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ASSESSMENT OF ZN, CU AND MN NANOPARTICLE USAGE ON THE GROWTH AND YIELD OF RICE

Francis Aibuedefe IGIEBOR[1*],Beckley IKHAJIAGBE[2]

[1]Department of Biological Sciences, College of Science and Computing, Wellspring University, Benin City, Nigeria
[2]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Abstract: Nanotechnology has emerged as a sustainable tool for enhancing crop productivity, especially through the application of green-synthesized nanoparticles (NPs). This study evaluated the effects of biosynthesized zinc (ZnNP), copper (CuNP), and manganese (MnNP) nanoparticles from *Carica papaya*, *Azadirachta indica*, and *Hibiscus sabdariffa* leaf extracts on the growth performance of Nerica rice grown in ferruginous soil. The experiment was conducted in the Botanic Garden, University of Benin, Nigeria. Ferruginous soil was collected and characterized for physicochemical properties. Aqueous extracts of the selected plant leaves were used to biosynthesize ZnNPs, CuNPs, and MnNPs, which were characterized using UV-Vis spectrophotometry. Nerica rice seeds were sown in perforated bowls arranged in a completely randomized design. Synthesized nanoparticles were foliar-applied four weeks after sowing, with a booster application two weeks later. Growth parameters, photosynthetic pigments, physiological parameters, and chlorosis were assessed over an 11-week period. Soil and plant biochemical analyses followed standard protocols. Data were analyzed using SPSS v21. Ferruginous soil had low organic matter (1.12 %), high iron content (442.7 mg/kg), acidic pH (5.8), and was sandy loam in texture. Application of ZnNP, CuNP, and MnNP significantly enhanced plant height (93.2 cm with 25 % CuNP from *Azadirachta indica*), leaf number, grain yield, chlorophyll content, and antioxidant parameters compared to the control. Therefore, biosynthesized metal nanoparticles positively influenced rice growth and yield in ferruginous soil, indicating their potential as eco-friendly agricultural inputs. Future research should focus on field-scale validation and nanoparticle uptake mechanisms.

Keywords: ferruginous soil, nanoparticles, *Oryza sativa*, plant extracts, growth, yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food crop that sustains more than half of the global population, particularly in Africa and Asia (Igiebor *et al*., 2019; Ikhajiagbe *et al*., 2021). Its productivity is influenced by various factors including soil fertility, water availability, and pest or disease pressures. Among these, micronutrient deficiencies especially of zinc (Zn), copper (Cu), and manganese (Mn) are widely recognized as critical limitations to optimal growth and grain yield in rice cultivation. These micronutrients play essential roles in enzymatic activation, photosynthesis, protein synthesis, and stress tolerance in plants.

Traditional methods of micronutrient supplementation often involve chemical fertilizers, which are not only costly but also associated with environmental hazards such as leaching, bioaccumulation, and degradation of soil health (Igiebor *et al*., 2023). In response to these challenges, nanotechnology offers an innovative and eco-friendly solution through the application of nanoparticles as nano-fertilizers. Nanoparticles possess unique properties such as high surface area, enhanced reactivity, and controlled release, which make them suitable for efficient nutrient delivery to plants (Igiebor *et al*., 2021). Recent advances in green synthesis of nanoparticles using plant extracts have provided a sustainable alternative to physical and chemical methods. Phytochemicals present in plant extracts act as reducing and capping agents, enabling the formation of stable nanoparticles with minimal toxicity (Igiebor *et al*., 2023). Several studies have demonstrated the efficacy of biosynthesized nanoparticles in improving plant growth, photosynthetic efficiency, and nutrient uptake in various crops (Ikhajiagbe *et al*., 2021).

Despite the growing interest in the use of nanoparticles in agriculture, limited studies have been conducted to evaluate the effect of green-synthesized Zn, Cu, and Mn nanoparticles on rice growth and productivity under field-like conditions. This study, therefore, aimed to assess the impact of biosynthesized ZnNPs, CuNPs, and MnNPs on the morphological, physiological, and yield parameters of rice cultivated in ferruginous soil. The findings of this study will provide valuable insights into the potential application of plant-based nanotechnology in sustainable rice farming systems.

MATERIALS AND METHODS

Description of the Study Site

The experiment was conducted at the University of Benin's Department of Plant Biology and Biotechnology Botanic Garden in Nigeria. The land was mostly covered with weeds until it was cleaned for utilisation. The study site's GPS coordinates are N6º23'51.8º, 5º36'56.6ºE.

Collection of soil, leaves and seeds used for the study

A shovel was used to collect ferruginous soil from a reddish soil area of the Botanic Garden, Department of

***Correspondence**: Francis Aibuedefe Igiebor, Department of Biological Sciences, College of Science and Computing, Wellspring University, Benin City, Nigeria, Tel. +234-(0) 8033809330, email: francis.igiebor@lifesci.uniben.edu*

Plant Biology and Biotechnology, University of Benin, Benin City, at a depth of 0 - 10 cm. The rice variety utilised was Nerica, sourced from Raymos Guanah Farms Limited in Ughelli, Delta State. The leaves of *Carica papaya* L. (pawpaw) and *Azadiracta indica* A. Juss (neem) were collected from their respective trees in the botanic garden, while the leaves of *Hibiscus sabdariffa* L. (red sorrel) were bought from New Benin Market, Benin City, Nigeria.

Preparation of leaves extracts of plants used for the study

The leaf samples were repeatedly cleaned with deionised water to eliminate dust before being dried in the shade. The dried leaves were cut into smaller pieces using a knife and then ground into a powder with a mortar and pestle. Five grammes (5 g) of each sample powder were boiled in 100 millilitres (mL) of distilled water at 80 degrees Celsius for ten minutes and filtered through Whatman No. 1 filter paper. Finally, the obtained extract solution was cooled to 4 °C and kept for future nanoparticle production (Anandalakshi and Venugobal, 2017).

The stock solution (100 % of each extract) was made from the extracts previously prepared for each of the three leaves and diluted with distilled water to reach the three concentrations (v/v). The concentrations were as follows: 5 % (5 ml of extracts), 25 % (25 ml of extracts), and 50 % (50 ml of extracts).

Synthesis of Green Nanoparticles

A one-millimole (1 mM) aqueous solution of zinc sulphate (ZnSO4) was produced, and an aqueous leaf extract was employed to synthesise zinc nanoparticles. Twenty (20) ml of leaf extract was added to an 80 ml aqueous solution of 1 mM zinc sulphate. It was stirred continuously for two hours at room temperature using a magnetic stirrer. ZnNP was characterised using a UV-visible spectrophotometer (Devasenan *et al*., 2016).

Ten (10) ml of plant leaf extract was added to 90 ml of 10mM copper sulphate solution and stirred for continuous mixing at room temperature. Visual observation of the solution reveals a colour shift, which confirms CuNP production (Wu *et al*., 2020). The characterisation of CuNP was carried out using ultraviolet-visible (UV-Vis) spectrophotometer from 200 to 600 nm (Wu *et al*., 2020).

The approach of Paul *et al*. (2017) was used with minor modifications in the manufacture of manganese oxide nanoparticles. Five millilitres (5 mL) of aqueous leaf extract were added to 50 mL of an aqueous solution of 0.2 M potassium permanganate (KMnO4) while heating and stirring at 70 ^{0}C and pH 7 for 30 minutes. With the production of precipitate, the solution changed hue from colourless to brown. The precipitate was then centrifuged at 3000 rpm for 15 minutes and rinsed with distilled water three times. This was characterised according to the approach outlined by Chatterjee *et al*. (2017), using a UV-visible spectrophotometer at 250-800 nm.

Experimental Design and Treatment

The designated site inside the botanical garden was cleaned to bare earth, and all rubbish was removed. The experimental bowls (20 L) were perforated and filled with 20 kg of soil. The experimental design used was a completely randomised design, with the experimental plot assumed to be homogeneous. The ferruginous soil taken from multiple designated locations was combined to form composite samples. Each treatment had replicas. Twenty (20) rice seeds were put in each bowl and trimmed to 5 stands after two weeks. The biosynthesized nanoparticles were foliar sprayed directly over each treatment's leaves using a spray pump. This spraying was done in the evening, but between 3 and 4 hours after synthesis (the peak active phase). Morphological traits were examined and parameters obtained at 2, 5, 8, and 11 weeks.

Maintaining soil moisture

Since the setup was outside, it was primarily dependent on rainfall. Nonetheless, the USDA (2009) Hand-feel technique was always used to maintain the soil moisture. Using a spade, the soil sample was taken in the root zone. Each soil sample's water deficit was calculated at many depths between 0 and 2 inches/feet by touching the soil and determining its moisture content based on its texture. This is identifiable by a wet outline when pressed, some blackness from insufficient moisture, hard baking, cracking, and occasionally loose crumbs on the surface (wilting point).

Application of biosynthesized nanoparticles

Four weeks after sowing of the rice, nanoparticles synthesized in the laboratory within that same day was applied to the rice stands by foliar spray within 3 – 4 hours after synthesis. Two weeks following the initial treatment, a booster dosage was administered (Ikhajiagbe *et al*., 2021). Up to the experiment's conclusion, care was taken to guarantee that weeds were consistently eliminated from each experimental bowl.

Growth parameters

The methods outlined by Ikhajiagbe (2016) and Özalkan *et al*. (2010) were used to monitor plant growth and morphological responses. The following growth parameters were measured: plant height, leaf length, sheath length, stem girth, number of leaves, panicle branch, length of grain, diameter of grain, weight of grain, number of grains, weight of panicle, length of panicle, weight of dried roots, number of roots, root depth, and grain yield..

Soil Physicochemical Parameters

A glass-calomel electrode (MP 220 AFAB Lab, LLC) with a 1:2.5 soil-to-water ratio was used to potentiometrically determine the pH of the soil. Titrimetric techniques were used to determine the amount of organic matter in the soil by multiplying the organic carbon content by 1.724. Sims and Haby (1971) used the Walkley-Black wet oxidation technique with potassium dichromate and loss on ignition at 360 °C. Carbon was also measured by entire combustion at temperatures higher than 1,000°C using infrared/conductivity detection. The Kjeldahl

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technique, which includes distillation, titration, and digestion in sulphuric acid with $CuSO_4/TiO_2$ catalysts, was used to measure the total nitrogen concentration (AOAC, 1999a). Colorimetric detection of 'Molybdenum Blue' at 882 nm and 0.5 M sodium bicarbonate extraction (pH 8.5) were used to determine the phosphorus concentration. In order to determine the total exchangeable acidity, soil was saturated with 1 M KCl, titrated with 0.02 M HCl, and the exchangeable H^+ and Al^{3+} fractions were calculated. In accordance with APHA (2008) procedures, the concentration of iron (Fe) was ascertained by acidifying a soil-water solution, reducing it with hydroxylamine hydrochloride, buffering it, reacting it with 1,10-phenanthroline, and measuring it spectrophotometrically at 510 nm. The pipette method was used to assess the texture of the soil (clay, silt, and sand concentrations). Timed aliquots were sampled after settling times to determine fractions after sodium hexametaphosphate was used to disseminate the soil. Aliquot weights were used to compute percentage compositions. A 1:5 (w/v) soil-water combination was shaken, allowed to settle, and the electrical conductivity in the supernatant was measured without disturbing the sediment.

Photosynthetic Pigments

Standard spectrophotometric techniques were used to identify the photosynthetic pigments (lycopene, tocopherol, carotenoids, chlorophyll a, and chlorophyll b). By macerating 1 g of fresh leaf tissue in 80 % acetone and measuring absorbance at 645 nm and 663 nm, chlorophyll a and b were calculated in accordance with Arnon (1949) and Maxwell and Johnson (2000). After 0.5 g of the material was saponified with alcoholic KOH, extracted with petroleum ether, and absorbance measurements were taken at 450 nm and 503 nm, total carotenoids and lycopene were calculated in accordance with Zakaria and Simpson (1979). According to Rosenberg (1992) and Ayodele *et al*. (2014), the Emmerie–Engel reaction was used to determine the amount of tocopherol. This reaction was based on absorbance at 460 and 520 nm following reaction with ferric chloride and dipyridyl reagent. Using a conventional leaf colour chart, chlorosis was visually evaluated; yellow colours denoted chlorosis.

Physiological Parameters

Ascorbate concentration, proline content, and nitrogen absorption were measured in accordance with conventional protocols. By breaking down plant material into ammonium sulphate with sulphuric acid at 373 °C and then distilling the liberated ammonia into an acid trap (HCl or H_2SO_4), the amount of nitrogen was determined by measuring the ammonia concentration. Using the Bates *et al*. (1973) and Marin *et al*. (2006) methods, proline content was extracted and measured by boiling plant material in ethanol and water, then centrifuging the mixture. By measuring the drop in absorbance at 290 nm in a potassium phosphate buffer reaction system, the ascorbate content was ascertained in accordance with Sarker and Oba (2018).

Data Analysis

Using SPSS Statistical Package version 21, the data gathered from the analysis were statistically analysed using descriptive, association, and inferential statistics. Since the soil used in the experiment was homogenised and the homogeneity of the entire plot was assumed, analysis of variance was done on a single factor basis. GraphPad Prism version 6 was used to plot the graphs.

RESULTS AND DISCUSSION

The chemical and physical properties of the ferruginous soil used in this investigation were illustrated by the data shown in Table 1. The pH value was 6.47 ± 0.21, which means it was almost neutral. Clay was 7.92 ± 1.42 %, silt was 5.74 ± 2.76 %, sand was 85.10 ± 18.24 %, total organic carbon was 0.41 ± 0.11 %, total nitrogen was 0.64 ± 0.19 %, exchange acidity was 0.21 ± 0.13 meq/100 g, and Fe was 813 ± 21.44 mg/kg. The conductivity of electricity was 301.913 ± 1.23 s/cm.

ZnNPs biosynthesised using pawpaw, sorrel, and neem leaf extracts are shown to absorb between 300 and 600 nm in Figure 1. The fourth hour following production occurred when Pawpaw-ZnNP and Neem-ZnNP showed the highest peak activity, whereas the third hour was when Sorrel-ZnNP showed the highest peak. CuNPs biosynthesised using pawpaw, sorrel, and neem leaf extracts are shown to absorb between 300 and 600 nm in Figure 2. The fourth hour following production was when Pawpaw-CuNP and Sorrel-CuNP activity peaked, whereas the third hour was when Neem-CuNP activity peaked. The absorption of MnNPs biosynthesised using leaf extracts from pawpaw, sorrel, and neem, measured between 300 and 600 nm, is depicted in Figure 3. The fourth hour following production saw the highest peak activity for pawpaw-MnNP, sorrel-MnNP, and neem-MnNP.

Table 1.

Physical and chemical properties of soils used in the study. These are background mean concentrations

Parameters	Ferruginous soil (n = 3)
pH	6.47 ± 0.21
Electric conductivity (µs/cm)	301.91 ± 31.23
Total organic carbon (%)	0.41 ± 0.11
Total Nitrogen (%)	0.64± 0.19
Exchangeable acidity (meq/100 g)	0.21 ± 0.13
Clay (%)	7.92 ± 1.42
Silt (%)	5.74 ± 2.76
Sand (%)	85.10 ± 18.24
Fe (mg/kg)	813 ± 21.44

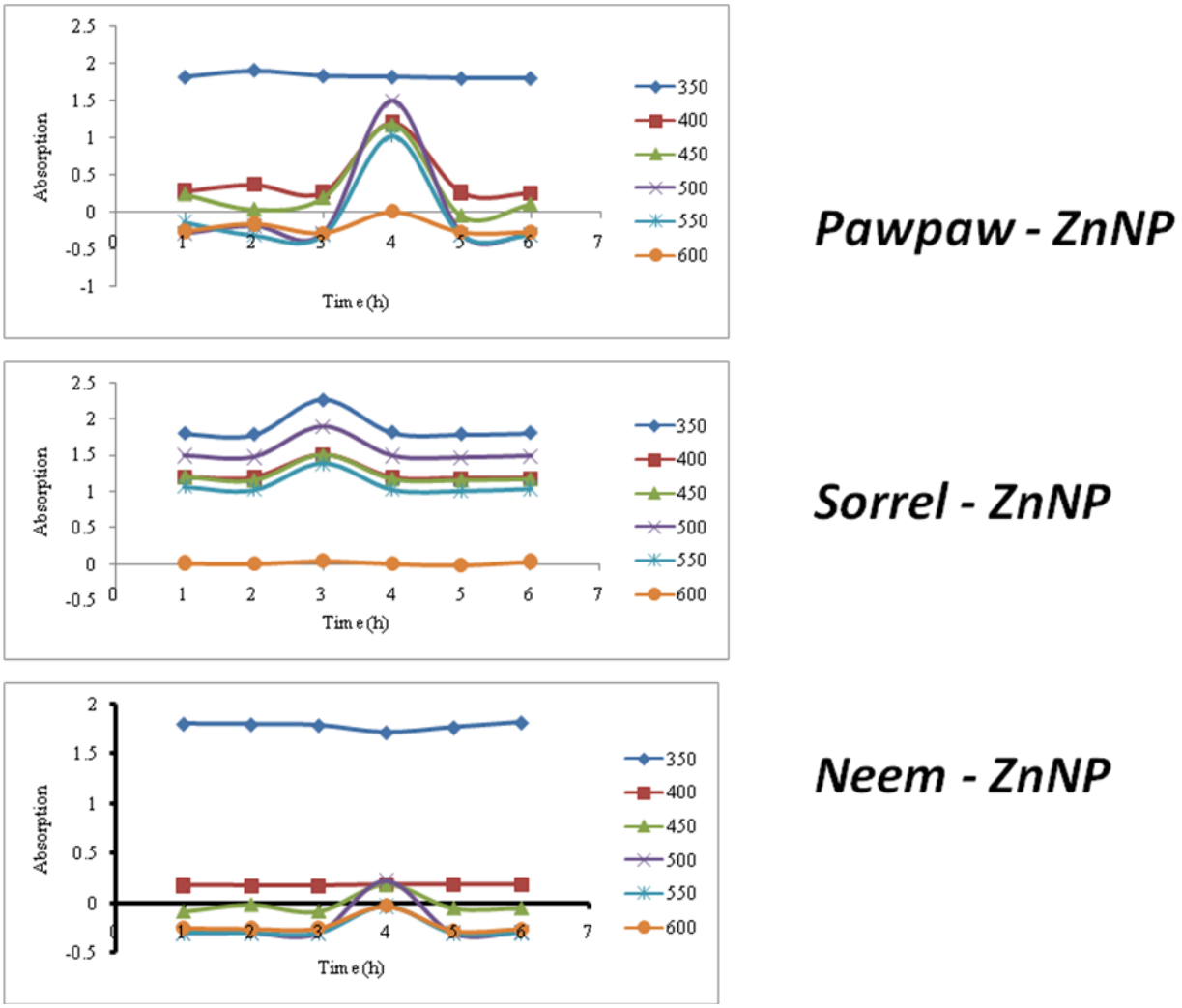

Fig. 1. Absorption of Zinc nanoparticles biosynthesized with pawpaw, sorrel and neem leaf extracts and measured at wavelengths of 300 - 600 nm.

Figure 2 depicts the overall grain yield brought about by the use of nanoparticles during the evaluated period of growth. In contrast to the control, where grain weight was less than 200 g, it was shown that ZZ3 (50 %) plants, PZ1 (5 %) plants, and NZ1 (5 %) plants recorded weights of roughly 800 g per plant. In general, the treatments varied significantly in the weight of grain produced per plant, varied significantly with treatments.

The grain yield as a result of nanointervention during the evaluated period of growth is shown in Figure 3. Approximately 900 grains per plant were reported in neem extracts alone, neem-mediated ZnNPs, and pawpaw-mediated ZnNPs, all at 5 % concentration. In contrast to the control, ZnNPs caused by red sorrel recorded about 900 grains per plant.

Table 2 presents rice yield parameters brought about by the use of nanoparticles during the investigated period of growth. In general, there were significant differences ($p>0.05$) in yield parameters between the treatments. In comparison to the control, PCI (40) had the most panicle branch and ZZ1 (3) had the lowest (20). The length of the grain, which runs from 0.7 to 0.8 cm, and its diameter, which is between 0.4 and 0.8 cm, were not significantly different ($p<0.05$) across the treatments. The grain ranged in weight from 0.1 to 1.8 g/DW. PZ1 (136) had the most grains, followed by NZ1 (134), ZZ2, PZ2, and ZZ3 (133), whereas PC2 (22) had the fewest grains in comparison to the control, which had 80. Panicle weight varied from 0.2 to 1.3 g/DW. When compared to the control, the maximum panicle length was observed in NM2 (25 %) and the lowest in NZ3 (50 %)

Table 3 compares the yield increase caused by synthesised nanoparticles mediated by red sorrel extracts to plants subjected to just extracts. The grain was reported to vary in length from 14.3 to 28.6. It was reported that the diameter ranged from 16.7 to 33.3. The largest number of grains was reported in ZZ2 (141.8), followed by ZZ3 (90.9), and the lowest number was observed in ZC3 (-56.4) when compared to the control (45.5). The maximum weight of grain per plant was ZZ3 (1613.3), followed by ZZ2 (535.5), and the lowest weight was ZC1 (-28.7) compared to the control (123.8). There was a significant difference in the number of grains produced by each plant. ZZ2 (302.9) had the most grains per plant, followed by ZZ3 (218.2), and ZM3 had the fewest (-39.4). The number of leaves differed significantly.

Table 4 compares the yield increase caused by synthesised nanoparticles mediated by pawpaw leaf extracts to plants only exposed to extracts. The diameter of the grain varied from 25 to 50, but there was no significant variability in the length of the grain.

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The most grains were noticed in PZ1 (338.7), followed closely by PZ2 (306.5), while the fewest were observed in PC2 (-29) when compared to the control (158.1). The weight of the grains did differ significantly, though. In comparison to the control, PZ1 had the largest grain weight (5099.4), followed by PZ2 (1480.6), and PC3 (945.4). In comparison to the control, PZ1 (629.2) and PZ2 (576), respectively, had the highest recorded numbers of grains per plant. The number of leaves and their length varied significantly.

Table 5 compares the yield increase brought on by synthesised nanoparticles mediated by neem leaf extracts to plants only exposed to extracts. The length and diameter of the grains did not significantly change across the treatments. In comparison to control, NZ1 (5 %) had the maximum number of grains (139.3) and NC1 (5 %) had the lowest (-39.3). The weight and number of grains produced per plant were highest in NZ1 (5 %), followed by NZ2 (25 %) and NZ3 (5 0%) but lowest in NM2 (25 %) as compared to control.

Table 6 shows the number of rice roots occasioned by nanoparticle intervention during the investigated growth period. The highest dried root weight was 14.0 g (PC3) and the lowest weight was 1.5 g (ZZ1). The highest number of root was recorded in ZC3 (108) followed by PC3 and NEXTR (100) while the lowest was recorded in PZ3 (28). The highest root depth was recorded in control (44) while the lowest was recorded in ZM2 (15).

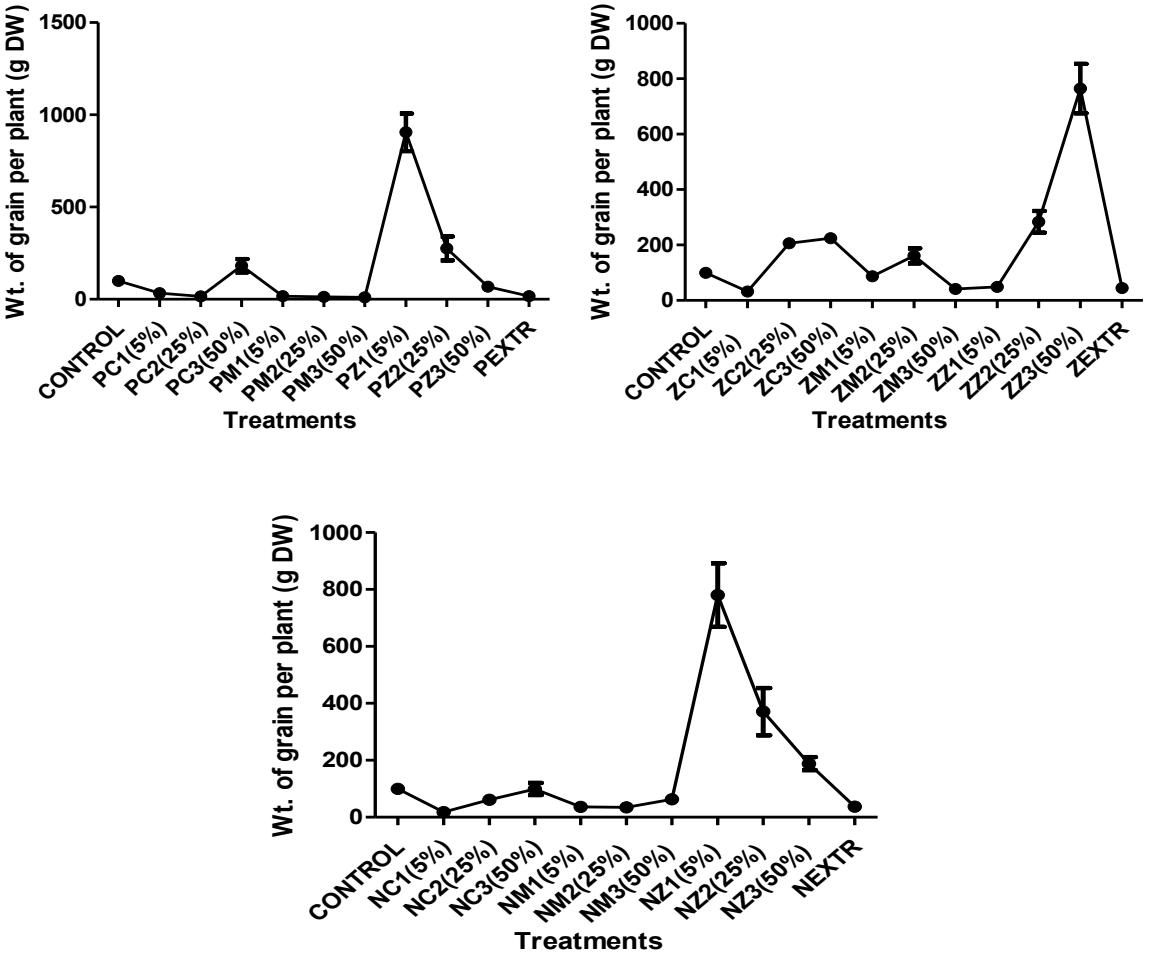

Fig. 2. Total grain yield occasioned by application of nanoparticle over assessed period of growth.
KEY: ZC1=5 % *Hibiscus sabdariffa*-mediated CuNPs, ZC2=25 % *Hibiscus sabdariffa*-mediated CuNPs, ZC3=50 % *Hibiscus sabdariffa*-mediated CuNPs, ZM1=5 % *Hibiscus sabdariffa*-mediated MnNPs, ZM2=25 % *Hibiscus sabdariffa*-mediated MnNPs, ZM3=50 % *Hibiscus sabdariffa*-mediated MnNPs, ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PC1=5 % *Carica papaya*-mediated CuNPs, PC2=25 % *Carica papaya*-mediated CuNPs, PC3=50 % *Carica papaya*-mediated CuNPs, PM1=5 % *Carica papaya*-mediated MnNPs, PM2=25 % *Carica papaya*-mediated MnNPs, PM3=50 % *Carica papaya*-mediated MnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, NC1=5 % *Azadiracta indica*-mediated CuNPs, NC2=25 % *Azadiracta indica*-mediated CuNPs, NC3=50 % *Azadiracta indica*-mediated CuNPs, NM1=5 % *Azadiracta indica*-mediated MnNPs NM2=25 % *Azadiracta indica*-mediated MnNPs, NM3=50 % *Azadiracta indica*-mediated MnNPs, NZ1=5 % *Azadiracta indica*-mediated ZnNPs, NZ2=25 % *Azadiracta indica*-mediated ZnNPs, NZ3=50 % *Azadiracta indica*-mediated ZnNPs, ZEXTR= *Hibiscus sabdariffa* extracts only, PEXTR=*Carica papaya* extract only, NEXTR= *Azadiracta indica* extract only, Control=No application of NPs.

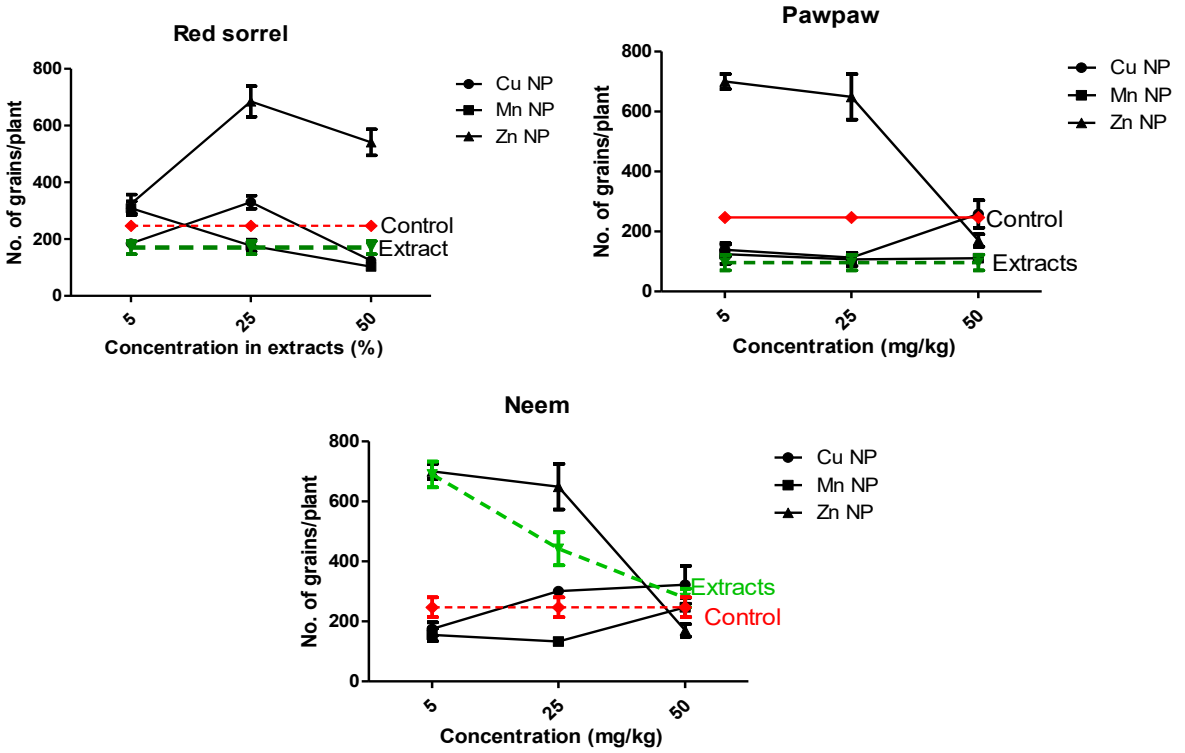

Fig. 3. Grain yield gain due to application of nanoparticle over assessed period of growth.

Table 2.

Yield parameters of rice occasioned by application of nanoparticle over assessed period of growth

Treatment	Panicle branch	Len. of grain (cm)	Diameter of grain (cm)	Weight of grain (g/DW)	No of grains	Weight of panicle (g/DW)	Length of panicle (cm)
ZC1(5%)	20.0	0.8	0.6	0.2	36.0	0.3	16.5
ZC2(25%)	12.0	0.9	0.6	0.6	64.0	1.1	22.2
ZC3(50%)	17.0	0.8	0.6	1.8	24.0	0.4	13.2
PC1(5%)	40.0	0.8	0.6	0.2	27.0	0.4	12.9
PC2(25%)	27.0	0.8	0.5	0.1	22.0	0.2	10.5
PC3(50%)	15.0	0.8	0.8	0.7	50.0	1.2	20.6
NC1(5%)	15.0	0.8	0.6	0.1	34.0	0.3	21.5
NC2(25%)	25.0	0.8	0.6	0.2	65.0	0.3	21.9
NC3(50%)	15.0	0.8	0.7	0.3	68.0	0.3	15.2
ZM1(5%)	17.0	0.7	0.8	0.3	75.0	0.3	20.4
ZM2(25%)	13.0	0.8	0.8	0.9	43.0	0.2	16.5
ZM3(50%)	7.0	0.5	0.7	0.4	25.0	0.2	19.3
PM1(5%)	10.0	0.7	0.8	0.1	30.0	0.3	20.5
PM2(25%)	6.0	0.8	0.8	0.1	26.0	0.3	22.8
PM3(50%)	8.0	0.8	0.8	0.1	27.0	0.2	15.0
NM1(5%)	20.0	0.7	0.8	0.2	50.0	0.3	21.5
NM2(25%)	16.0	0.7	0.8	0.2	43.0	0.2	23.3
NM3(50%)	21.0	0.7	0.6	0.3	60.0	1.0	19.3
ZZ1(5%)	3.0	0.7	0.4	0.2	63.0	0.6	8.0
ZZ2(25%)	9.0	0.7	0.7	0.4	133.0	1.3	12.2
ZZ3(50%)	10.0	0.7	0.7	1.4	105.0	1.0	13.2
PZ1(5%)	16.0	0.8	0.8	1.3	136.0	1.3	13.1
PZ2(25%)	20.0	0.7	0.8	0.4	126.0	1.2	11.5
PZ3(50%)	6.0	0.8	0.5	0.4	33.0	0.3	14.4
NZ1(5%)	11.0	0.8	0.7	1.1	134.0	1.3	14.1
NZ2(25%)	13.0	0.7	0.6	0.8	87.0	0.7	10.6
NZ3(50%)	9.0	0.8	0.5	0.7	54.0	0.6	6.3
ZEXTR	20.0	0.7	0.6	0.3	55.0	0.2	20.0
PEXTR	25.0	0.7	0.4	0.2	31.0	0.2	10.1
NEXTR	25.0	0.7	0.6	0.2	56.0	0.3	11.5
CONTROL	20.0	0.8	0.6	0.4	80.0	0.5	30.0

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Treatment	Panicle branch	Len. of grain (cm)	Diameter of grain (cm)	Weight of grain (g/DW)	No of grains	Weight of panicle (g/DW)	Length of panicle (cm)
F-statistic	1.009	0.741	1.548	1.345	2.174	9.641	6.278
p-value	0.031	0.396	0.223	0.256	0.151	0.004	0.018
LSD(0.05)	4.7	0.2	0.2	0.1	18.2	0.2	5.3

KEY: ZC1=5 % *Hibiscus sabdariffa*-mediated CuNPs, ZC2=25 % *Hibiscus sabdariffa*-mediated CuNPs, ZC3=50 % *Hibiscus sabdariffa*-mediated CuNPs, ZM1=5 % *Hibiscus sabdariffa*-mediated MnNPs, ZM2=25 % *Hibiscus sabdariffa*-mediated MnNPs, ZM3=50 % *Hibiscus sabdariffa*-mediated MnNPs, ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PC1=5 % *Carica papaya*-mediated CuNPs, PC2=25 % *Carica papaya*-mediated CuNPs, PC3=50 % *Carica papaya*-mediated CuNPs, PM1=5 % *Carica papaya*-mediated MnNPs, PM2=25 % *Carica papaya*-mediated MnNPs, PM3=50 % *Carica papaya*-mediated MnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, NC1=5 % *Azadiracta indica*-mediated CuNPs, NC2=25 % *Azadiracta indica*-mediated CuNPs, NC3=50 % *Azadiracta indica*-mediated CuNPs, NM1=5 % *Azadiracta indica*-mediated MnNPs NM2=25 % *Azadiracta indica*-mediated MnNPs, NM3=50 % *Azadiracta indica*-mediated MnNPs, NZ1=5 % *Azadiracta indica*-mediated ZnNPs, NZ2=25 % *Azadiracta indica*-mediated ZnNPs, NZ3=50 % *Azadiracta indica*-mediated ZnNPs, ZEXTR= *Hibiscus sabdariffa* extracts only, PEXTR=*Carica papaya* extract only, NEXTR= *Azadiracta indica* extract only, Control=No application of NPs

Table 3.

Yield gain due to red sorrel extracts-mediated synthesized nanoparticles compared to plants exposed to only extracts

Treatment	Length of grain	Diameter of grain	No. of grains	Weight of grain per plant	No of grains/plant	Leaf length (cm)	No. of leaf
Control	14.3	0	45.5	123.8	45.3	12.2	27.8
ZC1(5%)	14.3	0	-34.5	-28.7	8.8	69.4	88.9
ZC2(25%)	28.6	0	16.4	362.5	94.1	116.7	83.3
ZC3(50%)	14.3	0	-56.4	403.5	-27.1	100.6	55.6
ZM1(5%)	0	33.3	36.4	95.8	81.8	100.6	27.8
ZM2(25%)	14.3	33.3	-21.8	260.8	4.1	91.1	77.8
ZM3(50%)	-28.6	16.7	-54.5	-6.8	-39.4	127.8	72.2
ZZ1(5%)	0	-33.3	14.5	10.1	90.6	2.8	-22.2
ZZ2(25%)	0	16.7	141.8	535.5	302.9	17.2	27.8
ZZ3(50%)	0	16.7	90.9	1613.3	218.2	13.3	33.3
ZEXTR	0	0	0	0	0		0
Δ Percentage gain (+) or loss (-) compared to when seeds were treated with only plant extracts							

KEY: ZC1=5 % *Hibiscus sabdariffa*-mediated CuNPs, ZC2=25 % *Hibiscus sabdariffa*-mediated CuNPs, ZC3=50 % *Hibiscus sabdariffa*-mediated CuNPs, ZM1=5 % *Hibiscus sabdariffa*-mediated MnNPs, ZM2=25 % *Hibiscus sabdariffa*-mediated MnNPs, ZM3=50 % *Hibiscus sabdariffa*-mediated MnNPs, ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, ZEXTR= *Hibiscus sabdariffa* extracts only, , Control=No application of NPs.

Table 4.

Yield gain due to pawpaw leaf extracts-mediated synthesized nanoparticles compared to plants exposed to only extracts

Treatment	Length of grain	Diameter of grain	No. of grains	Weight of grain per plant	No of grains/plant	Leaf length (cm)	No. of leaf
Control	14.3	50	158.1	473.5	157.3	30.3	27.8
PC1(5%)	14.3	50	-12.9	93.5	44.8	108.4	61.1
PC2(25%)	14.3	25	-29	-8	17.7	107.7	33.3
PC3(50%)	14.3	100	61.3	945.4	168.8	94.2	38.9
PM1(5%)	0	100	-3.2	0.4	29.2	145.2	66.7
PM2(25%)	14.3	100	-16.1	-25.4	11.5	160	88.9
PM3(50%)	14.3	100	-12.9	-35.5	15.6	101.3	72.2
PZ1(5%)	14.3	100	338.7	5099.4	629.2	49.7	50
PZ2(25%)	0	100	306.5	1480.6	576	54.8	44.4
PZ3(50%)	14.3	25	6.5	294.3	77.1	36.8	22.2
PEXTR	0	0	0	0	0	0	0

Δ Percentage gain (+) or loss (-) compared to when seeds were treated with only plant extracts

KEY: PC1=5 % *Carica papaya*-mediated CuNPs, PC2=25 % *Carica papaya*-mediated CuNPs, PC3=50 % *Carica papaya*-mediated CuNPs, PM1=5 % *Carica papaya*-mediated MnNPs, PM2=25 % *Carica papaya*-mediated MnNPs, PM3=50 % *Carica papaya*-mediated MnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, PEXTR=*Carica papaya* extract only, Control=No application of NPs

Table 5.

Yield gain due to neem leaf extracts-mediated synthesized nanoparticles compared to plants exposed to only extracts

Treatment	Length of grain	Diameter of grain	No. of grains	Weight of grain per plant	No of grains/plant	Leaf length (cm)	No. of leaf
Control	14.3	0	42.9	172.1	42.8	16.8	21.1
NC1(5%)	14.3	0	-39.3	-51.8	1.2	113.9	52.6
NC2(25%)	14.3	0	16.1	66.9	74	116.2	73.7
NC3(50%)	14.3	16.7	21.4	169.9	86.1	126.6	57.9
NM1(5%)	0	33.3	-10.7	-1	-10.4	71.1	57.9
NM2(25%)	0	33.3	-23.2	-6.7	-23.1	79.8	73.7
NM3(50%)	0	0	7.1	70.5	42.8	39.9	47.4
NZ1(5%)	14.3	16.7	139.3	2027.1	298.8	41	0
NZ2(25%)	0	0	55.4	911.1	155.5	12.7	31.6
NZ3(50%)	14.3	-16.7	-3.6	412.8	60.7	27.7	-10.5
NEXTR	0	0	0	0	0	0	0

Δ Percentage gain (+) or loss (-) compared to when seeds were treated with only plant extracts

KEY: NC1=5 % *Azadiracta indica*-mediated CuNPs, NC2=25 % *Azadiracta indica*-mediated CuNPs, NC3=50 % *Azadiracta indica*-mediated CuNPs, NM1=5 % *Azadiracta indica*-mediated MnNPs NM2=25 % *Azadiracta indica*-mediated MnNPs, NM3=50 % *Azadiracta indica*-mediated MnNPs, NZ1=5 % *Azadiracta indica*-mediated ZnNPs, NZ2=25 % *Azadiracta indica*-mediated ZnNPs, NZ3=50 % *Azadiracta indica*-mediated ZnNPs, NEXTR= *Azadiracta indica* extract only, Control=No application of NPs.

Table 6

Root parameters of rice occasioned by application of nanoparticle over assessed period of growth

Treatment	Weight dried roots (g)	No of roots	Root depth (cm)
ZC1(5%)	2.7	53.0	19.0
ZC2(25%)	4.6	86.0	25.9
ZC3(50%)	2.6	108.0	28.0
PC1(5%)	1.8	50.0	29.0
PC2(25%)	5.4	31.0	28.0
PC3(50%)	14.0	100.0	30.0
NC1(5%)	9.9	60.0	26.0
NC2(25%)	6.0	80.0	29.0
NC3(50%)	6.8	76.0	30.0
ZM1(5%)	1.8	58.0	28.5
ZM2(25%)	4.9	64.0	15.0
ZM3(50%)	3.3	43.0	27.0
PM1(5%)	3.9	52.0	25.0
PM2(25%)	3.1	42.0	29.0
PM3(50%)	4.2	30.0	18.0
NM1(5%)	2.4	68.0	28.0
NM2(25%)	4.6	49.0	28.0
NM3(50%)	4.7	59.0	27.0
ZZ1(5%)	1.5	44.0	16.0
ZZ2(25%)	3.3	52.0	19.0
ZZ3(50%)	1.8	31.0	18.0
PZ1(5%)	2.0	43.0	19.0
PZ2(25%)	4.2	34.0	18.0
PZ3(50%)	1.7	28.0	20.0
NZ1(5%)	4.6	54.0	16.0
NZ2(25%)	4.0	35.0	19.0
NZ3(50%)	4.4	47.0	20.0
ZEXTR	6.7	92.0	20.0
PEXTR	6.2	47.0	16.2
NEXTR	4.1	100.0	29.8
Control	5.1	86.0	44.0
F-statistic	1.191	2.398	6.047
p-value	0.284	0.132	0.020
LSD(0.05)	1.2	15.7	6.5

KEY: ZC1=5 % *Hibiscus sabdariffa*-mediated CuNPs, ZC2=25 % *Hibiscus sabdariffa*-mediated CuNPs, ZC3=50 % *Hibiscus sabdariffa*-mediated CuNPs, ZM1=5 % *Hibiscus sabdariffa*-mediated MnNPs, ZM2=25 % *Hibiscus sabdariffa*-mediated MnNPs, ZM3=50 % *Hibiscus sabdariffa*-mediated MnNPs, ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs,

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ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PC1=5 % *Carica papaya*-mediated CuNPs, PC2=25 % *Carica papaya*-mediated CuNPs, PC3=50 % *Carica papaya*-mediated CuNPs, PM1=5 % *Carica papaya*-mediated MnNPs, PM2=25 % *Carica papaya*-mediated MnNPs, PM3=50 % *Carica papaya*-mediated MnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, NC1=5 % *Azadiracta indica*-mediated CuNPs, NC2=25 % *Azadiracta indica*-mediated CuNPs, NC3=50 % *Azadiracta indica*-mediated CuNPs, NM1=5 % *Azadiracta indica*-mediated MnNPs NM2=25 % *Azadiracta indica*-mediated MnNPs, NM3=50 % *Azadiracta indica*-mediated MnNPs, NZ1=5 % *Azadiracta indica*-mediated ZnNPs, NZ2=25 % *Azadiracta indica*-mediated ZnNPs, NZ3=50 % *Azadiracta indica*-mediated ZnNPs, ZEXTR= *Hibiscus sabdariffa* extracts only, PEXTR=*Carica papaya* extract only, NEXTR= *Azadiracta indica* extract only, Control=No application of NPs.

At 11 weeks after planting, Table 7 presents the biochemical characteristics of rice exposed to biosynthesized nanoparticles. The chlorophyll levels in the leaves demonstrate that there were no significant differences in chlorophyll levels across treatments. The maximum concentration of chlorophyll a was found in PM3 (50 %) at 7.87 mg/L, while NC3 (50 %) had the lowest concentration at 1.4 mg/L. The maximum activity of chlorophyll b, however, was 10.63 mg/L, whereas the lowest was 1.69 mg/L in ZM1 (5 %). Lycopene levels in ferruginous soils were higher than the control. The lowest concentration was found in ZEXTR with 152 mg/L compared to control with 164 mg/L, while the highest concentration was observed in PM3 (50 %) with 257 mg/L. The amount of vitamin C in it was minimally significant. The NC3 (50 %) had the highest concentration (314.23 mg/dL), while the ZZ2 (25 %) had the lowest concentration (117.52 mg/dL, compared to 256.2 mg/dL for the control). The proline contents in the ZC2 (25 %), ZC3 (50 %), PC1 (5 %), PC2 (25 %), and PZ3 (50 %) treatments did not differ significantly from one another. Furthermore, there were no significant differences in the amounts of carotene.

As of 11 weeks after planting, Table 8 shows the nitrogen content of rice stands subjected to biosynthesized nanoparticles. Nitrogen levels in leaves ranged from 0.32 to 1.02 %, indicating the minimum and maximum limits. In stem, NM3 was found to have a higher nitrogen concentration with 1.42 % compared to the control with 0.31 %. The concentration in the roots ranged from 0.28 % in ZC3 to 0.58 % in PZ3.

Table 7.

Foliar biochemical characteristics of rice exposed to biosynthesized nanoparticles (Assay was conducted at 11 weeks after sowing)

Treatment	Chlorophyll-a (mg/L)	Chlorophyll-b (mg/L)	Lycopene (mg/L)	Vitamin C (mg/dL)	Proline (mg/L)	Carotene (mg/g)
ZC1(5%)	4.34	3.6	185	200.64	4.15	453.7
ZC2(25%)	3.52	3.88	182	198.45	3.41	361.4
ZC3(50%)	2.8	3.46	174	218.52	2.27	335.8
PC1(5%)	4.4	6.63	198	265.33	2.35	534.2
PC2(25%)	6.03	6.63	200	224.82	3.18	457.6
PC3(50%)	2.09	2.38	197	225.91	3.58	548
NC1(5%)	4.65	4.48	197	221.53	4.51	551.9
NC2(25%)	3.31	5.51	187	200.36	3.52	302.5
NC3(50%)	1.4	2.08	164	314.23	2.72	351.6
ZM1(5%)	1.55	1.69	173	265.69	4.59	223.9
ZM2(25%)	3.17	3.57	170	285.04	4.5	278.9
ZM3(50%)	2.64	4.3	161	240.88	3.3	361.4
PM1(5%)	3.51	3.91	181	119.34	4.49	447.8
PM2(25%)	5.27	7.45	193	179.56	5.25	523.4
PM3(50%)	7.87	10.63	257	141.24	5.21	492
NM1(5%)	3.88	5.49	159	175.18	5.12	310.3
NM2(25%)	3.29	4.57	161	212.04	5.55	186.6
NM3(50%)	3.73	2.99	189	155.11	3.49	284.8
ZZ1(5%)	4.9	4.36	164	261.31	4.63	355.5
ZZ2(25%)	1.78	2.68	183	117.52	5.17	606.9
ZZ3(50%)	2.04	2.01	159	166.42	4.96	377.1
PZ1(5%)	4.21	3.13	236	175.18	5.23	519.5
PZ2(25%)	3.83	3.92	197	79.56	3.69	502.8
PZ3(50%)	3.01	4.032	232	124.45	3.22	612.8
NZ1(5%)	2.73	3.84	211	278.1	5.08	923.1
NZ2(25%)	2.25	3.77	172	217.52	4.02	225.9
NZ3(50%)	2.04	2.01	162	152.19	4.31	210.1
ZEXTR	1.89	2.11	152	160.23	4.32	369.2
PEXTR	2.64	3.64	254	212.6	3.87	251.4

Treatment	Chlorophyll-a (mg/L)	Chlorophyll-b (mg/L)	Lycopene (mg/L)	Vitamin C (mg/dL)	Proline (mg/L)	Carotene (mg/g)
NEXTR	4.87	7.43	162	206.2	5.27	436
CONTROL	2.34	2.42	164	256.2	4.84	524.4
F-statistic	2.552	1.673	0.645	1.411	5.3	1.167
p-value	0.121	0.206	0.429	0.245	0.029	0.289
LSD (0,05)	1.56598	1.68602	65.22	98.23	1.498	153.2

KEY: ZC1=5 % *Hibiscus sabdariffa*-mediated CuNPs, ZC2=25 % *Hibiscus sabdariffa*-mediated CuNPs, ZC3=50 % *Hibiscus sabdariffa*-mediated CuNPs, ZM1=5 % *Hibiscus sabdariffa*-mediated MnNPs, ZM2=25 % *Hibiscus sabdariffa*-mediated MnNPs, ZM3=50 % *Hibiscus sabdariffa*-mediated MnNPs, ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PC1=5 % *Carica papaya*-mediated CuNPs, PC2=25 % *Carica papaya*-mediated CuNPs, PC3=50 % *Carica papaya*-mediated CuNPs, PM1=5 % *Carica papaya*-mediated MnNPs, PM2=25 % *Carica papaya*-mediated MnNPs, PM3=50 % *Carica papaya*-mediated MnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, NC1=5 % *Azadiracta indica*-mediated CuNPs, NC2=25 % *Azadiracta indica*-mediated CuNPs, NC3=50 % *Azadiracta indica*-mediated CuNPs, NM1=5 % *Azadiracta indica*-mediated MnNPs NM2=25 % *Azadiracta indica*-mediated MnNPs, NM3=50 % *Azadiracta indica*-mediated MnNPs, NZ1=5 % *Azadiracta indica*-mediated ZnNPs, NZ2=25 % *Azadiracta indica*-mediated ZnNPs, NZ3=50 % *Azadiracta indica*-mediated ZnNPs, ZEXTR= *Hibiscus sabdariffa* extracts only, PEXTR=*Carica papaya* extract only, NEXTR= *Azadiracta indica* extract only, Control=No application of NPs.

Table 8.

Nitrogen concentration of rice stands exposed to biosynthesized nanoparticles (Assay was conducted at 11 weeks after sowing)

Treatment	Nitrogen (leaf) (%)	Nitrogen (Stem) (%)	Nitrogen (Root) (%)
ZC1(5%)	0.43	0.32	0.34
ZC2(25%)	0.41	0.26	0.4
ZC3(50%)	0.32	0.21	0.28
PC1(5%)	0.35	0.48	0.61
PC2(25%)	0.35	0.34	0.31
PC3(50%)	0.41	0.39	0.47
NC1(5%)	0.53	0.35	0.35
NC2(25%)	0.55	0.39	0.4
NC3(50%)	0.55	0.37	0.39
ZM1(5%)	0.55	0.76	0.33
ZM2(25%)	0.63	0.41	0.47
ZM3(50%)	0.38	0.6	0.38
PM1(5%)	0.38	0.41	0.35
PM2(25%)	0.42	0.33	0.4
PM3(50%)	0.36	0.31	0.4
NM1(5%)	0.46	0.16	0.51
NM2(25%)	0.41	0.29	0.33
NM3(50%)	0.32	1.42	0.34
ZZ1(5%)	0.37	0.45	0.4
ZZ2(25%)	0.42	0.59	0.49
ZZ3(50%)	0.69	0.41	0.41
PZ1(5%)	0.38	0.36	0.4
PZ2(25%)	0.49	0.4	0.46
PZ3(50%)	0.56	0.44	0.58
NZ1(5%)	1.02	0.42	0.41
NZ2(25%)	0.55	1.05	0.39
NZ3(50%)	0.41	0.49	0.39
ZEXTR	0.44	0.46	0.4
PEXTR	0.42	0.38	0.49
NEXTR	0.47	0.39	0.41
CONTROL	0.38	0.31	0.15
F-statistics	2.195	0.239	0.320
p-value	0.149	0.629	0.576
LSD (0,05)	0.17	0.19	0.21

KEY: ZC1=5 % *Hibiscus sabdariffa*-mediated CuNPs, ZC2=25 % *Hibiscus sabdariffa*-mediated CuNPs, ZC3=50 % *Hibiscus sabdariffa*-mediated CuNPs, ZM1=5 % *Hibiscus sabdariffa*-mediated MnNPs, ZM2=25 % *Hibiscus sabdariffa*-mediated MnNPs, ZM3=50 % *Hibiscus sabdariffa*-mediated MnNPs, ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PC1=5 % *Carica papaya*-mediated CuNPs, PC2=25 % *Carica papaya*-mediated CuNPs, PC3=50 % *Carica papaya*-mediated CuNPs,

PM1=5 % *Carica papaya*-mediated MnNPs, PM2=25 % *Carica papaya*-mediated MnNPs, PM3=50 % *Carica papaya*-mediated MnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, NC1=5 % *Azadiracta indica*-mediated CuNPs, NC2=25 % *Azadiracta indica*-mediated CuNPs, NC3=50 % *Azadiracta indica*-mediated CuNPs, NM1=5 % *Azadiracta indica*-mediated MnNPs NM2=25 % *Azadiracta indica*-mediated MnNPs, NM3=50 % *Azadiracta indica*-mediated MnNPs, NZ1=5 % *Azadiracta indica*-mediated ZnNPs, NZ2=25 % *Azadiracta indica*-mediated ZnNPs, NZ3=50 % *Azadiracta indica*-mediated ZnNPs, ZEXTR= *Hibiscus sabdariffa* extracts only, PEXTR=*Carica papaya* extract only, NEXTR= *Azadiracta indica* extract only, Control=No application of NPs.

The Zn content of ZnNP-plant tissues is shown in Table 9. The amount of Zn was accumulating in leaves at higher percentages. Z1 (5 %) and Z3 (50 %) both had accumulation rates below 100 %. Except for treatments N2 and N3, which were found to be somewhat higher than the standard limit, the amount of Zn in the leaf was, however, within the standard limit of 60 mg/kg. P1, P3, N2, and N3 were found to have accumulation percentages that were more than 100 % in seeds. The seed's Zn content, however, was within the accepted level. Zn accumulation in P3, N2, and N3 was greater than 100 % in residual soil. Nevertheless, only N2 was found to be above the standard limit, with all other substances falling within the range. The residual soil's Zn content was within acceptable ranges, with the exception of P3, N2, and N3, which were said to be over acceptable ranges.

The concentration of Cu in the tissues of CuNP-plants is shown in Table 10. The proportion of Cu accumulation in the leaf was greater than 100 % in P1, P2, P3, and N3, but less than 100 % in the other treatments. The accumulation in the leaf, however, did not exceed the statutory limit of 40 mg/kg. In general, the percentage accumulation in the seed was less than 55 %, and the seed's level of copper was within acceptable bounds. In addition to exceeding the standard limit for Cu in the soil across all treatments, the percentage buildup in residual soil was above 500 %.

The amount of Mn in tissues of MnNP-plants is presented in Table 11. The concentrations of Mn detected in plant tissues were often far lower than the recommended limit of 500 mg/kg. However, the higher percentage accumulation was recorded in N3, with 103.7 % for leaf, 126.4 % (P2), 105.3 % (N1), and 123.9 % in N3 for residual soil.

Table 9.

Level of Zn (mg/kg) in tissues of ZnNP-plants

Treatment	Leaf	Seed	Residual soil	Δ accumulation (%)		
				Leaf	**Seed**	**Residual soil**
Control	18.5	8.4	31.4	-	-	-
Z1(5%)	32.4	15.3	47.7	*75.1*	*82.1*	*51.9*
Z2(25%)	38.6	11.1	49.7	*108.6*	*32.1*	*58.3*
Z3(50%)	34.5	15.3	49.8	*86.5*	*82.1*	*58.6*
P1(5%)	37.2	17.3	54.5	*101.1*	*106.0*	*73.6*
P2(25%)	39.4	11.3	50.7	*113.0*	*34.5*	*61.5*
P3(50%)	46.3	24.2	70.5	*150.3*	*188.1*	*124.5*
N1(5%)	37.4	15.4	52.8	*102.2*	*83.3*	*68.2*
N2(25%)	69.2	34.2	103.4	*274.1*	*307.1*	*229.3*
N3(50%)	62.8	28.4	71.2	*239.5*	*238.1*	*126.8*
Mean Square	465.394	147.454	955.77	-	-	-
F-statistics	5.673	4.151	4.907	-	-	-
p-value	0.022	0.048	0.032	-	-	-
LSD (0,05)	12.22	6.82	22.4	-	-	-
Standard (Veg.) = 60 mg/kg (WHO; codex alimentarius commission, 1991)						

Table 10.

Level of Cu (mg/kg) in tissues of CuNP- plants

Treatment	Leaf	Seed	Residual soil	Δ accumulation (%)		
				Leaf	**Seed**	**Residual soil**
Control	8.5	7.3	18.5	-	-	-
Z1(5%)	10.0	6.2	171.84	*17.6*	*-15.1*	*828.9*
Z2(25%)	15.2	7.3	154.88	*78.8*	*0.0*	*737.2*
Z3(50%)	16.2	9.2	186.56	*90.6*	*26.0*	*908.4*
P1(5%)	17.5	7.4	193.28	*105.9*	*1.4*	*944.8*
P2(25%)	19.3	11.2	126.08	*127.1*	*53.4*	*581.5*
P3(50%)	21.3	8.2	180.16	*150.6*	*12.3*	*873.8*
N1(5%)	13.5	6.9	123.84	*58.8*	*-5.5*	*569.4*
N2(25%)	11.4	6.4	131.84	*34.1*	*-12.3*	*612.6*
N3(50%)	18.4	7.6	227.84	*116.5*	*4.1*	*1131.6*
Mean Square	63.043	2.312	16202.7	-	-	-
F-statistics	8.793	1.07	13.305	-	-	-

Treatment	Leaf	Seed	Residual soil	Δ accumulation (%)		
				Leaf	**Seed**	**Residual soil**
p-value	0.007	0.415	0.002	-	-	-
LSD (0,05)	5.6	3.8	46.41	-	-	-

Standard = 40 mg/kg (WHO/FAO (FAO/ WHO, codex general standard for contamination and toxin in foods, 1996)

Δacc (%) Percentage increase (+) or decrease (-) in nutrient concentration compared to control

Table 11.

Level of Mn (mg/kg) in tissues of MnNP- plants

Treatment	Leaf	Seed	Residual soil	Δ accumulation (%)		
				Leaf	**Seed**	**Residual soil**
Control	13.4	6.7	28.4	-	-	-
Z1(5%)	11.3	8.3	36.7	*-15.7*	*23.9*	*29.2*
Z2(25%)	12.3	5.7	34.2	*-8.2*	*-14.9*	*20.4*
Z3(50%)	19	9.6	48.3	*41.8*	*43.3*	*70.1*
P1(5%)	13.2	7.2	33.5	*-1.5*	*7.5*	*18.0*
P2(25%)	10.3	6.7	64.3	*-23.1*	*0.0*	*126.4*
P3(50%)	19.5	10.4	49.5	*45.5*	*55.2*	*74.3*
N1(5%)	11.3	8.4	58.3	*-15.7*	*25.4*	*105.3*
N2(25%)	16.4	5.2	46.2	*22.4*	*-22.4*	*62.7*
N3(50%)	27.3	9.4	63.6	*103.7*	*40.3*	*123.9*
Mean Square	12.376	0.167	469.5	-	-	-
F-statistics	0.418	0.046	5.01	-	-	-
p-value	0.745	0.986	0.03	-	-	-
LSD (0,05)	7.8	3.1	18.2	-	-	-

Standard =500 mg/kg (WHO (Codex Alimentarius Commission, Joint FAO/WHO, 2001 and codex alimentarius commission, 1994)

Δacc (%) Percentage increase (+) or decrease (-) in nutrient concentration compared to control

DISCUSSION

In this study, the use of only neem extracts and biosynthesized CuNPs greatly increased the quantity of roots. This finding contradicts that of El-temash and Joner (2012) study, which found that AgNPs reduced the amount of ryegrass roots and impeded seed germination.

According to this study, Zn may have a considerably more significant impact than other treatments on the nitrogen content of rice stands subjected to biosynthesized NPs, increasing it to 1.02 %. In plant tissue, the addition of Zn increased the accumulation of amino acids and nitrogen metabolism (Sudha and Stalin, 2015).

The administration of NPs did not significantly alter the vitamin C content in this investigation, which may indicate that the application of NPs causes additional physiological stress as seen by the vitamin C and proline contents.

The amount of yield obtained in this study is a result of the significant increase in grain production. Compared to controls, plants subjected to biosynthesized NPs showed a significant difference. The beneficial and complementary effects of iron and zinc, which by increasing Zn absorption would improve yields, may be related to the increases in grain yields with the application of Zn in this study. The synergistic interactions eventually boosted the absorption of these elements in the plant tissue (Ghasemi *et al*., 2014). It is indeed remarkable to note that the application of biosynthesized ZnNP via foliar spray through the use of pawpaw leaf extracts can increase grain yield and profitability in a variety of rice production systems. According to Saha *et al*. (2017), foliar zinc spray resulted in the maximum increase in zinc concentration in rice grains by resulting in optimal grain production, which supports the findings of the current study.

The results showed that exposure to various concentrations of biosynthesized NPs significantly increased the amounts of all photosynthetic pigments (lycopene, caretene, chlorophyll a, and b). The findings support Farghaly and Nafady (2015) and Latif *et al*. (2017) who reported that NPs greatly boosted photosynthesis. In addition, it was shown in this study that a larger concentration of photosynthetic pigments enhanced the rate of photosynthesis, which in turn boosted plant growth and rice grain weight. According to studies by Govorov and Carmeli (2017), metal nanoparticles can increase the effectiveness of photosynthetic systems' ability to produce chemical energy.

Micronutrients, including zinc, copper, and manganese, are crucial for plant development. Each micronutrient has a specific role to play in plant development. Zinc is crucial for the health of membranes and the functions of phytochromes. While manganese is needed for disease resistance, electron transport, and enzyme activation, copper is needed for growth (Hemalatha and Venkatesan, 2011). Mn over abundance in plant tissues can alter a variety of functions, including translocation, enzyme activity, and the use of other mineral elements (Ducic and Polle, 2007; Lei *et al*., 2007). This study findings showed that

at 11 weeks (harvest day), Mn uptake did not significantly differ between the treatments. The standard deviation for all treatments, including the control, was below 500 mg/kg, demonstrating that the soil Mn level is adequate for plant development. In contrast, only the N2 and N3 treatments showed noticeably different zinc uptake. This supports Tariq's (2007) findings, which similarly noted a considerable rise in Zn and Mn concentrations in plant leaves following foliar spraying. Also, the Cu concentration of the leaves reported in this study was below the acceptable limit of 40 mg/kg. Furthermore, Thennakoon *et al*. (2020) reported a similar result. With the right concentration, a copper nanoparticle application might enhance plant growth and development (Hafeez *et al*., 2015). Treatments with Cu levels below the threshold level performed poorly and produced lower yields. In this investigation, the amount of Zn, Cu, and Mn in the rice grain significantly decreased. This is in contrast to Zhang *et al*. (2021) report, which claimed an increase in rice grain.

CONCLUSIONS

This study has demonstrated that the application of biosynthesized nanoparticles (NPs), particularly zinc (ZnNPs) and copper (CuNPs), as well as neem extracts, can significantly influence various physiological and biochemical parameters of rice plants. Notably, neem extracts and CuNPs enhanced root growth, while biosynthesized ZnNPs had a substantial impact on nitrogen accumulation and photosynthetic pigment content, which are directly linked to improved grain yield. However, the observed decrease in Zn, Cu, and Mn concentrations in the harvested grains suggests that while NPs promote vegetative and reproductive growth, they may not necessarily enhance micronutrient accumulation in edible parts. This discrepancy highlights the need for further investigation into the long-term implications of NP applications on nutritional quality. Therefore, the study underscores the potential of green-synthesized NPs, especially ZnNPs delivered via pawpaw leaf extract, as sustainable agronomic tools for improving rice productivity while minimizing environmental and physiological stress.

AUTHORS CONTRIBUTIONS

*C*onceptualization: F.A.I. and B.I.; methodology, B.I.; data collection F.A.I.; data validation, F.A.I. and B.I.; data processing B.I.; writing—original draft preparation, F.A.I.; writing—review and editing, F.A.I. and B.I.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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