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RESEARCH ARTICLE

Application of Zinc Oxide Nano Particles in the Performance of *Jatropha curcas* Grown in Cement Waste Contaminated Soil

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Abstract

The aim of this study was to investigate the efficacy of ZnO nanoparticles in the performances of *Jatropha curcas (J.curcas)* grown in cement waste contaminated soil in replicated pot trials. There were 5 levels of nano treatments (20ppm, 40ppm, 60ppm, 80ppm and 100ppm) and one level of Zinc salt and inorganic liquid fertilizers while seeds without treatments served as control. The eight different treatments were replicated 5 times each with a total of 40 experimental units of pots. A 7-day seed germination experiment was conducted in petri dishes consisting of different concentrations of nano particles except the control dish. Result showed that 100ppm Zn nano enhanced seed germination by 50% as against 0% seed germination in petri dishes without nano treatments after 7 days. The effects of nano particles on the growth of *Jatropha curcas* showed that, those planted in the cement contaminated medium treated with nano particles had average height (12.13 cm) with approximately ten (10) leaves with the leaf length and width measuring optimally (8.910 cm) and (8.43 cm) respectively whereas the stem diameter and the plant vigour were measured to be (3 cm) each on average. The effects of nano particles on the emergence of plant and height showed that the emergence of the J. curcas was more rapid (2.750 ± 0.957 cm) following the treatment with fertilizer while the treatment with nano particles at 60 ppm had the highest mean plant height (21.250 ± 4.193 cm). The effect of nano particles on the number of leaves and length of J. curcas revealed that plants in the control experiment had the lowest number and length of leaves (8.500 ± 1.291) and (8.500 ± 0.707cm) respectively while the treatment with nano particles at 60 ppm had the highest mean number of leaves (16.750 ± 4.113) and leaf length (9.250 ± 0.500 cm). Inferences drawn from the study suggest that addition of zinc oxide (ZnO) nano particles to cement waste contaminated soil increased growth, some biochemical aspects, and yield attributes of J. curcas.

Keywords: *Jatropha curcas*- Contaminated soil- Zinc Oxide nanoparticles- Plant- Fertilizer

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Introduction

Jatropha curcas is an important source of biofuel, grown primarily in the tropics and subtropics. The plant is native to Central and South America but is now widely distributed, with two main centers of diversity; the primary center in Mexico and Central America, and the secondary center in Brazil. It has now been introduced in other regions including South and Southeast Asia, Africa, and the Caribbean [1].

Jatropha curcas is a perennial shrub found in tropical and subtropical regions of the world. It belongs to the Euphorbiaceae family and is commonly known as physic nut or Barbados nut. The Species is highly valued for its oil content, which is used for various applications, including biodiesel production. Various research have highlighted the potent nutritional [2], medicinal and ethnobotanical [3, 4] as well as industrial and agricultural values [5] exhibited by this plant.

Several varieties of J. curcas have been identified based on their morphological and genetic characteristics. For example, the Brazilian J. curcas variety is known for its high oil yield and is tolerant to drought conditions. In contrast, the Peruvian variety has higher yields in moist conditions. The Indian variety is known for its ability to grow in arid and semi-arid regions, while the African

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variety is resistant to pests and diseases [6].

However, sustainable production of *Jatropha curcas* faces several challenges. Climate change, drought, soil erosion, degradation, and pests and diseases are some of the factors that affect its production. In addition, competition for land and water resources due to pollution, the lack of government support, and low market prices for *Jatropha curcas* seeds also impact plant production [7].

Cement waste contaminated soil is a significant issue affecting agricultural productivity worldwide. The presence of pollutants such as heavy metals and pesticides in cement waste contaminated soil poses severe health risks to humans, animals, and the environment. Additionally, it limits plant growth, leading to reduced crop yield and quality. Jatropha curcas, is grown on marginal lands, which are more susceptible to cement waste contaminated soil. Therefore, the growth and success of *Jatropha curcas* is significantly affected by cement waste contaminated soil. The development of an efficient and sustainable means of enhancing plant growth in contaminated soil is crucial for sustainable biofuel production and environmental remediation [8].

The utilization of nanotechnology in agriculture has gained significant attention in recent years due to its potential to address environmental challenges and enhance crop productivity. Among various nano materials, zinc oxide (ZnO) nanoparticles have emerged as a promising candidate for improving plant growth and yield. According to Hossain et al. [9] ZnO nanoparticles have unique physicochemical properties, such as high surface area, small size, and reactivity, which make them suitable for agricultural applications. Several studies have demonstrated the positive impact of ZnO nanoparticles on plant growth, including enhanced photosynthesis, nutrient uptake, and stress tolerance. These nanoparticles can interact with plant systems at the molecular level, influencing various physiological and biochemical processes.

In the context of soil contaminated with cement waste, *Jatropha curcas* faces multiple stressors, including altered soil pH, reduced nutrient availability, and potential heavy metal toxicity. Cement waste often contains elements like chromium, cadmium, and lead, which can adversely affect plant growth. ZnO nanoparticles, with their ability to act as soil amendments and plant stimulants, offer a potential solution to mitigate these challenges [10]. Mohamed et al [11] also highlighted the role of ZnO nanoparticles in improving soil quality and nutrient availability. These nanoparticles can modify soil pH, enhance nutrient retention, and facilitate the release of essential elements for plant uptake.

The effectiveness of ZnO nanoparticles in ameliorating heavy metal stress in plants, demonstrates their potential in addressing contamination issues associated with cement waste. The interaction between ZnO nanoparticles and plant roots plays a crucial role in promoting growth. The nanoparticles can enhance root development, increase root surface area, and improve nutrient absorption efficiency. This is particularly relevant for Jatropha curcas, which relies on robust root systems to thrive in challenging soil conditions. As we delve into the application of ZnO nanoparticles in cement waste-contaminated soil for *Jatropha curcas* cultivation, it is essential to consider the broader implications for sustainable agriculture and biofuel production. By addressing soil contamination challenges and promoting plant growth, this research contributes to the development of eco-friendly approaches for enhancing crop productivity in degraded environments [12].

The production of biofuels from crops such as *Jatropha curcas* has been identified as a sustainable option for reducing carbon emissions and enhancing energy security. However, the growth and yield of *Jatropha curcas* are limited by cement waste contaminated soil. The application of ZnO nanoparticles has been reported to enhance the growth and yield of crops in contaminated soil. However, the effectiveness of ZnO nanoparticles in enhancing the growth and yield of *Jatropha curcas* in contaminated soil is yet to be fully explored [13].

Materials and Methods

Study Area

This research work was carried out in the Department of Botany, Joseph Sarwuan Tarka University, Makurdi, Benue State. Makurdi is located in the guinea savanah vegetation zone and its geographical coordinates are; 80, 53’ 0” North, 70 73’ 0”east for longitude and latitude respectively (Google Earth, 2024). Makurdi has a minimum temperature range of 25.710C ± 3.4-30C, It is a 16km radius circle, covering 804km2 lands mass. Makurdi has an estimated population of 500,797 [14]. 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. Mean Monthly Relative Humidity in Makurdi LGA varies between 43% in January to 81% in July-August period [15]. Makurdi L.G.A. falls within the middle belt

Materials Used

Seed of Jatropha curcas, Synthesized ZnO nanoparticles, Distilled water, Petri dishes. Agar powder (Bacteriological), Micropipette Graduated cylinders, Potting soil, Polythene leather Watering can, Weighing scale, Meter rule, Notebooks, Gloves, Lab coat, and Fertilizer (NPK).

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 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 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 [16].

Synthesis of ZnO nanoparticles

ZnO 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 zinc nitrate hexahydrate was 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 zinc oxide was obtained and they were ready for characterization.

Collection of Seed

Seeds of tobacco (Nicotiana tabacum L.) were obtained from seed stores of department of plant breeding and seed science of Joseph Sarwuan Tarka University.

Collection of Soil Sample

Surface soil sample were collected from fallow land of the botanical garden of the department of Botany, Joseph Sarwuan Tarka 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 40 pots.

Collection of Cement Wastes

Cement wastes were gotten from Benue cement industry at Gboko Benue state.

Soil contamination procedures

The collected soil samples were spiked with cement kiln waste collected from Dangote Cement Company Gboko, Benue State, Nigeria. Exactly 2kg of cement waste was added to each pot containing 25kg of soil sample. All soil samples in the 40 pots were contaminated with equal amount of cement kiln waste. Each mixture was thoroughly mixed together and allowed to stand for 7 days before seed sowing.

Seed Germination Test in Petri Dishes

Agar broth was prepared following standard aseptic practices and transferred equally into 10 petri dishes to solidify. A set of five petridishes labeled To, T1, T2, T3 and T4 contained the following concentrations of ZnO nano particles: 0ppm (control), 10ppm, 25ppm, 50ppm and 100ppm respectively. These were replicated for the second set of petri dishes. Four seeds of *Jatropha curcas* were placed on the surfaces of the agar media in each of the petri dish. The experiment was allowed to stand for 7 days. Number of germinated seeds and day to germination were recorded.

Experimental Design

A completely randomized design with 5 replicates was used to assign treatments to investigate the growth responds of Jatropha Curcas. They were randomly assigned to different treatment groups ensuring unbiased comparisons and allowing for accurate assessment of their respective performance in terms of growth rate. At various treatment levels: Control, Fertilizer, Salt, 20, 40, 60, 80 and 100ppm were used. There were 40 experimental units. Four (4) seeds were sown at 3cm depth manually in each pot on the 12^{th} of September, 2023 and were thinned to three per pot after seedling establishment.

Determination of Growth Parameters

Plant height (cm)

Average height of plants was determined by measuring the height of five randomly selected plants from the ground surface to the top of the main stem in centimeter at maturity.

Numbers of leaves

The numbers of leaves was determined by counting the total leaves per plant per pot.

Leaf length

Leaf length was determined by measuring the distance from the base to the apex of the leaf.

Stem diameter

Stem diameter was determined by using the ruler to measure the distance between the widest part of the stem.

Numbers of branches

The numbers of branches was determined by counting the numbers of the branches from each plant per pot.

Yield Parameters Determination

Wet Plant biomass (g)

The wet plant biomass was determined by using the weighing scale to measure the weight of the entire plant harvested, including the stems, leaves, pods, and seeds immediately without drying it.

Dry plant biomass (g)

The dry plant biomass was determined by using the oven to dry the entire plant to remove the moisture, and once completely dried, the weight was obtained by weighing the entire plant material.

Determination of Physiological Yield Parameters

Moisture content determination

Moisture content was determined using the air oven method [17]. 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;

% Moisture =(w2-w3)/(w2-w1) x 100/1

w1=weight of the empty moisture can

w2=weight of can and sample before drying

w3=weight of can and sample after drying

Chlorophyll content determination

0.1g of fresh tobacco 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 formular:

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

Statistical Analysis

Minitab 16.0 was used in analysing the results. The following tools were applied: Descriptive statistics (mean, standard error,), 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).

Results

Effect of ZnO Nano Treatment on Jatropha curcas

Table 1 shows the effects of different concentrations of ZnO nano particles on the seed germination performances of J. curcas in petri dishes. Among the five treatment levels, T1 (10ppm) initiated the germination of one seed (25%) at day 6 while T4 (100ppm) gave the highest seed germination of 50% where 2 out of 4 seeds germinated at day 6.

The result of the applications of zinc oxide (ZnO) nano particles in the enhancement 0f growth and yield of J. curcas in cement waste contaminated soil showed variable levels of adaptability of the plant to the contaminated soil. The results are expressed as means of duplicate experiments plus or minus the standard deviation. Table 2 shows the effects of nano particles on the growth of J. curcas. The result showed that, The J. curcas planted in the cement contaminated medium treated with nano particles had average height (12.13 cm) with approximately ten (10) leaves with the leaf length and width measuring optimally (8.910 cm) and (8.43 cm) respectively whereas the stem diameter and the plant vigour were measured to be (3 cm) each on average.

The effects of nano particles on the emergence of plant and height is shown in Table 3. The result showed that the emergence of the J. curcas was more rapid (2.750 ± 0.957 cm) following the treatment with fertilizer while the treatment with nano particles at 60 ppm had the highest mean plant height (21.250 ± 4.193 cm). Table 4 shows the effect of nano particles on the number of leaves and length of J. curcas. The results revealed that plants in the control experiment had the lowest number and length of leaves (8.500 ± 1.291) and (8.500 ± 0.707cm) respectively while the treatment with nano particles at 60 ppm had the highest mean number of leaves (16.750 ± 4.113) and leaf length (9.250 ± 0.500 cm)

Table 5 shows the effects of nano treatments on plant biomass and moisture. The result showed that plants treated with nano particles at 40 ppm had the highest chlorophyll content and percentage moisture of (9.828 ± 7.739) and (90.100 ± 1.068) respectively while plants in control experiment had the highest wet plant biomass (19.850 ± 0.420) whereas the lowest dry plant biomass was recorded from treatments with nano particles at 60 ppm (1.375 ± 0.050^{d}).

Discussion

Nanotechnology has a dominant position in transforming agriculture and food production. Nanotechnology has a great potential to modify conventional agricultural practices, Most of the agrochemicals applied to the crops are lost and do not reach the target site due to several factors including leaching, drifting, hydrolysis, photolysis, and microbial degradation. Nanoparticles and nanocapsules provide an efficient means to distribute pesticides and fertilizers in a

Table 1. Seed Germination Trial at Different Concentrations of Zno Nano Particles

Petridish code	Nano Concentration (ppm)	No of seed inoculated	Day of emergence after inoculation	Number of emergence	% survival
T0	0	4	0	0	0
T1	10	4	6	1	25
T2	25	4	0	0	0
T3	50	4	0	0	0
T4	100	4	6	2	50

Table 2. Effect of Nano Particles on the Growth of *Jatropha curcas*

Character	Mean	Mean	t-statistic	p-value
Plant Height	12.131	18.154	-8.718	0
No. Plants	2.077	2.103	-0.131	0.897
No. Leaves	9.897	10.821	-0.983	0.332
Leaf Length	7.538	8.91	-4.307	0
Leaf Width	7	8.436	-4.125	0
Stem Diameter	3.321	3.769	-2.433	0.02
Plant Vigour	3.923	4.231	-1.306	0.2

Table 3. Effect of Nano Particles on the Emergence of Plant and Height

Treatments	Number of Emergence	Plant Height
	Mean ± SD	Mean ± SD
Control	1.750 ± 0.957	16.500 ± 3.000
Fertilizer	2.750 ± 0.957	17.250 ± 5.058
Salt	1.500 ± 0.577	20.250 ± 0.957
Nano 20 ppm	2.500 ± 1.291	17.750 ± 1.708
Nano 40 ppm	2.250 ± 1.258	17.500 ± 4.041
Nano 60 ppm	3.000 ± 0.000	21.250 ± 4.193
Nano 80 ppm	2.000 ± 1.155	17.750 ± 5.315
Nano 100 ppm	2.000 ± 1.155	17.750 ± 2.217
F (Treatment)	14	23

controlled fashion with high site specificity thus reducing collateral damage. Farm application of nanotechnology is gaining attention by efficient control and precise release of pesticides, herbicides, and fertilizers. They can also detect level of soil moisture and soil nutrients. Plants can rapidly absorb nanofertilizers. Nanoencapsulated slow release fertilizers can save fertilizer consumption and minimize environmental pollution

The result obtained in this study revealed that application or treatment of cement waste contaminated soil with zinc oxide (ZnO) nano particles (NPs) has greatly improved the development and yield of *Jatropha curcas* as evident in the mean emergence and plant height. The result of this study corroborated with the findings of Prasad et al. [18] who reported that Zinc oxide NPs have potential to boost the yield and growth of food crops. They treated peanut seeds with different concentrations of zinc oxide nanoparticles. Zinc oxide nanoscale treatment (25 nm mean particle size) at 1000 ppm concentration was used which promoted seed germination, seedling vigor, and plant growth and these zinc oxide nanoparticles also proved to be effective in increasing stem and root growth in peanuts.

In this study, ZnO nano particles inoculation enhanced photosynthetic pigments of J. curcas over those plants grown without ZnO addition. This result is in agreement with the result of Rady et al. [19] and Kortei and Quansah [20] who independently found that ZnO addition to soil increased photosynthetic pigment on common bean and lettuce plants, respectively. This stimulating effect of ZnO nano particles is consistent with ZnO effects on stomatal opening. As well as, the processes involved by ZnO nano particles lead to increased rates of photosynthesis and of carbon compounds to the plants and increase the contents of chlorophyll which increase the rate of photosynthesis and carbohydrate synthesis. Moreover, the increases in chlorophyll contents might be attributed to an increase in the rate of the uptake of elemental zinc and other nutrient elements and thus increases in chlorophyll content.

With respect of ZnO effect on photosynthetic pigments, similar results were obtained by Sofy [21]. Such increase in photosynthetic pigment content in the leaves of plants may be attributed to the enhancing effect of ZnO on chlorophyll accumulation through the useful role of Zn on plant growth. Raliya and Tarafdar [22] also reported that ZnO NPs induced a significant improvement in chlorophyll synthesis. With regard to Zn nanoparticles treatment, these obtained results are in harmony with those obtained by Babaeiaet al. [23] and Tawfik et al. [24].

Moreover, the exogenous application of ZnO NPs in this study improves photosynthetic pigments of J. curcas; they reflected these increases to the role of Zn element in keeping chlorophyll synthesis via protection of sulfhydryl group. According to Gonorov and Carmeli [25], metal nanoparticles can induce the efficiency of chemical energy production in photosynthetic systems. However, higher content of photosynthetic pigments, i.e., chlorophyll a, chlorophyll b, and carotenoids, would increase the rate of photosynthesis; due to which, there was more production of photosynthesis process, which in turn increased the weight and growth of plant as it was observed in this study. The results obtained in this study also showed that *Jatropha curcas* treated with Zinc

Table 4. Effect of Nano Particles on the Number of Leaves and Length

	Number of leaves	Leaf length(cm)
Treatments	Mean ± SD	Mean ± SD
Control	8.500 ± 1.291	8.500 ± 0.707
Fertilizer	13.000 ± 5.033	9.125 ± 0.750
Salt	8.750 ± 0.957	9.250 ± 1.190
Nano 20 ppm	11.750 ± 4.113	9.250 ± 0.500
Nano 40 ppm	12.500 ± 5.972	8.125 ± 1.436
Nano 60 ppm	16.750 ± 4.113	9.250 ± 0.500
Nano 80 ppm	11.750 ± 7.274	8.500 ± 1.472
Nano 100 ppm	8.250 ± 4.717	9.125 ± 0.750
F (Treatment)	26	16

Table 5. Effects of Nano Treatments on Plant Biomass and Moisture

Treatment	Chlorophyll content	Wet plant biomass	Dry plant biomass	Percentage moisture
Control	2.393 ± 0.499	19.850 ± 0.420[a]	2.300 ± 0.000[bc]	27.930 ± 39.519[b]
Fertilizer	4.268 ± 3.606	12.750 ± 1.905[cd]	3.850 ± 0.173[a]	70.850 ± 2.170[a]
Salt	3.688 ± 0.632	9.550 ± 0.574[d]	2.700 ± 1.334[b]	73.125 ± 11.528[a]
Nano 20 ppm	6.210 ± 1.193	11.425 ± 5.584[cd]	1.925 ± 0.250[bcd]	74.825 ± 9.728[a]
Nano 40 ppm	9.828 ± 7.739	14.175 ± 1.684[bc]	1.500 ± 0.200[cd]	90.100 ± 1.068[a]
Nano 60 ppm	4.223 ± 1.469	13.250 ± 0.173[c]	1.375 ± 0.050[d]	89.625 ± 0.403[a]
Nano 80 ppm	2.808 ± 0.453	16.850 ± 0.645[ab]	2.000 ± 0.566[bcd]	88.000 ± 3.461[a]
Nano 100 ppm	3.840 ± 1.737	13.500 ± 0.294[c]	1.875 ± 0.574[bcd]	86.150 ± 4.207[a]
F (Treatment)	12	27	21	72

oxide (ZnO) nano particles enhances rapid emergence of the plant this result agrees with the findings of Lopez et al. [26] who reported that seed germination of six higher plant species (radish, rape, ryegrass, lettuce, corn, Jatropha and cucumber) increases when treated with five types of NPs (multiwalled carbon nanotubes, aluminum, alumina, zinc, and zinc oxide).

The treatment of plants with zinc oxide (ZnO) nano particles in this study at different concentrations of (20, 40, 60, 80 and 100 mg/ L) increased markedly dry weight of *Jatropha curcas* as compared with untreated plants. Data clearly show that 40mg/L was the most effective concentration of ZnO or nano ZnO on most of growth parameters of plant grown in cement waste contaminated soil.

In conclusion, from the present study, it could be concluded that seed gerimantion was enhanced at 100pmm ZnO. Also, addition of zinc oxide (ZnO) nano particles to cement waste contaminated soil increased growth and physiological yield attributes of Jatropha curcas. In comparison, between control and treatment nano ZnO effect on enhancing growth and yield of *Jatropha curcas* plant, data clearly show that nano ZnO have stimulating effects on growth and yield of *Jatropha curcas* via enhancement of photosynthetic pigments (chlorophyll content), number of leaves and leaf length, wet and dry plant biomass and well as percentage moisture of *Jatropha curcas* plant. Therefore, ZnO nano particle may be applied to increase the performances of J, curcas when grown to remediate cement polluted soil.

Recommendations

Based on the outcome of the study, the following recommendations are given

1. ZnO nano particles should be used to initiate seed germination of *J.curcas* in the field
2. ZnO nano particles should be used to improve growth performances of *J.curcas* in the field
3. ZnO nano particles should be used to improve physiological yield performances and the overall productivity of *J.curcas* in the field
4. The full potentials of *J.curcas* can now be harnessed using nano particles as growth and yield enhancers. This may include the aspect of phytoremediation of polluted soil (such as cement contaminated soil), biodiesel production and carbon sequestration among other benefits.

Acknowledgments

Statement of Transparency and Principals

- Author declares no conflict of interest
- Study was approved by Research Ethic Committee of author affiliated Institute.
- Study's data is available upon a reasonable request.
- All authors have contributed to implementation of this research.

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