**Effect of ZnO Nano Treatments on the Germination and Growth of Two Varieties of Soybean (TGX1904-6F AND TGX1951-3F)**

**Abstract**

The exploration of ZnO nanoparticles and their impact on Soybean (Glycine max L.) growth and productivity is driven by the urgent need to develop sustainable agricultural practices capable of addressing food security challenges in the face of climate change and soil nutrient depletion. A comprehensive understanding of the potential benefits and risks associated with the application of zinc oxide (ZnO) nanoparticles in agriculture is essential for advancing sustainable crop management strategies, addressing global food security challenges, and optimizing the productivity of key crops such as soybean (*Glycine max* L.). Seeds of soybean were obtained from the seed store of the department of plant breeding and seed science of Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria. ZnO NPs were prepared utilizing green synthesis method by means of Jatropha species extract. A completely randomized design with 5 replicates was used to assign treatments to investigate the growth and yield difference between two varieties of plant (Vigna radiata L.). 5mL of the prepared plant extract was put into a beaker and heated gradually. The effect of ZnO nanoparticles on percentage seed germination of the two varieties of soybeans was determined as those seed were made to germinate on sterilized agar solution, supplemented with different concentrations of ZnO nanoparticles. 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 were used for mean separation at 95% confident limit (P value =0.05 limit). The result for TGX1904-6F showed that nano 80ppm more significantly increased plant height compared to salt and fertilizer, suggesting an optimal concentration promoting growth. The plant vigor of soybean remains consistent across all nano treatments for both varieties (TGX1904-6f and TGX1951-3f), indicating that none of the treatments has a significant effect on the overall vigour of the two varieties of soybean (TGX1904-6F and TGX1951-3F). This can also be an indication that the concentrations of ZnO nano treatments selected did not exert a substantial negative impact on the overall health and growth of soybean plants. Adaptability of soybean plants to the applied treatments might be responsible for the lack of significant differences in plant vigor among treatments indicating their resilience under the experimental conditions.

**Keywords: Nanotechnology, Germination, Soybeans and ZnO nanoparticles.**

**Introduction**

Nanotechnology has brought a lot of changes in many industrial and agricultural sectors through the use of modern and forward-looking methods of promoting the yield and quality of crops (Elham *et al.,*2022; Basit *et al*. 2023). Seleman *et al*., (2020) described Nanoparticles (NPs) as organic or inorganic materials of various sizes ranging from 1 to 100 nm, and recently they have found application worldwide. Different types of nanomaterials possess great displayed assurance in the progress or growth of sustainable agriculture since they aid in increasing productivity by improving the effectiveness of agricultural inputs and reducing losses (Qayyum *et al*., 2022). Metal and metal oxide nanoparticles, including those containing silver, gold, copper, and zinc, are increasingly recognized for their significant potential in agriculture. These nanoparticles offer remarkable opportunities to enhance crop productivity, improve stress tolerance, and increase nutrient-use efficiency. Studies have shown that the application of such nanoparticles can increase yields by up to 20%, reduce disease incidence by 50%, decrease nutrient leaching by 30%, and boost soil carbon sequestration by 15% (Fayomi *et al.,* 2024)

So far, the use of Nanoparticles (NPs) is bringing impelling force in modern agriculture through the visible showed results in increasing crop yield, increasing soil fertility, quality and productive farming, and availability of good crops (Singh *et al*., 2021; Fayomi *et al.,* 2024). Many authors have reported tomato, wheat, cucumber and eggplant to have indicated that nanoparticles possess a good influence on plants response to drought conditions. Drought tolerance is a complex character of high importance for the potato crop. Therefore, nanotechnology techniques can provide clear guidelines for enhancing potato drought tolerance and yield sustainability (Zaki and Radwan 2022; Alowaiesh *et al.,* 2024).

Soybean of the kingdom Plantae belongs to the phylum: angiosperms (flowering plant), class: eudicots (plants with two seed leaves or cotyledons), order: fabales, family: fabaceae (Leguminosae), subfamily; Papilionideae., genus: Glycine and species: *glycine max* L. (Doyle *et al*., 2017; Sadiq *et al.,* 2020). Praneeta *et al.,* (2013) documented that several varieties of soybean are in existence due to series of breeding activities and some of which include:Medium Maturing Varieties (MMV) such as TGX1479-2F, Samsoy-2, TGX1670-1F and TGX 1440-1E. Early Maturing Varieties (EMV) include TGX1479-2F, TGX 1681-3F, TGX 10192 EB, TGX 1649-11F, TGX 1485-1D and TGX 1740-7F. Others are: TGX 18055-F, TGX 923-2E, TGX 1740 and TGX 849-313D (Praneeta *et al.,* 2013).

Soybean has been widely grown in Nigeria for long period of time, but maximum yield has not been obtained. This might be as a result of non-usage of improved varieties, and studies has conducted has shown possibility of maximizing soybean yield potential through application of several practices among which are; use of rhizobia inoculant, improved varieties, and sowing at the right date (Sadiq *et al.,* 2020). Panasiewick *et al*., (2024) and Serafin-Andrzejewska *et al*., (2024) reported that cultivation of soybean has significant contributions to sustainable agriculture through its capacity to fix atmospheric nitrogen in union with nitrogen-fixing bacteria. This natural nitrogen-fixing ability promotes soil fertility cutting down the call for synthetic nitrogen fertilizers and encouraging environmentally friendly farming practices (Serafin-Andrzejewska *et al*., 2024; Antoine Harf 2025). According to Mannem *et al.,* (2024), Soybean contains approximately 37-41% protein, 18-21% oil, 30-40% carbohydrates and 4-5% ash content. Soybean has emerged to be one of the world’s leading sources for vegetable oil and plant protein that are both well accustomed to the routine diet of everyday people. Outside the agricultural and nutritional significance of soybean, it also plays a key role in the global economy by revenue/income generation essentially through trade and processing industries (Majidion *et al.,* 2024). Soybean as well aids the livelihoods of many farmers, traders and workers. According to Majidian *et al*., (2024), Soybean is used widely by many in the production of several products such as animal feed, biodiesel, and bio-based materials, contributing to sustainable energy and environmental conservation. Its usage in many industries underscore its significance in the global market (Majidian *et al*., 2024). However, soybean production encounters several challenges, such as nutrient deficiencies, abiotic stress, and pest infestations, all of which can reduce its yield potential (Mannem *et al.*, 2024).

Zinc is a crucial nutrient for plant growth and development. Its deficiency can lead to stunted growth, significantly reduced crop yields, and chlorosis (Wang *et al*., 2023). Within this framework, zinc oxide (ZnO) nanoparticles have emerged as a viable alternative due to their nanoscale physiochemical characteristics, which facilitate enhanced nutrient absorption and assimilation in plant systems (Wang *et al.*, 2023).

The exploration of ZnO nanoparticles and their impact on Soybean (*Glycine max* L.) growth and productivity is driven by the urgent need to develop sustainable agricultural practices capable of addressing food security challenges in the face of climate change and soil nutrient depletion. ZnO nanoparticles, due to their high surface area and reactivity, have demonstrated the potential to improve nutrient use efficiency, particularly in zinc-deficient soils, by enhancing micronutrient uptake, photosynthetic activity, and stress tolerance in crops (Dimkpa and Bindraban, 2016: Mannem *et al.*, 2024; Singh *et al.,* 2024). According to Basit *et al.,* (2023) ZnO nanoparticles can mitigate the negative effects of chromium stress on soybean growth and development by enhancing antioxidant activity and reducing oxidative damage. As soybeans are a major source of protein and oil globally, improving their yield and resilience through nano-fertilizer applications is of considerable agronomic and economic importance (FAO, 2020).

A comprehensive understanding of the potential benefits and risks associated with the application of zinc oxide (ZnO) nanoparticles in agriculture is essential for advancing sustainable crop management strategies, addressing global food security challenges, and optimizing the productivity of key crops such as soybean (*Glycine max* L.).

**Materials and Methods**

**Study Area**

This study was conducted in Makurdi, the capital of Benue State, Nigeria, located approximately between Longitude 8°30′E and Latitude 7°30′N to 7°43′N. The city spans about 804 km², with an estimated population of over 500,000 according to the 2006 Nigerian Census and projections by (City Population 2023). Makurdi lies within the Guinea Savannah zone, a transitional vegetation belt between the southern rainforest and the northern Sudan Savannah. It is characterized by tall grasses and scattered deciduous trees, which shed leaves during the dry season (Fayomi *et al.,* 2024). Climatically, Makurdi falls within the tropical sub-humid climate zone, experiencing two distinct seasons: a wet season (April to October) and a dry season (November to March). The mean monthly relative humidity ranges from about 40% in January to over 80% in August ([NIMET, 2021](https://nimet.gov.ng/)). The soils of Makurdi are predominantly ferruginous tropical soils, classified as Luvisols, which are moderately fertile but prone to leaching and erosion ([FAO/ITPS, 2015](https://www.fao.org/3/i5199e/i5199e.pdf)).

**Collection of Seed**

Seeds of soybean were obtained from the seed store of the department of plant breeding and seed science of Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria.

**Collection and Preparation of Plant Materials (Jatropha Leaves Species)**

Jatropha species leaves were harvested from a local farm in Tarka L.G.A of Benue State and identified in the Department of Botany of Joseph Sarwuan Tarka University, Makurdi. Fresh leaves of Jatropha species were harvested, sorted and 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 and taken to the laboratory for analyses.

**Preparation of plant extract**

Fresh leaves of Jatropha species were washed with clean water to remove dirts and unwanted materials 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 (Chauhan *et al.* 2016; Zubir *et* *al*. 2020).

**Synthesis of ZnO nanoparticles**

ZnO NPs were prepared utilizing green synthesis method by means of Jatropha species extract. 5mL of the prepared plant extract was put into a beaker and heated gradually. At a temperature 60°C, 1 mM of zinc nitrate hexahydrate was added to the extract after which the mixture was stirred continuously, maintaining the temperature at 60°C, until the mixture turned to 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 and the residual was washed with ethanol and distilled water several times. The power was subsequently dried by heating at 1000C, after which zinc oxide was obtained and prepared for characterization.

**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 soybean (*Glycine max* 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, 0, 10, 25, 50 and 100ppm was used.

**Seed Germination Test on Two Varieties of Soybeans (TGX1904-6F and TGX1951-3F)**

The effect of ZnO nanoparticles on percentage seed germination of the two varieties of soybeans was determined as those seed were made to germinate on sterilized agar solution, supplemented with different concentrations of ZnO 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.

**Statistical Analysis**

Minitab 16.0 was used in analyzing the results. Descriptive statistics tool was used to calculate the total mean of each parameter across treatments.

**Results**

The application of zinc oxide nanoparticles (ZnO-NPs) in agriculture is a growing area of interest due to their potential to enhance seed germination, growth, nutrient uptake, and plant vigor and stress resilience. Zinc (Zn) is essential in plant development, playing a critical role in chlorophyll production, enzyme activation, protein synthesis and membrane integrity. However, the biological impact of ZnO-NPs is highly concentration-dependent, often producing beneficial effects at low concentrations and toxic or inhibitory effects at higher levels across plant species and growth stages.

The results on the effect of ZnO nanoparticle treatments on seed Germination and early growth, Percentage (%) survival, Average Length of plantlet, vigor and Average Root length of two varieties of soybean (TGX1904-6F and TGX1951-3F) are presented in table 1 and 2 below. The results focused on the impact of ZnO-nanoparticles treatments at varying concentrations on seed germination and growth two varieties of soybean (TGX1904-6F and TGX1951-3F) are presented in table 1 and 2.

**Effect of ZnO Nano Treatments on seed germination of TGX1904-6F variety**

The result of day 7 (table 1) revealed that ZnO mean treatment with concentration of 100ppm maintained the percentage survival (77.8%) as the value was equal to that of the control (77.8%). The average root length was suppressed by all concentrations of the nano treatment, although 10ppm had the highest value (13.0cm), and the control was 13.2cm. Likewise, the average length of plantlet was suppressed with treatment with all concentrations of the nano treatment, although 10ppm (15.6cm) followed by 100ppm (14.2cm) had a higher value compared to other treatments, and the control had a value of 24.3cm. However, all concentrations of the nano treatments maintained the plant vigor.

**Table 1: Effects of Nano Treatments on Germination of TGX 1904-6F**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | Concentration (ppm) | No of seed inoculated | Day of  emergence after inoculation | Number of emergences | Percentage (%) survival | Average Length of plantlet (cm) | Plant vigor | Average Root length  (cm) |
|  |  |  |  | **Day 7** | **Day 7** | **Day 7** | **Day 7** | **Day 7** |
| Mean T0 | 0 | 9 | 2 | 7 | 77.8 | 24.3 | 5 | 13.2 |
| Mean T1 | 10 | 9 | 2 | 6 | 66.7 | 15.6 | 5 | 13.0 |
| Mean T2 | 25 | 9 | 2 | 5 | 55.6 | 9.9 | 5 | 9.5 |
| Mean T3 | 50 | 9 | 2 | 5 | 55.6 | 10.1 | 5 | 9 |
| Mean T4 | 100 | 9 | 2 | 7 | 77.8 | 14.2 | 5 | 7 |

**Effect of ZnO Nano Treatments on seed Germination of TGX1951-3F variety**

From Table 2, ZnO mean treatment at concentration of 100ppm (88.9%) followed by 10ppm (77.8%) most effectively improved percentage survival as it had a value higher than the control (55.6%). All nano concentrations maintained plant vigor (5) (very good). However, the average length of plantlet and average root length were suppressed by all concentrations of the nano treatments used, although concentrations of 10ppm (17.9cm) and 10ppm (7.2cm) had the highest value, while the control had a value of 23.8cm and 13.2cm for average length of plantlet and average root length respectively.

**Table 2. Effects of Nano Treatments on Germination of TGX 1951-3F**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | Concentration (ppm) | No of seed inoculated | Day of  emergence after inoculation | Number of emergences | Percentage (%) survival | Average Length of plantlet (cm) | Plant vigor | Average Root length  (cm) |
|  |  |  |  | **Day 7** | **Day 7** | **Day 7** | **Day 7** | **Day 7** |
| Mean T0 | 0 | 9 | 2 | 5 | 55.6 | 23.8 | 5 | 13.2 |
| Mean T1 | 10 | 9 | 2 | 7 | 77.8 | 17.9 | 5 | 7.2 |
| Mean T2 | 25 | 9 | 2 | 5 | 55.6 | 18 | 5 | 6.5 |
| Mean T3 | 50 | 9 | 2 | 6 | 66.7 | 14.3 | 5 | 4.5 |
| Mean T4 | 100 | 9 | 2 | 8 | 88.9 | 17.5 | 5 | 5.2 |

**Discussion**

**Germination and Percentage Survival**

From table 1, the percentage survival rate for 100 ppm ZnO-nanoparticles (T4) matched the control group (77.8%), indicating that ZnO-NPs (ZnO-nanoparticles) at this concentration did not negatively affect germination success. Interestingly, while 10 ppm resulted in slightly lower survival (66.7%), the trend suggests that low to moderate concentrations do not severely hinder germination, though they don't significantly enhance it either in TGX1904-6F. This is in line with the study of Yuvaraj *et al.*, (2023) who stated that ZnO-NPs improved germination and early vigor in cereals and legumes at low concentrations. However, Basit *et al.,* (2023) emphasized that the response of soybean to ZnO-nanoparticles can be genotype-specific, with some cultivars (like TGX1904-6F) showing more resilience to nanoparticle-induced stress than others. From Table 1, result for TGX1904-6F showed that nano 80ppm more significantly increased plant height compared to salt and fertilizer, suggesting an optimal concentration promoting growth. Thus, the steady germination rate at 100 ppm in TGX1904-6F suggests moderate tolerance and adaptability to ZnO-NP exposure.

From table 2, the highest germination and survival rates were observed in the 100 ppm (88.9%) and 10 ppm (77.8%) ZnO-NP treatments. Both outperformed the control (55.6%), indicating that ZnO-NPs enhance seed germination, likely by improving zinc bioavailability in early growth phases, stimulating amylase and antioxidant enzymes, as reported in prior studies (Raliya et al., 2015) and enhancing water absorption and seed metabolism. This agrees with the findings of Raliya *et al*. (2015) who found that ZnO-nanoparticles improved germination and growth in *Vigna radiata* through enhanced enzyme activity and nutrient availability and also with that of Nasir *et al*. (2024) who reported that ZnO-nanoparticles improved seedling emergence in crops like cowpea and wheat. Fayomi *et al.,* (2024) had reported Zinc oxide nanoparticles (ZnO NPs) to exhibit concentration-dependent effects on soybean growth, with low doses (20 ppm) supporting normal growth, while moderate concentrations (40–60 ppm) significantly enhancing branching, attributing it to hormonal stimulation. Fayomi *et al.,* (2024) however, reported high concentrations (80–100 ppm) to cause growth inhibition and morphological stress, suggesting phytotoxicity. Their findings highlight the importance of optimal ZnO NP levels for improved plant performance without inducing toxicity. This is an indication that ZnO-nanoparticles, when applied at optimal concentrations (10–100 ppm), can boost seed germination in legumes like soybean.

**Shoot and Root Growth**

While germination was maintained, shoot and root growth were clearly suppressed across all nano-treated groups (table 1), control (T0) showed the highest values with plantlet having a length of 24.3cm and root length of 13.2cm, T1 (10ppm) showed the least suppression, with plantlet length of 15.6cm and root length of 13.0 cm, while T4 (100ppm) maintained survival but had a marked reduction in length with Plantlet and Root recording 14.2cm and 7.0cm in length respectively (table 1). This suppression suggests that while ZnO-nanoparticles didn’t inhibit seed emergence, they interfered with elongation growth, likely due to hormonal disruption (e.g., auxin transport), zinc accumulation in root cells impeding water/nutrient uptake, and oxidative stress from reactive oxygen species (ROS) at the root tips. This collaborate with the study of Rizwan *et al*. (2019) who reported that metal oxide nanoparticles often inhibit root elongation due to their toxicity at cellular levels, especially in roots where uptake is direct. Siddiqui and Al-Whaibi (2014) had observed similar trends in tomato seedlings, where nanosilicon treatments maintained germination but reduced root and shoot length due to altered water uptake and ion balance. ZnO nanoparticles have also exhibited the capacity to stimulate root growth, diversify the population of microorganisms in the rhizosphere, and contribute to the enhancement of soil structure and fertility (Wang *et al*., 2023). This indicates that nanoparticles improve some early-stage physiological activities but can limit elongation and biomass production, especially at higher doses.

Despite enhanced germination, plantlet and root lengths were reduced across all nano-treated groups compared to the control with the Control (T0) recording 23.8cm plantlet and 13.2cm root (the highest). T2 (10 ppm) recorded 17.9cm length in plantlet and 7.2cm in root, while T4 (100 ppm) recorded 17.5 cm plantlet and 5.2 cm root. This indicates that while ZnO-NPs promote germination, they may exert inhibitory effects on seedling elongation, possibly due to Zn toxicity at cellular levels, Interference with auxin signaling, crucial for root/shoot development and Nanoparticle-induced oxidative stress, limiting cell expansion. This result is in contrast with that of Rizwan *et al*. (2019) who highlighted that ZnO-Nanoparticles can reduce shoot and root length due to generation of reactive oxygen species (ROS) and membrane damage at high concentrations and also with that of Siddiqui and Al-Whaibi (2014) which showed that while nano-silicon enhanced germination, it also suppressed elongation in tomato seedlings, indicating a possible common trend among nanoparticles. Variability in media treatment with ZnO concentration and plant genotype may contribute to the observed alterations and differences number of emergences, percentage (%) survival, average length of plantlet and average root length response to ZnO nanoparticles. This study agrees with this dual effect that ZnO-nanoparticles stimulate early growth stages but may suppress elongation depending on concentration and duration.

**Plant Vigor**

From table 1, Despite reductions in shoot/root length, plant vigor scores remained uniformly at 5 (very good) across all treatments in both varieties (TGX1904-6F and TGX1951-3F). This might suggest that visual plant health (e.g., leaf turgor, color) was unaffected, internal stress responses did not manifest visibly at this stage and both varieties (TGX1904-6F and TGX1951-3F) might possess tolerance mechanisms that mask early stress signs. It can also This can also be an indication that the concentrations of ZnO nano treatments selected did not exert a substantial negative impact on the overall health and growth of soybean plants. Abubakar *et al.* (2018) found similar results where soybean plants maintained vigor ratings despite morphological suppression (height or biomass reduction) under ZnO-nanoparticles exposure. This result is also in line with that of Abubakar *et al*. (2018) who reported minimal effects on plant vigor in soybean by certain nano treatments and differs with the study by Tahir and Mathew (2021), Rivera *et al*., (2022) and Pandya *et al.*, (2024) who reported conflicting results, stating that certain treatments concentrations and types can influence plant vigor. This observation suggests that vigor scoring alone may not fully capture plant stress, and should be complemented by biochemical and physiological assessments or chlorophyll measurements to detect early stress indicators.

**Conclusion**

The study analyzed the effect of ZnO nano treatments on the germination and growth of two varieties of soybean (TGX1904-6F and TGX1951-3F) in Makurdi, Nigeria. from the result, from the obtained result it can be concluded that the germination rate remained unaffected and enhanced at the highest concentration (100 ppm) and lowest concentration (10ppm), indicating tolerance to ZnO-NPs in terms of survival, root and shoot lengths were negatively affected at all concentrations, suggesting concentration-dependent inhibition of elongation growth and the plant vigor remained stable across treatments, implying visual health was unaffected, possibly due to early-stage physiological compensation. Adaptability of soybean plants to the applied treatments might be responsible for the lack of significant differences in plant vigor among treatments indicating their resilience under the experimental conditions.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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