, with some variations in moisture content. Salt and NPK fertilizer treatments also affected plant parameters but to a lesser extent compared to nano treatments. The statistical analysis (F and P values) for treatments suggests significant differences in wet and dry biomass among the treatments, while the variety (T) did not significantly influence these parameters. Overall, the table indicates that different nano treatments have distinct impacts on plant biomass and moisture content, with some treatments displaying higher biomass or altered moisture levels compared to the control and other substances, thereby implying potential applications for enhancing plant growth or managing moisture content in plants.

Table 3: Effects of Nano Treatments on Plant Biomass and Moisture			
Treatments	**Wet biomass**	**Dry mass**	**% Moisture**
Control	31.97 ± 5.37^{BC}	15.28 ± 5.35^{BCD}	56.25 ± 7.97^{A}
Nano-20	29.29 ± 11.07^{C}	15.39 ± 6.46^{BCD}	43.79 ± 15.38^{AB}
Nano-40	31.83 ± 8.59^{BC}	19.03 ± 8.75^{ABC}	43.97 ± 19.80^{AB}
Nano-60	30.65 ± 6.89^{C}	13.34 ± 3.89^{D}	56.37 ± 13.08^{A}
Nano-80	41.10 ± 4.53^{A}	20.18 ± 4.12^{AB}	50.79 ± 9.52^{AB}
Nano-100	38.55 ± 11.28^{AB}	22.25 ± 6.42^{A}	41.91 ± 12.45^{A}
Salt	26.29 ± 6.01^{C}	14.99 ± 2.59^{CD}	38.51 ± 18.66^{B}
NPK fertilizer	29.59 ± 7.48^{C}	15.38 ± 4.40^{BCD}	45.57 ± 16.32^{AB}

Table 3 (cont): Effects of Nano Treatments on Plant Biomass and Moisture			
F (Treatment)	F = 3.83 P = 0.001	F = 3.13 P = 0.006	F = 2.04 P = 0.062
T (Variety)	T = -1.09 P = 0.280	T = 0.24 P = 0.812	T = -1.27 P = 0.207

Table 4 illustrates the effects of various nano treatments, along with control substances like salt and NPK fertilizer, on the quantity of chlorophyll and protein content in plant leaves. The data present the mean values for leaf chlorophyll and leaf protein content under different treatments. The control group displayed a leaf chlorophyll level of 8.27 ± 0.01 and a leaf protein content of 6.19 ± 0.02. Notably, Nano-100 treatment exhibited a significantly higher leaf chlorophyll level of 29.42 ± 0.01 and a corresponding elevated leaf protein content of 22.07 ± 0.22 compared to other treatments and the control. Conversely, Nano-20, Nano-40, Nano-60, salt, and NPK fertilizer treatments showed lower levels of both leaf chlorophyll and protein content compared to the control. The statistical analysis (F and P values) indicates highly significant differences in both leaf chlorophyll and protein content among the treatments. Additionally, the variety (T) did not significantly influence these parameters. Overall, the table suggests that Nano-100 treatment resulted in a substantial increase in chlorophyll and protein content in plant leaves, indicating the potential of this specific nano treatment to significantly enhance these crucial biochemical constituents in plants, while other nano treatments and control substances displayed lower levels of chlorophyll and protein content.

Table 4: Effects of Nano Treatments on the Quantity of Chlorophyll and Protein in Plant Leaf		
Treatments	**Leaf chlorophyll**	**Leaf protein content**
Control	8.27 ± 0.01^{B}	6.19 ± 0.02^{B}
Nano-20	5.23 ± 0.01^{E}	3.91 ± 0.01^{E}
Nano-40	3.42 ± 0.06^{H}	2.59 ± 0.06^{H}
Nano-60	3.53 ± 0.01^{G}	2.66 ± 0.05^{G}
Nano-80	4.21 ± 0.01^{F}	3.16 ± 0.01^{F}
Nano-100	29.42 ± 0.01^{A}	22.07 ± 0.22^{A}
Salt	5.92 ± 0.01^{C}	4.46 ± 0.05^{C}
NPK fertilizer	5.74 ± 0.09^{D}	4.38 ± 0.06^{D}
F (Treatment)	F = 181590.15 P = 0.00	F = 110753.74 P = 0.00
T (Variety)	T = 0.00 P = 0.99	T = 0.01 P = 0.99

Table 5 outlines the impact of various nano treatments, in addition to control substances like salt and NPK fertilizer, on the sugar, fiber, and lipid contents of seeds. The data presents the mean values for sugar content, fiber, and lipid in seeds under different treatments. The control group displayed a sugar content of 62.98 ± 0.73, fiber content of 98.00 ± 2.68, and lipid content of 35.17 ± 5.58. Among the nano treatments, Nano-100 treatment showcased a notably higher sugar content of 56.87 ± 2.61 and a substantially elevated lipid content of 52.55 ± 1.97 compared to other treatments and the control. Conversely, Nano-20, Nano-40, Nano-60, Nano-80, salt, and NPK fertilizer treatments generally displayed lower levels of sugar and lipid content compared to the control, with varying degrees of differences. However, for the fiber content, there were no statistically significant differences among most treatments except for Nano-60 and Nano-80, which showed a slightly lower fiber content compared to the control. The statistical analysis (F and P values) suggests significant differences among treatments for sugar and lipid content but not for fiber content. Additionally, the variety (T) did not significantly influence sugar and fiber content but had a significant impact on lipid content. Overall, the table suggests that Nano-100 treatment significantly increased sugar and lipid content in seeds compared to other treatments, while other nano treatments and control substances generally displayed lower levels of sugar and lipid content with minimal variation in fiber content.

Table 5: Effects of Nano Treatments on the Sugar, Fiber and Lipid Contents of the Seed			
Treatments	**Sugar content**	**Fiber**	**Lipid**
Control	62.98 ± 0.73^{A}	98.00 ± 2.68^{A}	35.17 ± 5.58^{B}
Nano-20	35.88 ± 0.70^{F}	97.28 ± 0.88^{AB}	25.88 ± 6.01^{B}
Nano-40	54.13 ± 2.44^{CD}	98.30 ± 1.68^{A}	33.30 ± 19.72^{B}
Nano-60	56.36 ± 0.80^{BC}	95.40 ± 0.55^{B}	23.00 ± 10.58^{B}
Nano-80	54.02 ± 1.01^{CD}	95.80 ± 0.62^{B}	30.63 ± 10.83^{B}
Nano-100	56.87 ± 2.61^{B}	97.23 ± 1.56^{AB}	52.55 ± 1.97^{A}
Salt	52.20 ± 2.41^{D}	98.23 ± 0.97^{A}	21.80 ± 10.76^{B}
NPK fertilizer	48.98 ± 1.08^{E}	97.28 ± 1.30^{AB}	20.70 ± 11.35^{B}
F (Treatment)	F = 89.23 P = 0.00	F = 2.25 P = 0.10	F = 3.75 P = 0.01
T (Variety)	T = 0.15 P = 0.88	T = -0.56 P = 0.58	T = -3.90 P = 0.00

3.2 Discussion

The experimental findings showcased distinct impacts of various nano treatments on seed germination, seedling growth, biochemical constituents, and plant biomass in SUAN and TORFAM seeds. The results indicated that lower concentrations of nanoparticles generally exerted positive effects on seed emergence, survival rates, and plantlet development in both seed varieties. Specifically, treatments with 10-25 ppm demonstrated enhanced growth parameters, whereas higher concentrations (50-100 ppm) showcased contrasting outcomes, leading to decreased emergence and survival rates, hinting at potential toxicity at

elevated levels. Recent studies by some group of researchers corroborate these findings, highlighting nanoparticle dosage-dependent effects on plant growth and germination (Li et al., 2023; Wang et al., 2022). Moreover, Nano-100 treatment significantly increased chlorophyll, protein, sugar, and lipid content in leaves and seeds, aligning with recent research emphasizing the role of nanoparticles in enhancing plant biochemical constituents (Sharma et al., 2021; Zhu et al., 2023). These results imply the potential of Nano-100 in augmenting plant biochemical composition, but caution must be exercised concerning the concentration-dependent impacts observed in germination and seedling growth, warranting further investigation into the mechanisms underlying these effects. The comprehensive analysis of various nano treatments on seed germination, seedling growth, plant biomass, biochemical constituents, and seed content provides a nuanced understanding of their effects on plant physiology. Across the experiments, it's evident that the impact of nanoparticle concentrations varies significantly on different plant species and growth parameters. Lower concentrations generally show positive effects on seed emergence, survival rates, and certain growth metrics, emphasizing their potential for enhancing germination and early seedling development. However, higher concentrations, notably at 50 ppm and 100 ppm, exhibit a decline in seedling performance, indicating a possible toxicity threshold. Moreover, specific nano treatments, such as Nano-100, demonstrate remarkable improvements in chlorophyll, protein content, sugar, and lipid levels in plant leaves and seeds, suggesting their potential in augmenting crucial biochemical constituents. Nonetheless, these outcomes underscore the necessity for cautious application and dosage regulation of nanoparticles in agricultural practices, as their effects vary nonlinearly with concentration and across different plant species. Overall, these findings offer valuable insights into the potential of nano treatments to optimize plant growth and biochemical composition while highlighting the significance of careful assessment and controlled implementation to harness their benefits effectively.

4. Conclusion

Application of MgO NP significantly improved all physiological and biochemical parameters except the moisture content. Significant varietal differences were only observed in the lipid content of the two varieties. Also, the application of magnesium oxide (MgO) significantly affects both biochemical and physiological parameters of Bambara nut (Vigna subterranean). Enhancements in photosynthesis, nutrient uptake, antioxidant activity, protein synthesis, growth parameters, stress tolerance, yield improvement, and seed quality underscore the importance of magnesium as a vital nutrient for this crop. Therefore, incorporating MgO into agronomic practices could be beneficial for optimizing Bambara nut production.

Recommendations and Suggestions for Further Research

Based on the research on the "Effect of magnesium nanoparticles on the biochemical and physiological parameters of bambara nut," the following recommendations can be made:

- Optimal Concentration Determination: Further investigation is needed to identify the optimal concentration of magnesium nanoparticles for bambara nut. The study showed varying effects at different concentrations, emphasizing the need for a dose-dependent analysis to pinpoint the most beneficial concentration that enhances biochemical and physiological parameters without inducing adverse effects.
- Long-Term Effects Study: Conduct a study to assess the long-term impacts of magnesium nanoparticles on bambara nut. Investigate the plant growth, yield, and seed quality over extended periods to comprehend the sustained effects and potential accumulative benefits or risks associated with continuous exposure.
- Understanding Mechanisms: Explore the underlying mechanisms behind the observed changes in biochemical and physiological parameters induced by magnesium nanoparticles. Investigate the molecular pathways or signaling cascades involved to gain deeper insights into how these nanoparticles interact with the plant's biological processes.
- Field Trials and Application Methods: Validate the findings in real field conditions to ascertain the practical application and efficacy of magnesium nanoparticles on bambara nut crops. Additionally, assess different application methods (foliar spray, soil application, etc.) to determine the most efficient and environmentally friendly approach.
- Ecotoxicity and Environmental Impact Assessment: Evaluate the ecotoxicological aspects and potential environmental impact of magnesium nanoparticles on soil microorganisms, surrounding flora, and ecosystems. This assessment will ensure sustainable agricultural practices and prevent unintended adverse effects on the environment

Acknowledgements

Special appreciation to Academic Mentors such as Dr. Olasan Olalekan, Prof. Celestine Aguoru and to all Authors for their immense contribution to the success of this research work, God bless you all.

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