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COMPARATIVE ASSESSMENT OF BIOSYNTHESIZED Zn, Cu, AND Mn NANOPARTICLES ON GERMINATION AND EARLY GROWTH OF *Oryza sativa* IN FERRUGINOUS SOIL

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ABSTRACT: The need for sustainable farming practices to address environmental challenges like elevated soil metal levels, which can affect agricultural productivity, has been emphasized. This study investigates the biosynthesis of nanoparticles (ZnNPs, CuNPs, and MnNPs) using leaf extracts of *Carica papaya*, *Azadiracta indica*, and *Hibiscus sabdariffa*, and their effects on the growth of rice (*Oryza sativa*). The experiment was conducted at the Department of Plant Biology and Biotechnology Botanic Garden, University of Benin, Nigeria, using ferruginous soil from the same site. Nanoparticles were synthesized by mixing plant extracts with respective metal salt solutions under controlled conditions and characterized using UV-visible spectrophotometry. Rice plants were foliar sprayed with the biosynthesized nanoparticles at varying concentrations, and growth parameters such as plant height, leaf length, and number of leaves was monitored over 11 weeks. Soil analysis showed near-neutral pH (6.47) and a sand-dominated texture. Nanoparticles demonstrated distinct absorption peaks, with the highest activity observed at 4 hours post-synthesis. The results revealed significant enhancement in growth compared to the control, with CuNPs showing the greatest increase in plant height and leaf length at a 25 % concentration. ZnNPs and MnNPs showed more modest improvements, with the 25 % concentration of ZnNPs yielding the highest leaf length. This study concludes that biosynthesized nanoparticles positively influenced rice growth, suggesting their potential as natural growth enhancers in agriculture. Future research should explore the mechanisms of nanoparticle uptake and their long-term effects on soil and plant health.

Keywords: biosynthesis, nanoparticles, rice growth, nanoparticle concentration, plant growth enhancers.

INTRODUCTION

The global pursuit of food security has emphasized the necessity for sustainable agricultural practices, especially in developing countries where population growth and urbanization intensify food demand (Igiebor *et al*., 2019). However, environmental challenges such as elevated levels of heavy metals in soils resulting from anthropogenic or natural activities pose significant threats to agricultural productivity (Osawaru *et al*., 2013a,b; Ikhajiagbe and Ogwu, 2020).

Rice (*Oryza sativa*), identified by the Food and Agriculture Organization (FAO, 2003) as a staple for over 50% of the world's population, ranks third in global crop production after maize and wheat. It provides vital nutrients, including carbohydrates, vitamins, and minerals, contributing significantly to global caloric intake. Annual per capita rice consumption ranges from 100 kg to 240 kg globally (Bruinsma, 2003). As its significance grows, FAO (2001) projected global rice production to increase from 586 million metric tonnes (MMT) to 756 MMT by 2030 to meet rising demand.

In Nigeria, rice is both a staple food and a major cash crop. Domestic consumption has surged due to evolving dietary preferences, with a growth rate of approximately 10 % per annum (Akande, 2003). Once trailing Egypt and Madagascar in rice output, Nigeria is now the leading producer in Africa (Ekugbe, 2021). After a peak production of 3.4 million tonnes in 1990, rice output fluctuated, but by 2006 reached 3.8 million tonnes. However, demand consistently outpaced production, prompting heavy reliance on imports. This trend shifted when the government implemented border closures and policies promoting the consumption of local rice, leading to a production increase to 8.44 million tonnes in 2019 (Igiebor *et al*., 2019). By 2021, Nigeria's rice output had grown to nine million tonnes (Orjiude, 2022).Given the anticipated rise in rice consumption due to population growth and urbanization, there is an urgent need to enhance local production to bridge the supply-demand gap and ensure food security. This also presents an opportunity for Nigeria to generate foreign exchange through rice exports (Osawaru and Ogwu, 2020). Technological innovations and improved agronomic practices are essential for achieving this goal (Nwite *et al*., 2010).

In recent years, nanoparticles (NPs) have emerged as promising tools in sustainable agriculture due to their potential to enhance plant growth and nutrient use

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efficiency (Igiebor *et al*., 2021). NPs, defined as particles with dimensions less than 100 nm, have been shown to influence plant structural development and alleviate micronutrient deficiencies (Ngo *et al*., 2014; Ikhajiagbe *et al*., 2021). Micronutrients such as zinc (Zn), copper (Cu), and manganese (Mn) are vital for plant metabolism and yield, even though required in trace quantities (Tripathi *et al*., 2022). Green synthesis of NPs using plant-based methods under mild, eco-friendly conditions has gained traction due to its non-toxic nature and environmental compatibility. The application of biosynthesized NPs in crops like *Oryza sativa* offers a potential alternative to synthetic fertilizers, promoting improved plant growth and productivity without environmental compromise (Benzon *et al*., 2015; Ikhajiagbe *et al*., 2021). Nevertheless, soil type and composition, particularly ferruginous soils characterized by high iron content and potential nutrient imbalances, may influence the effectiveness of such interventions. Therefore, the objective of this study is to comparatively assess the effects of biosynthesized zinc, copper, and manganese nanoparticles on the germination and early growth of *Oryza sativa* cultivated in ferruginous soil.

MATERIALS AND METHODS

Description of the Study Site

The experiment was carried out at the Department of Plant Biology and Biotechnology Botanic Garden of the University of Benin, Nigeria. Prior to being cleared for usage, the area was primarily covered with weeds. The Global Positioning System (GPS) coordinate of the study site is N6º23'51.8º; 5º36'56.6ºE.

Collection of soil used for the study

Ferruginous soil was obtained from reddish soil portion at the Botanic Garden, Department of Plant Biology and Biotechnology, University of Benin, Benin City using a shovel at a depth of 0 - 10 cm (Igiebor *et al*., 2019).

Collection of Plant species for extract

Leaves of *Carica papaya* L.(pawpaw) and *Azadiracta indica* A. Juss (neem) were obtained from their respective trees in the botanic garden while the leaves of *Hibiscus sabdariffa* L. (red sorrel) were obtained from the market.

Preparation of leaves extracts of plants used for the study

The leaves samples were repeatedly rinsed in deionized water to remove the dust before being dried in the shade. The dried leaves were chopped into smaller bits with knife and crushed into a powder with mortar and pestle. Five gram (5g) of each sample powder was boiled in 100 mL of distilled water at 80 °C for 10 mins and filtered in Whatman No: 1 filter paper. Finally, the prepared extract solution was cooled at 4 °C and stored for further synthesis of nanoparticles (Anandalakshi and Venugobal, 2017).

The stock solution (100 % of each extract), produced from the extracts previously prepared for each of the three leaves, was further diluted with distilled water to achieve the three concentrations (v/v). These concentrations were 5 % (5 ml of the extracts), 25 % (25 ml of the extracts), and 50 % (50 ml of the extracts).

Synthesis of Zinc Nanoparticles (ZnNP)

One millimole (1mM) aqueous solution of Zinc sulphate ($ZnSO_4$) was prepared and aqueous extract of leaf used for the synthesis of zinc Nanoparticles. Twenty (20) ml of leaf extract was added into 80 ml of aqueous solution of 1 mM zinc sulphate. It was placed on magnetic stirrer for 2 hours at room temperature for continuous stirring (Devasenan *et al*., 2016). ZnNP was characterized by using UV–Visible spectrophotometer (Devasenan *et al*., 2016).

Synthesis of Copper Nanoparticles (CuNP)

Ten (10) ml of plant leaf extract was added into 90 ml of 10mM of copper sulphate solution and kept in stirring for constant mixing under room temperature. A colour change of the solution noticed by visual examination, therefore confirm the synthesis of CuNP (Wu *et al*., 2020). The characterization of CuNP was carried out using ultraviolet- visible (UV-Vis) spectrophotometer from 200 – 600nm (Wu *et al*., 2020; Ikhajiagbe *et al*., 2021).

Synthesis of Manganese Nanoparticles (MnNP)

The method according to Paul *et al*. (2017) was adopted with slight modification in the synthesis of manganese oxide nanoparticles. Five milliliters (5 mL) of aqueous leaves extract of the leaves were added to50 mLof aqueoussolution of0.2 MPotassium permanganate ($KMnO_4$) while, heating and stirring at 700C and pH 7 for 30 min. The solution changed from colourless to brown with formation of precipitate. The precipitate was thereafter centrifuged at 3000 rpm for 15 min and washed with distilled water 3 times. This was characterized according to the method described by Chatterjee *et al*. (2017) using UV-visible spectrophotometer at 250 – 800nm.

Rice variety Used

Rice variety used was Nerica obtained from Raymos Guanah Farms Limited, Ughelli, Delta State (Igiebor *et al*., 2019).

Experimental Design and Treatment

The marked plot inside the botanical garden was cleared to bare ground and all debris removed. The experimental bowls which was perforated (20 L) were filled with 20 kg of soil. The experimental design used was a completely randomised design, with the experimental plot assumed to be homogeneous. All the ferruginous soil collected from various designated positions was bulked together to create composite samples. Each treatment were in replicates

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Twenty (20) rice seeds were sown per bowl and thinned down to 5 stands after 2 weeks. The biosynthesized nanoparticles were foliar sprayed by spray pump directly at the leaves of each treatment. This spraying was done in the evening, however between 3 – 4 hours after synthesis (the period where the highest peak activity was observed). Morphological characteristics such as were observed and parameters taken at 2, 5, 8 and 11 weeks.

Maintaining soil moisture

The set up was in the open and as such relied basically on rainfall. However, the soil moisture was always maintained using the Hand-feel method described by USDA (2009). The soil sample was collected in the root zone with a spade. The water deficit was estimated for each soil sample at several depths ranging from 0 - 2 inches/feet was taken by feeling the soil and judging the soil moisture using the texture. This can be detected when there is wet outline on hand when squeezed or having some darkness due to unavailable moisture, hard baked, cracked, sometimes has loose crumbs on 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. A booster dose was applied after two weeks of initial application (Ikhajiagbe *et al*., 2021).

Management

Care was taken to ensure weeds were removed from each experimental bowl on a constant basis up till the end of the experiment.

Growth parameters

Plant growth and morphological responses were monitored according to methods described by Ikhajiagbe (2016) and Özalkan *et al* (2010). Some of the growth parameters measured included Plant height, leaf length, number of leaves, stem girth, sheath length, 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

The soil pH was measured potentiometrically using a glass-calomel electrode (MP 220 AFAB Lab, LLC) with a 1:2.5 soil-to-water ratio. Soil organic matter was estimated via titrimetric methods, calculating from organic carbon content by multiplying by 1.724. Loss on ignition at 360 °C and the Walkley-Black wet oxidation method using potassium dichromate were employed (Sims and Haby, 1971). Additionally, total combustion at temperatures above 1,000°C with infrared/conductivity detection was used for carbon quantification. Total nitrogen content was determined by the Kjeldahl method involving digestion in sulfuric acid with $CuSO_4/TiO_2$ catalysts, distillation, and titration. Phosphorous content was analyzed using 0.5M sodium bicarbonate extraction (pH 8.5) and colorimetric detection of 'Molybdenum Blue' at 882 nm. Total exchangeable acidity was assessed by saturating soil with 1 M KCl, titrating with 0.02 M HCl, and calculating exchangeable H^+ and Al^{3+} fractions. Iron (Fe) concentration was determined following APHA (2008) protocols: soil-water suspension was acidified, reduced with hydroxylamine hydrochloride, buffered, reacted with 1,10-phenanthroline, and measured spectrophotometrically at 510 nm. Soil texture (clay, silt, and sand contents) was evaluated using the pipette method. Soil was dispersed with sodium hexametaphosphate, and timed aliquots were taken after settling periods to determine fractions. Percentage compositions were calculated based on aliquot weights. Electrical conductivity was determined by shaking a 1:5 (w/v) soil-water mixture, allowing it to settle, and measuring conductivity in the supernatant without disturbing sediment.

Photosynthetic Pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, lycopene, and tocopherol) were determined using standard spectrophotometric methods. Chlorophyll a and b were estimated following Arnon (1949) and Maxwell and Johnson (2000) by macerating 1 g of fresh leaf tissue in 80 % acetone and measuring absorbance at 645 nm and 663 nm. Total carotenoids and lycopene were determined according to Zakaria and Simpson (1979) after saponification of 0.5 g of sample with alcoholic KOH, followed by extraction with petroleum ether and absorbance readings at 450 nm and 503 nm. Tocopherol content was analyzed using the Emmerie–Engel reaction, as described by Rosenberg (1992) and Ayodele *et al*. (2014), based on absorbance at 460 nm and 520 nm after reaction with dipyridyl reagent and ferric chloride. Chlorosis was assessed visually using a standard leaf colour chart, with yellow hues indicating chlorosis.

Data Analysis

The experiment's data was analyzed using SPSS software to identify central tendencies and patterns. GraphPad Prism was used to visualize treatment effects and trends, providing insights into the interaction between nanoparticle treatments and rice plant growth and physiological responses.

RESULTS

Physicochemical Characteristics of Soil Used in the Study

The results reported in Table 1 demonstrated the chemical and physical characteristics of the ferruginous soil employed in this study. The pH level was 6.47 ± 0.21, indicating that it was just nearly neutral. Total organic carbon was 0.41 ± 0.11 %, total nitrogen was 0.64 ± 0.19 %, exchange acidity was 0.21 ± 0.13 meq/100g, clay was 7.92 ± 1.42 %, silt was 5.74 ± 2.76 %, sand was 85.10 ± 18.24 %, and Fe was 813 ± 21.44 mg/kg. Electric conductivity was 301.913 ± 1.23 s/cm.

Nanoparticle Production and Retention Time

Figure 1 shows the absorption of ZnNPs biosynthesized with pawpaw, sorrel and neem leaf extracts measured between 300 – 600 nm. The highest peak activity for pawpaw-ZnNP and Neem-ZnNP was at the 4th hour after production, while Sorrel-ZnNP highest peak was recorded at the 3rd hour. Figure 2 shows the absorption of CuNPs biosynthesized with pawpaw, sorrel and neem leaf extracts measured between 300 – 600 nm. The highest peak activity for pawpaw-CuNP and Sorrel-CuNP was at the 4th hour after production, while Neem-CuNP highest peak was recorded at the 3rd hour. Figure 3 shows the absorption of MnNPs biosynthesized with pawpaw, sorrel and neem leaf extracts measured between 300 – 600 nm. The highest peak activity for pawpaw-MnNP, Sorrel-MnNP and Neem-MnNP were at the 4th hour after production.

Morphological Growth Parameters

At harvest week 11, growth parameters (Figure 4) showed variations in plant height with plant-treated NPs. ZC3 (50 %) and ZC2 (25 %) had the highest heights in Cu-biosynthesized NPs, whereas NC3 (50 %) had the lowest heights. In Mn-biosynthesized NPs, PM1 (5 %) had a height of 25 cm, followed by control with a height of 25 cm, and ZM3 (50 %) had the lowest height. The plant height in Zn-biosynthesized NPs varied from 20 cm to 30 cm. The highest was recorded NZ2 (25 %), followed by NZ3 (50 %). The *Carica papaya* extract application bowl alone yielded the lowest height.

The leaf length of biosynthesised NPs is depicted in Figure 5. In comparison to the control, there was a considerable increase in leaf length. In CuNPs, it was observed that NC3 had the highest leaf length with 40 cm, followed by ZC2 with 39 cm and PC1 with 39 cm but the lowest length was recorded in PC3 with 30 cm. This was similar in MnNPs. However, for ZnNPs, it was observed that the highest leaft length was 25 cm (NZ1) while the lowest was recorded in PEXTR with 15 cm.

The number of leaves (Figure 6) varied from 15 (ZZ1) to 35 (ZC1). The Cu- biosynthesized NPs showed that ZC1 (5 %), ZC2 (25 %) and NC2 (25 %) had the highest number of leaves and control had the lowest numbers of leaves, respectively. Comparing the Mn-biosynthesized NPs to the control, PM2 (25 %) had the highest number of leaves, whereas ZM1 (5 %), and NM2 (25 %) had the fewest. The maximum number of leaves in Zn-biosynthesized NPs were found in PZ1 (5 %), followed by PZ2 (25 %) and ZZ1 (5 %), which had the fewest compared to the control.

After exposure to biosynthesized NPs, stem girth often increased significantly (Figure 7). In ZnNPs intervention, NZ2 had the maximum stem girth (6 cm), ZZ1 had 4.3 cm while NZ3 had 4.1 cm respectively. However, in CuNPs intervention, it was observed that. there were decrease across the treatments from ZC1 to NC3. Although in MnNPs intervention, the stem girth was highest in control with 4.9 cm and lowest in ZM1 with 2.3 cm.

After nano intervention, the sheath length significantly increased (figure 8). For CuNPs intervention, the NC1 sheath length was the largest (30 cm), while the shortest was recorded in control with 18 cm. For ZnNPs, the largest was reported in PZ1 (20 cm) while the shorted in PZ3 (14 cm). But for MnNPs, the largest was reported PM2 (25 cm) while the shortest was recorded in ZM3 (16 cm).

Assessment of plants stress parameters

Chlorosis in leaves (Figures 9 –10) between week 2 and week 7 has been recorded in this study. ZC3 (5 %) had the highest incidence (50 %) compared to the control, followed by PC3 (50 %) and NC1 (5 %), whereas the control had a considerably higher incidence of chlorotic growth than the treatment group. However, chlorotic activity was at its highest in PEXTR for the extract-only intervention.

Figures 11 and 12 show variations in foliar colour. It was reported that PC3 (50 %) and NEXTR had the maximum foliar colour intensity (dark green). Generally, after being exposed to nanoparticles over an 11-week period, there were noticeable changes in the intensity of the foliar coloration between the treatments. The colour during the first week was olive drab, possibly the clearest sign that the plants were starting to get chlorotic. However, after the application of nanoparticles, the leaf's colour improved to a dark green from its original predominant colour of olive drab in just 4 weeks.

Table 1.

Physical and chemical properties of soils used in the study (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/100g)	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

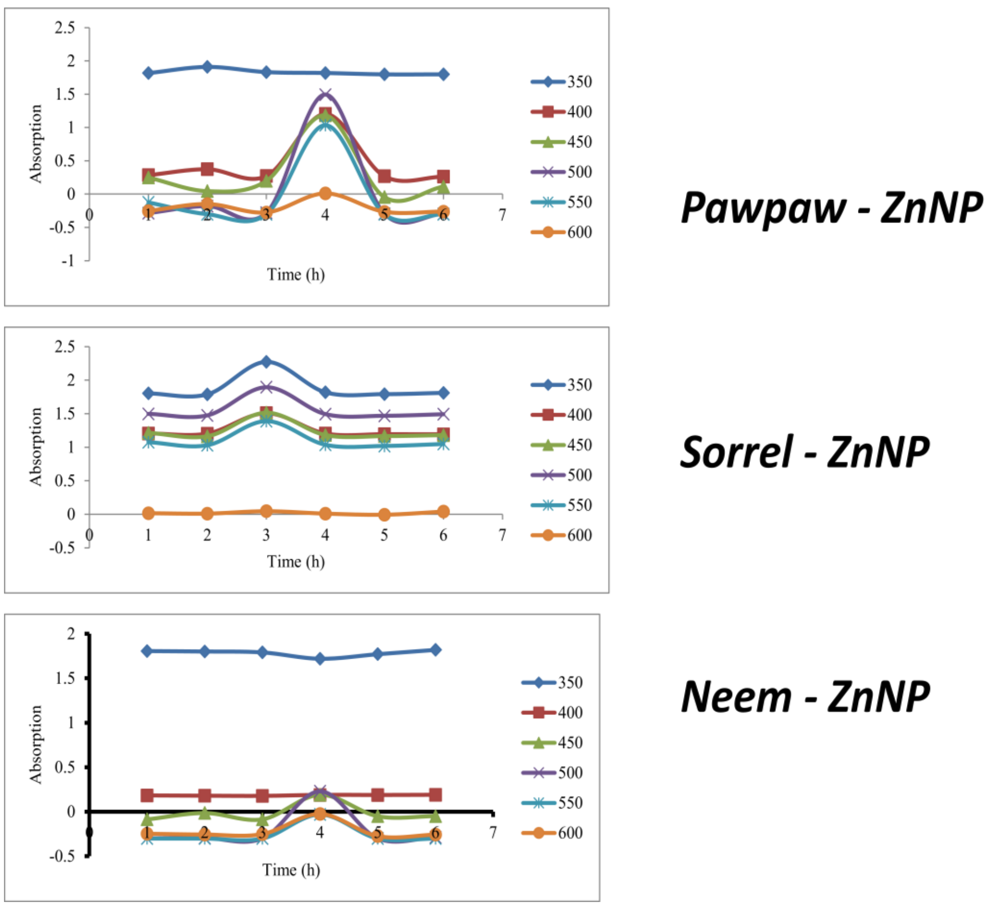


Fig. 1. Absorption of Zinc nanoparticles biosynthesized with pawpaw, sorrel and neem leaf extracts and measured at wavelengths of 300 - 600 nm.

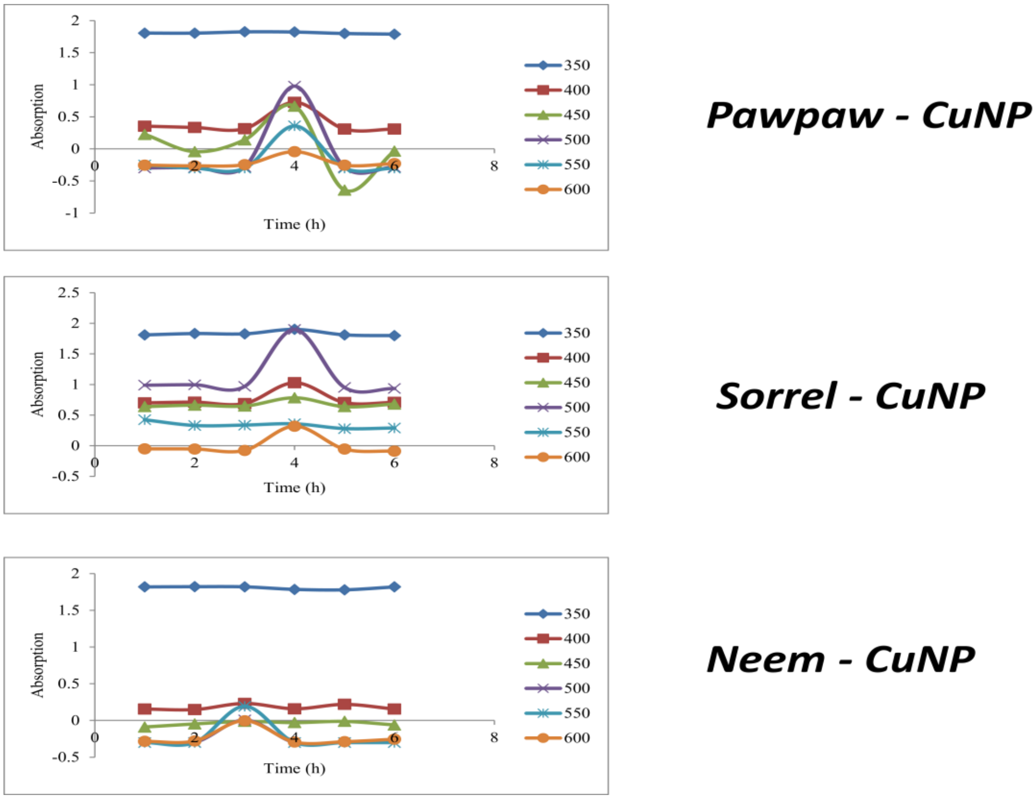


Fig. 2. Absorption of copper nanoparticles biosynthesized with pawpaw, sorrel and neem leaf extracts and measured at wavelengths of 300 - 600 nm.

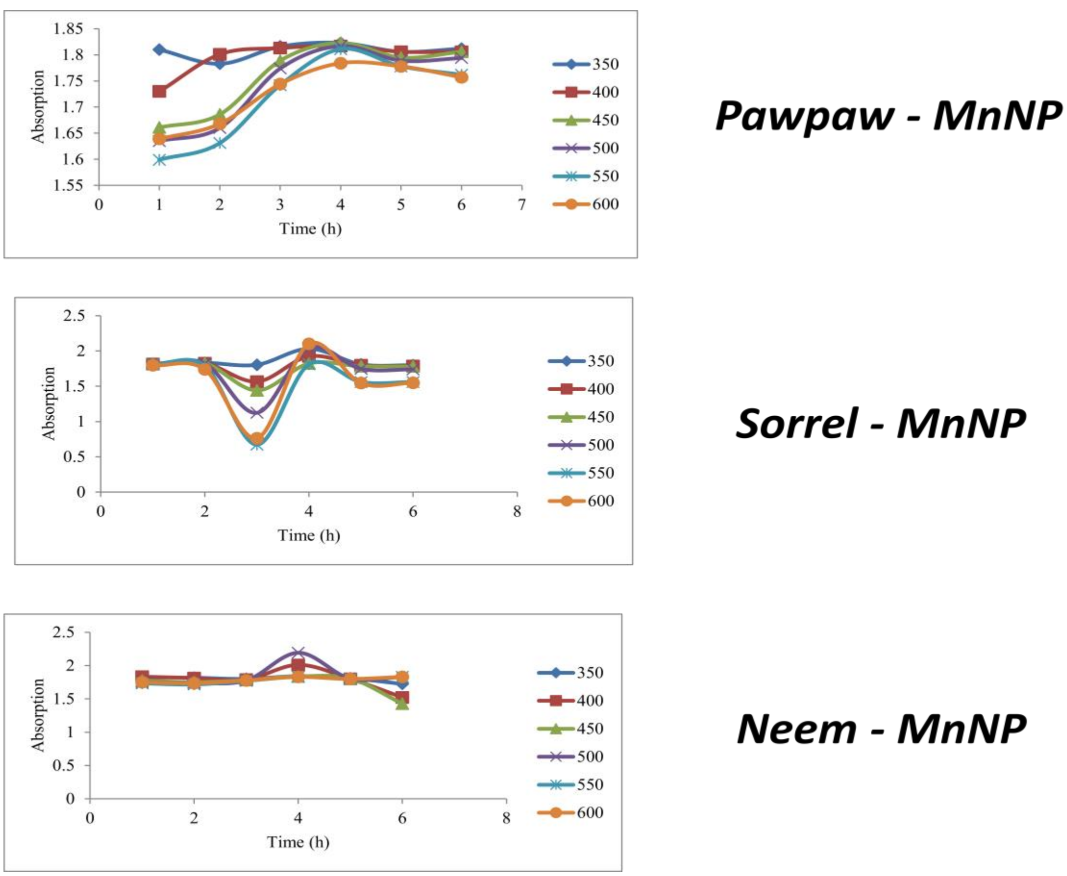


Fig. 3. Absorption of manganese nanoparticles biosynthesized with pawpaw, sorrel and neem leaf extracts and measured at wavelengths of 300 - 600 nm.

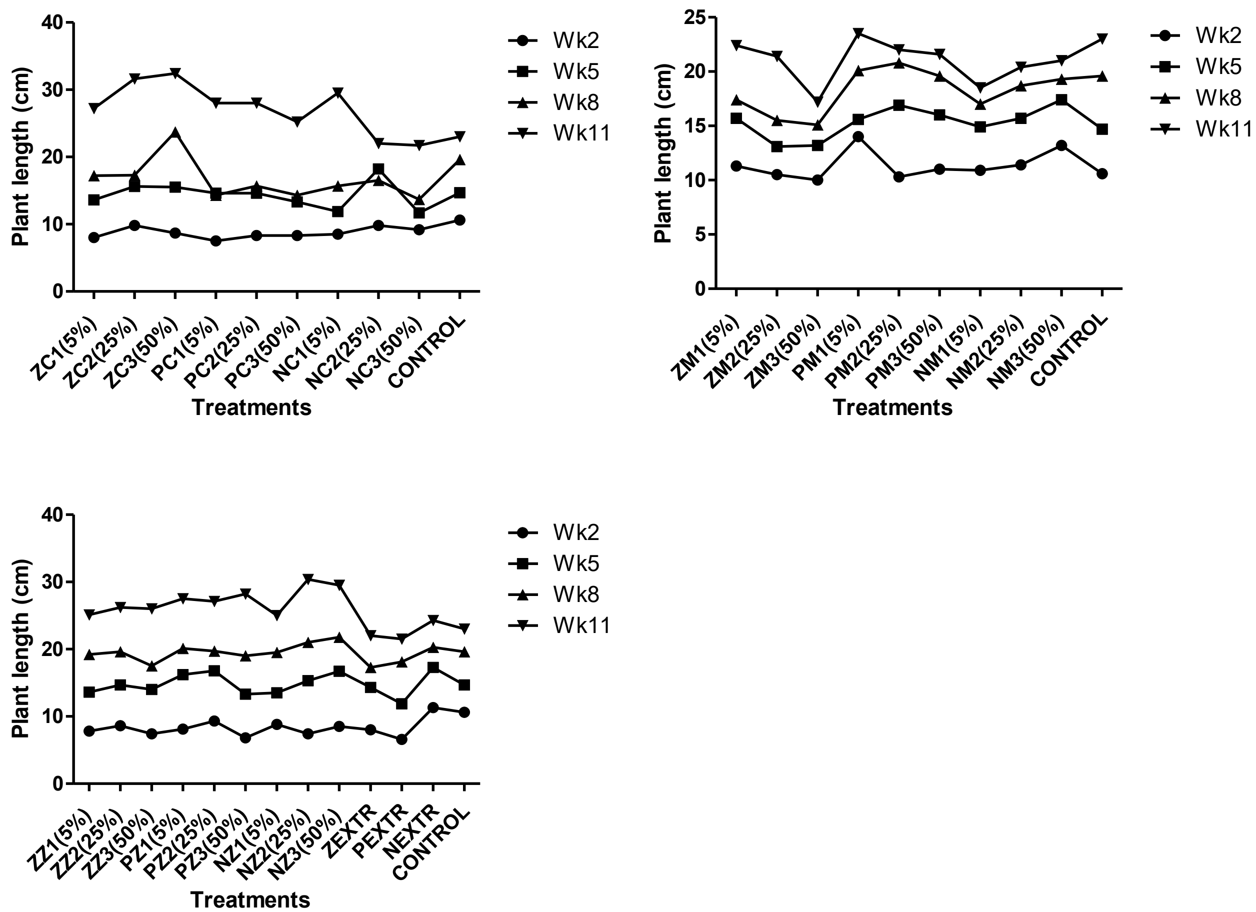


Fig. 4. Impact of NP treatments on plant height over assessed period of growth.

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.

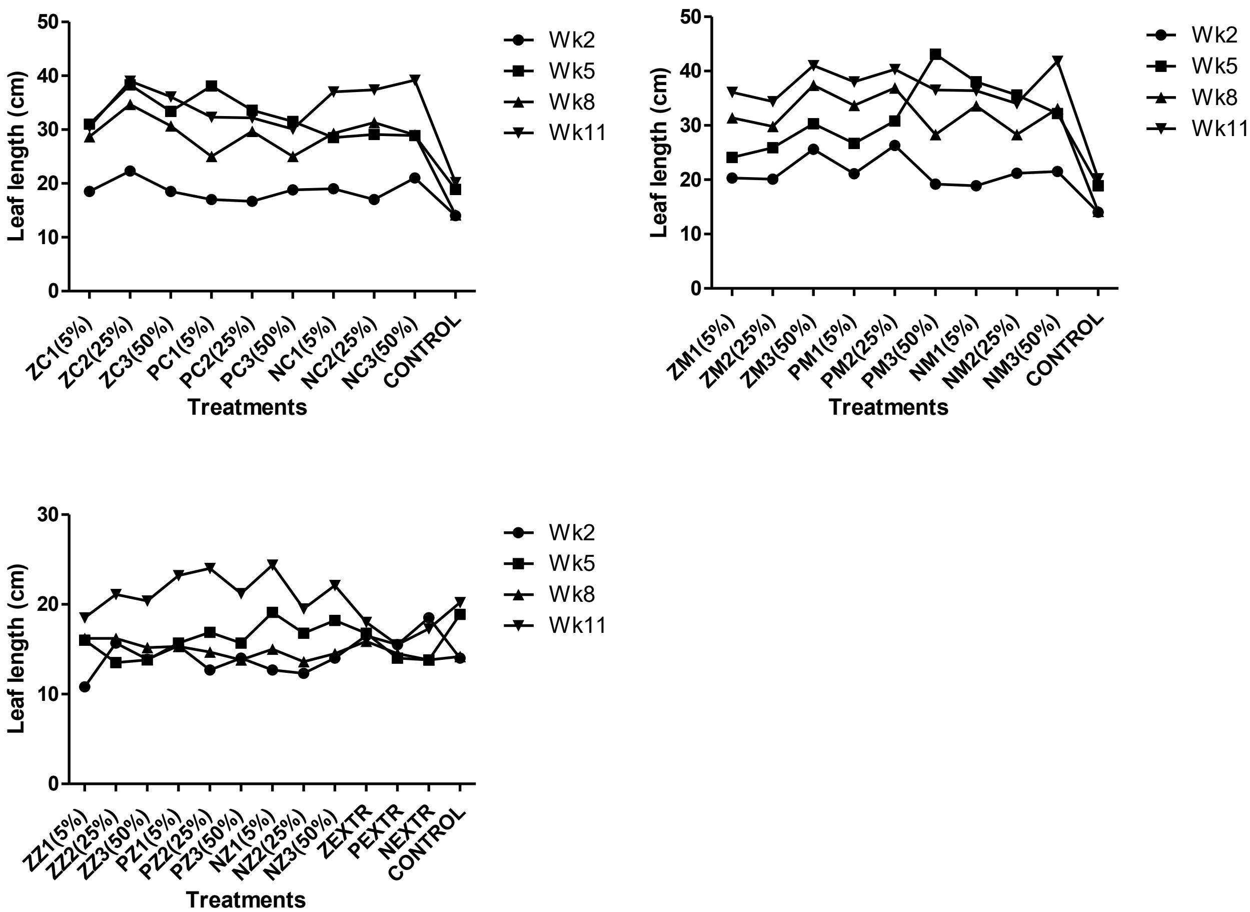


Fig. 5. Impact of NP treatments on leaf length over assessed period of growth.

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.

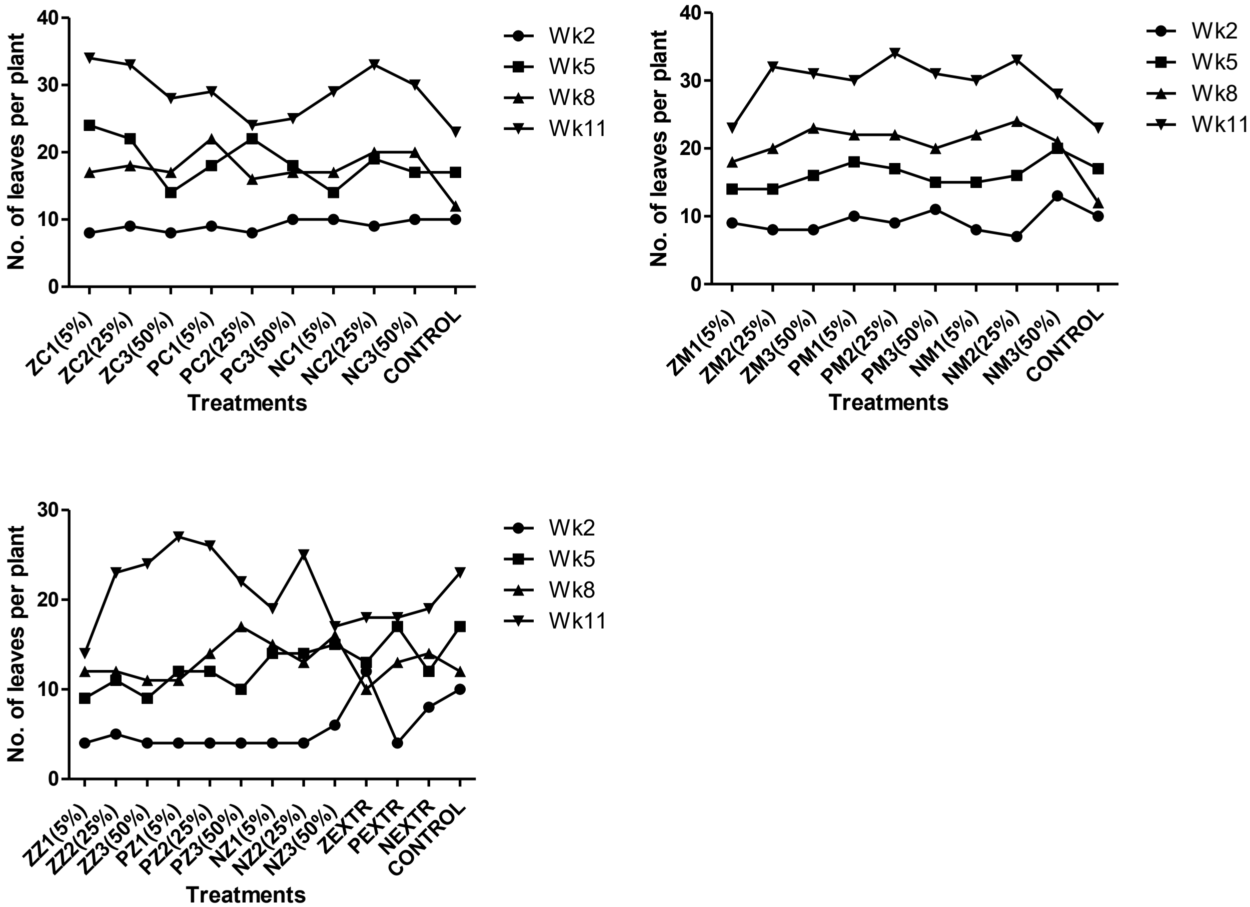


Fig. 6. Impact of NP treatments on number of leaves over assessed period of growth.

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.

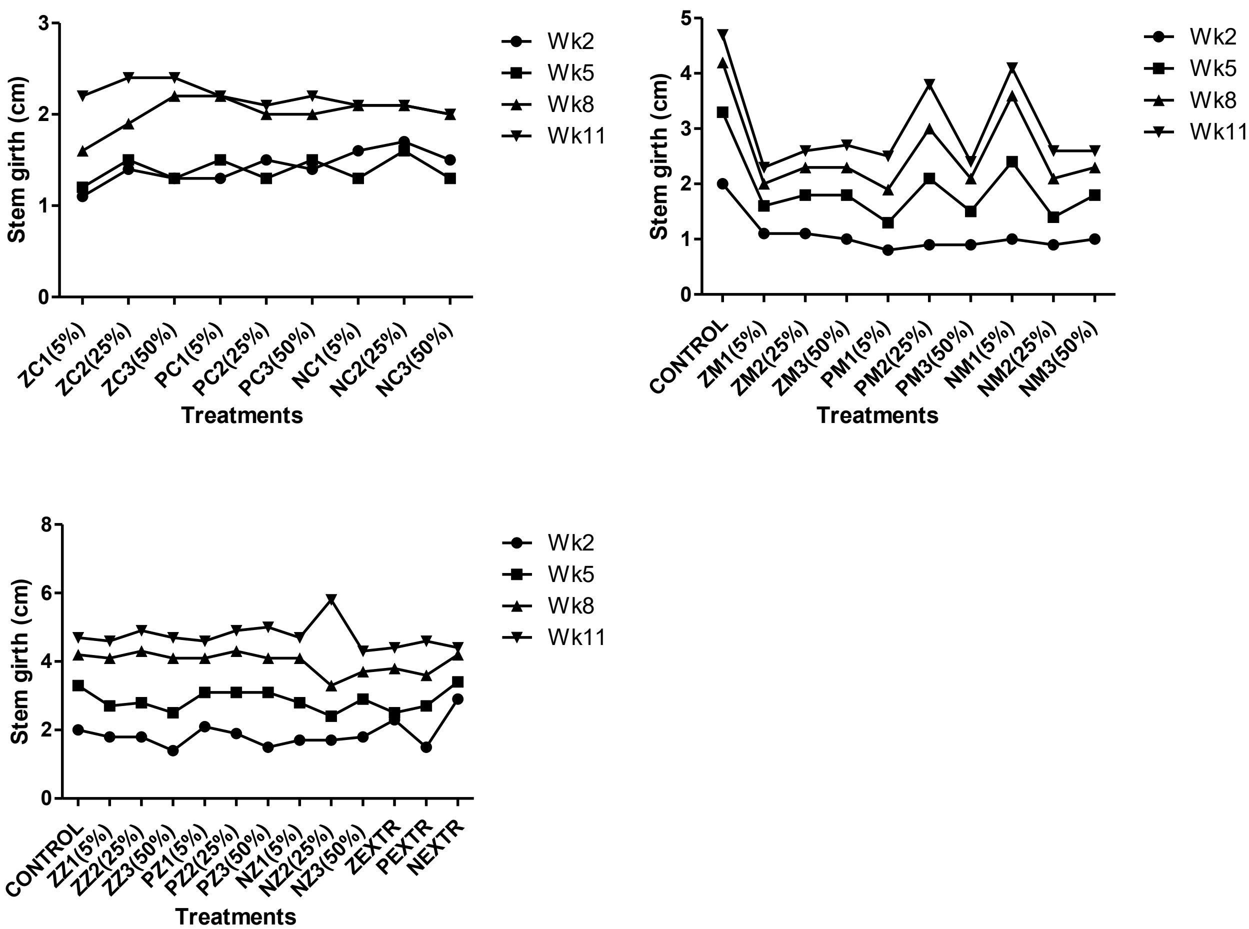


Fig. 7. Impact of NP treatments on stem girth over assessed period of growth.

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.

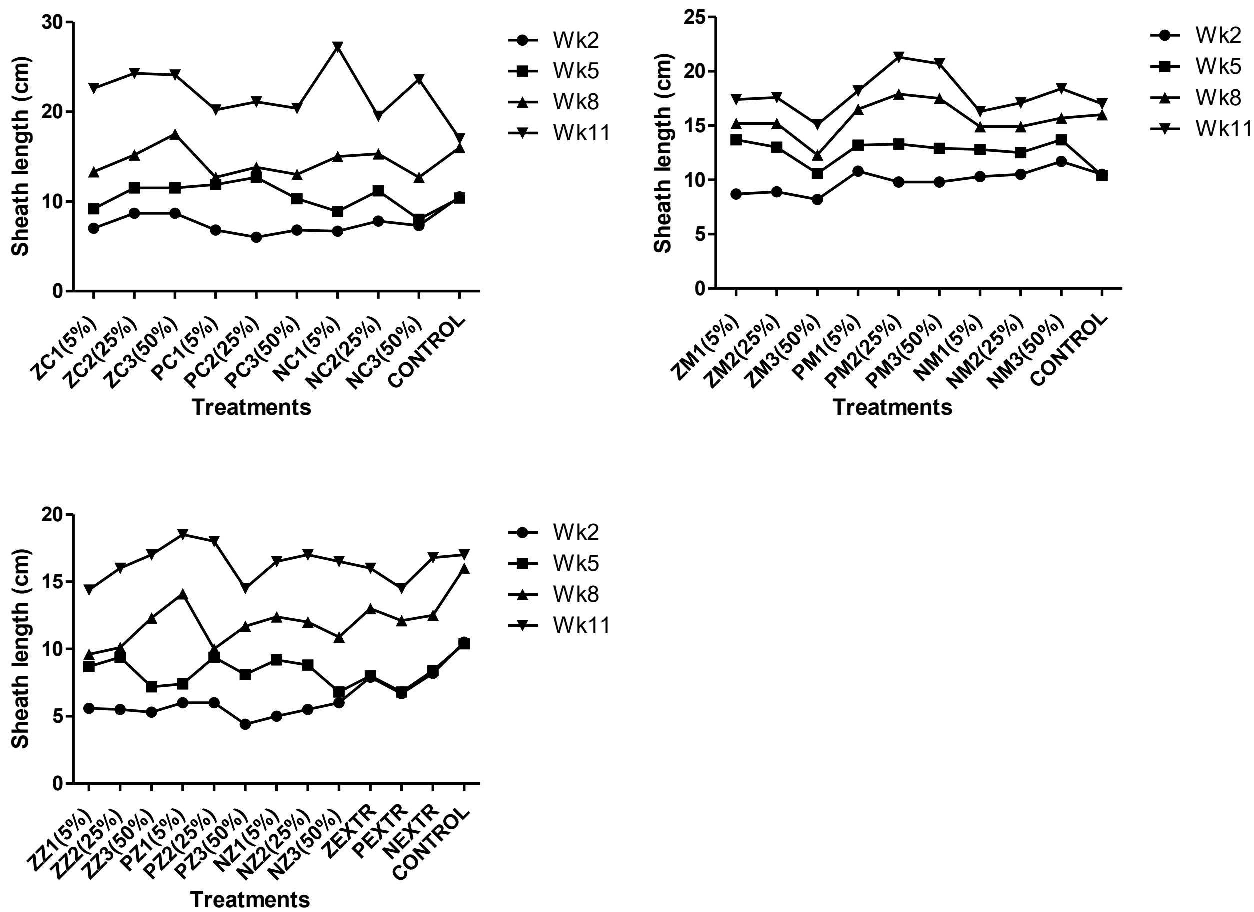


Fig. 8. Impact of NP treatments on sheath length over assessed period of growth.

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.

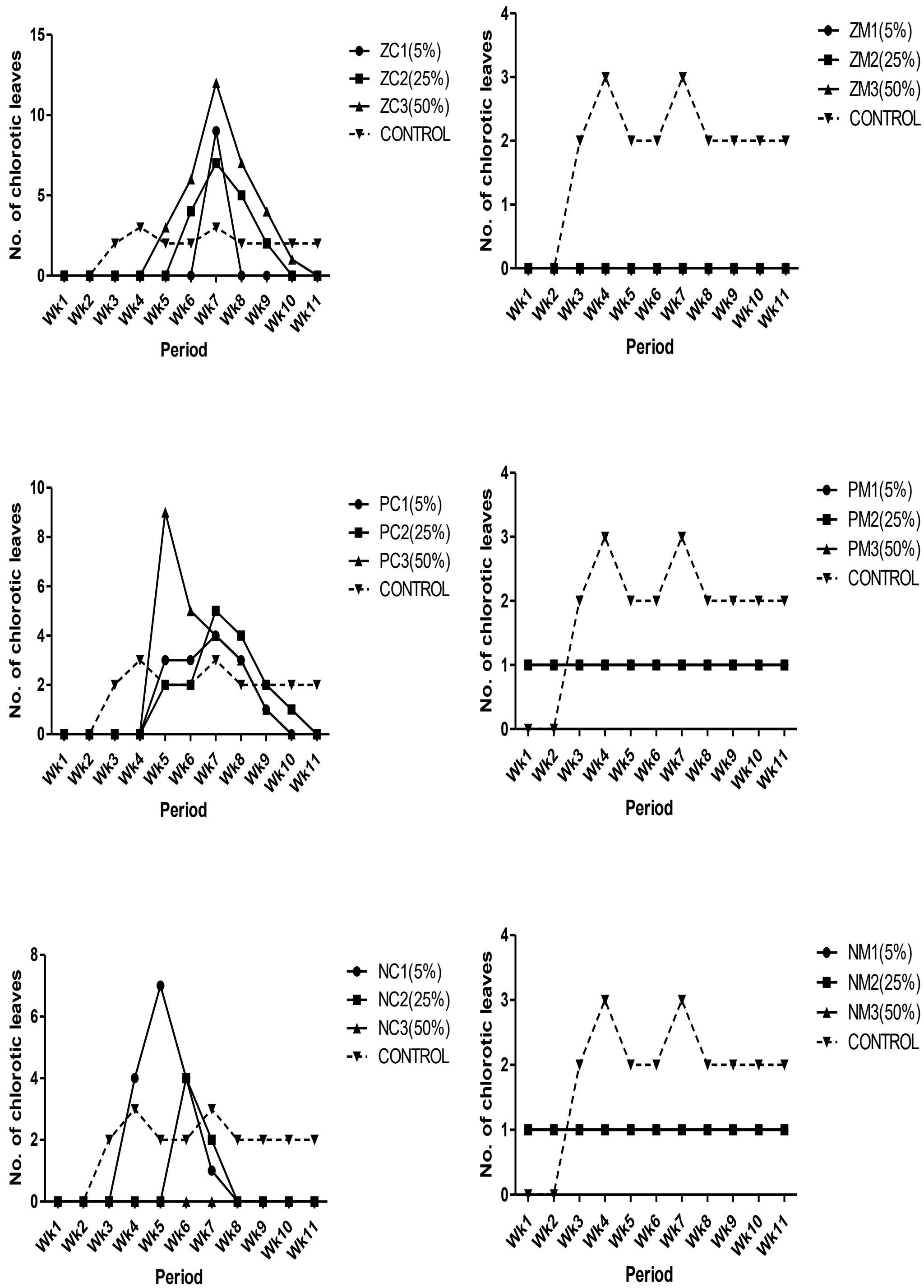


Fig. 9. Occurrence of foliar chlorosis occasioned by application of nanoparticle over assessed period of growth.

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, 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, , 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, , Control=No application of NPs.

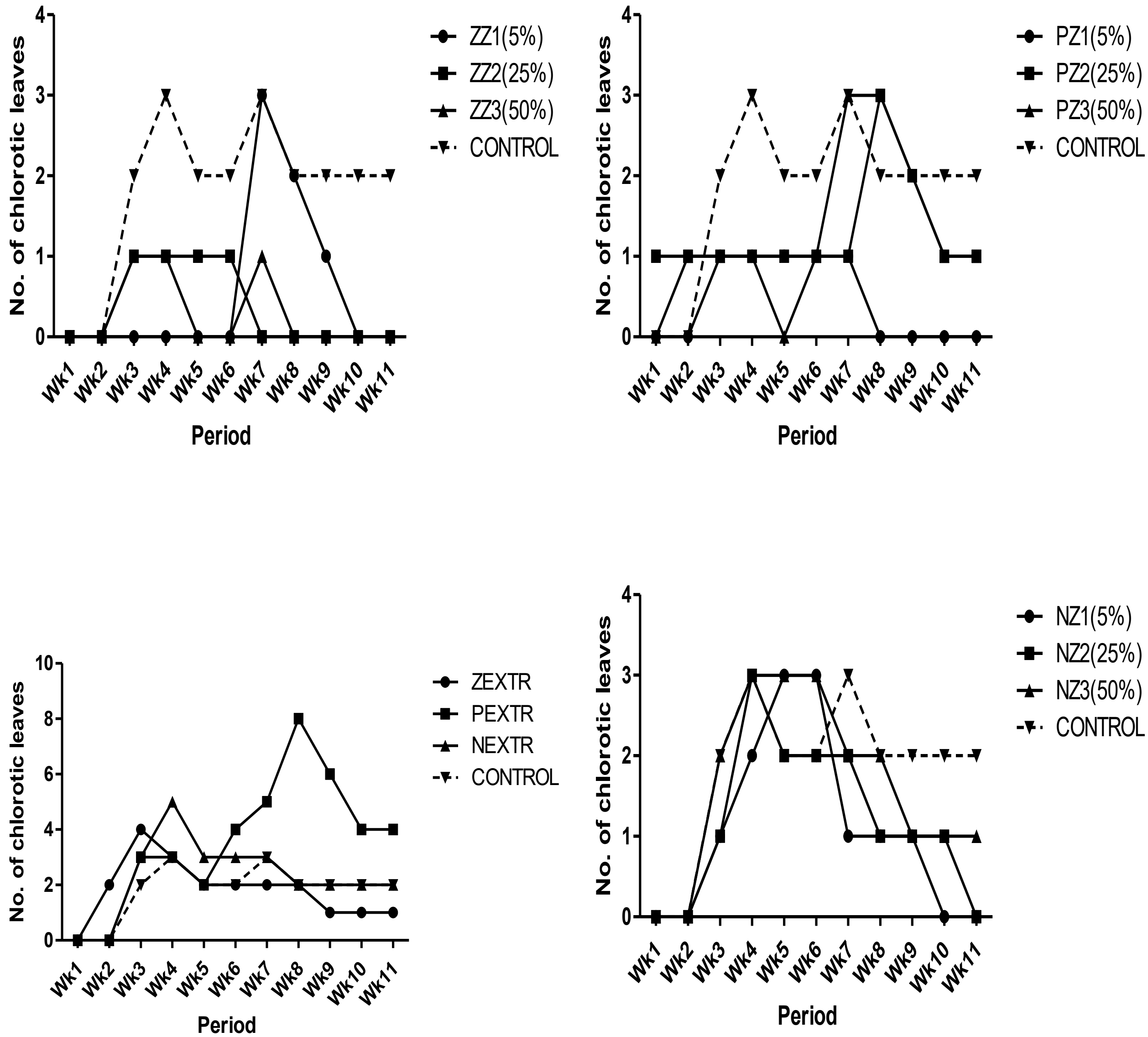
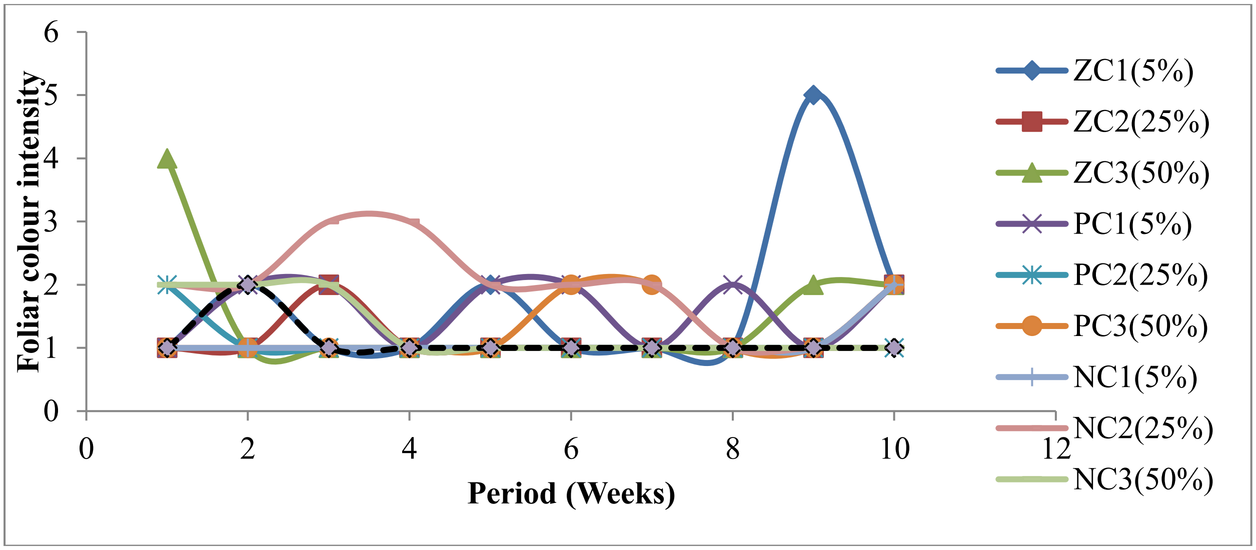
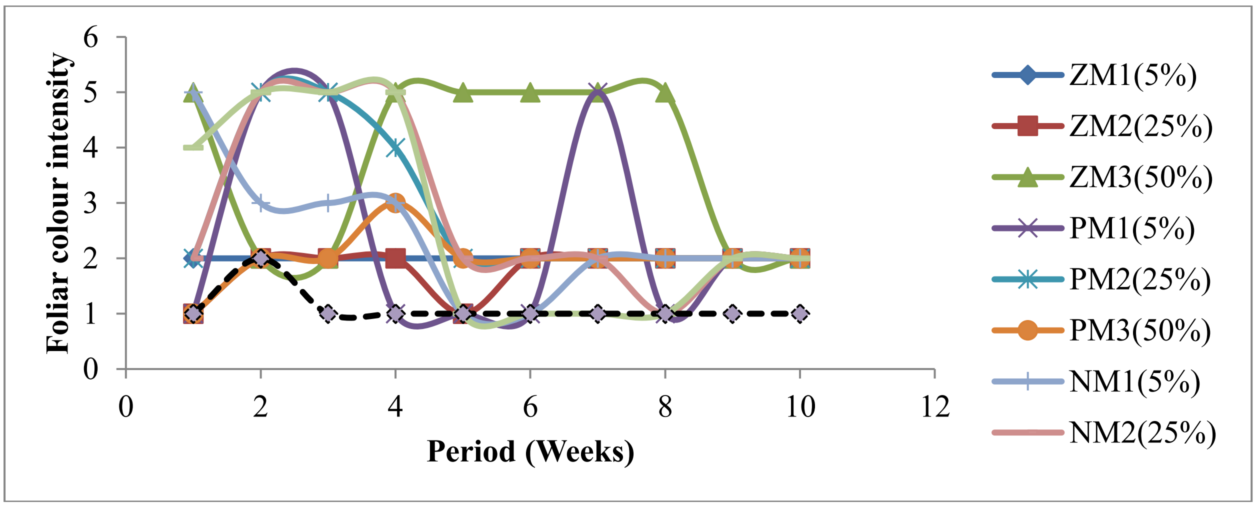


Fig. 10. Occurrence of foliar chlorosis occasioned by application of nanoparticle over assessed period of growth.

KEY: ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, 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.

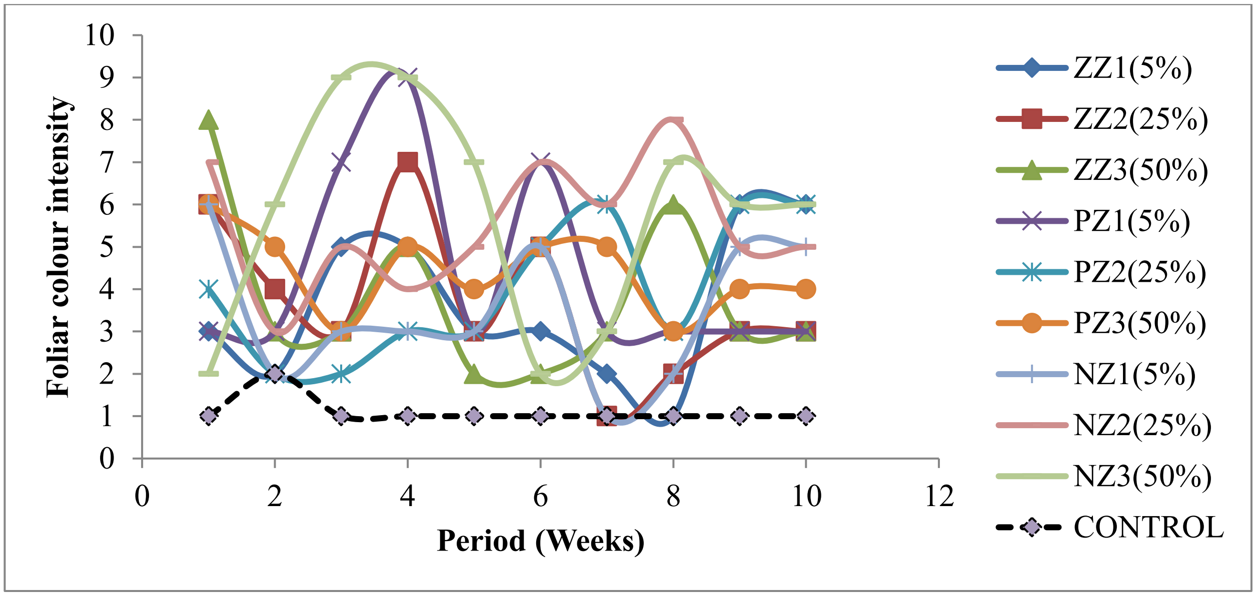


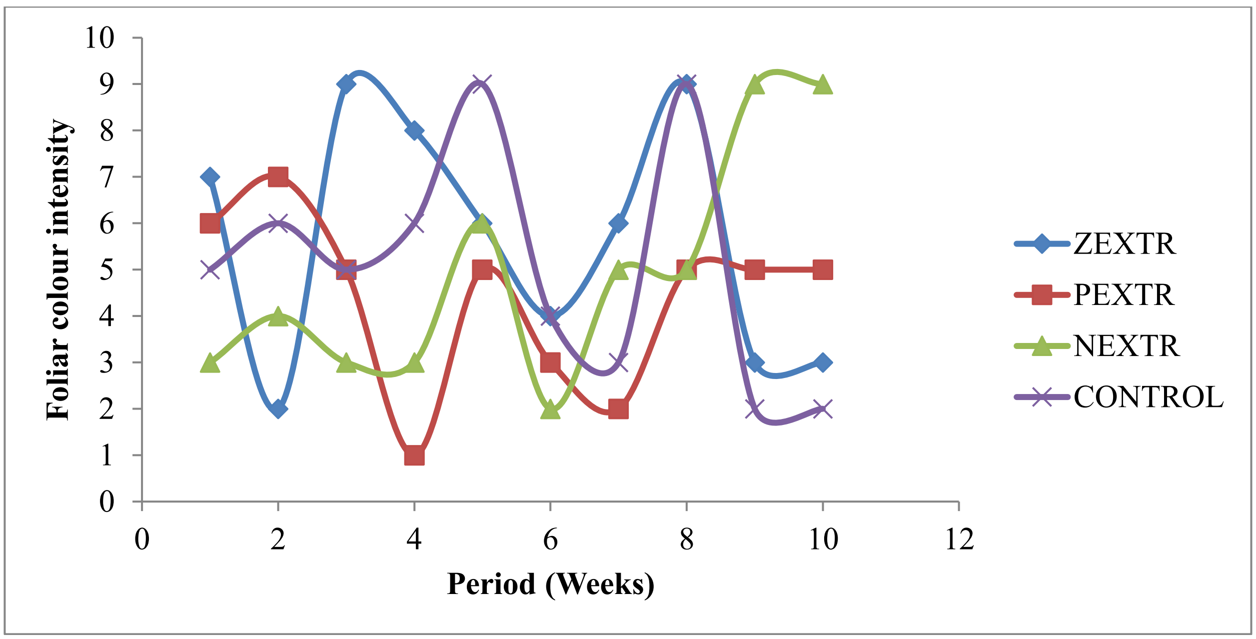


Foliar Colour changes - Colour intensity (0 – Olive drab green, 9 – dark green)

Fig. 11. Foliar Colour changes over the 11-week period following exposure to nanoparticles.

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, 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, , 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, , Control=No application of NPs.





Colour intensity (0 – Olive drab green, 9 – dark green)

Fig. 12. Foliar Colour changes over the 11-week period following exposure to nanoparticles.

KEY: ZZ1=5 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ2=25 % *Hibiscus sabdariffa*-mediated ZnNPs, ZZ3=50 % *Hibiscus sabdariffa*-mediated ZnNPs, PZ1=5 % *Carica papaya*-mediated ZnNPs, PZ2=25 % *Carica papaya*-mediated ZnNPs, PZ3=50 % *Carica papaya*-mediated ZnNPs, 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.

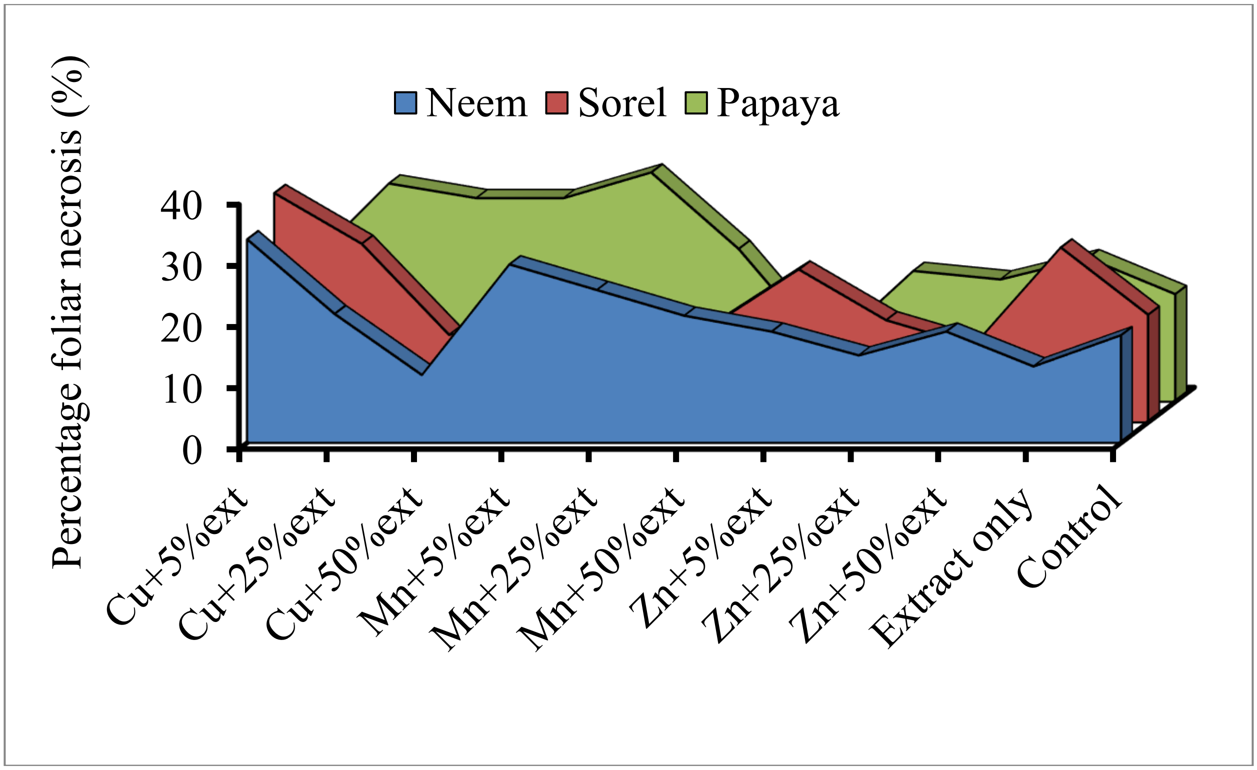


Fig. 13. Total occurrence of foliar necrosis occasioned by application of nanoparticle (Calculated as a total over a period of 11 weeks).

Necrosis

Figure 13 shows the total number of times foliar necrosis occurred during the course of the experiment. Evident spots of necrosis first surfaced and then spread throughout the plant, eventually affecting the entire leaf. When compared to the control, they were only seen in the Cu+5%ext, Mn+5%ext, Zn+50%ext, and extract.

DISCUSSION

This study successfully demonstrated the green synthesis of Zn, Cu, and Mn nanoparticles (NPs) using leaf extracts of *Carica papaya*, *Hibiscus sabdariffa*, and *Azadirachta indica*. The UV-visible spectroscopic analysis revealed that peak nanoparticle formation occurred within 3 to 4 hours post-synthesis, indicating rapid nanoparticle generation and stability, which is crucial for timely foliar application. The absorption of these nanoparticles was measured at wavelengths between 300 and 600 nm over this period, suggesting a potential improvement in bioactivity and supporting their concurrent application.

The application of these biosynthesized nanoparticles significantly influenced the germination and early seedling development of *Oryza sativa* var. Nerica. Morphological parameters such as plant height, leaf length, number of leaves, stem girth, root development, and dry weight of roots were notably enhanced compared to the control. This finding aligns with earlier reports by Ikhajiagbe*et al*. (2021); Wang *et al*. (2023) and Freire *et al*. (2024), who observed similar growth stimulation in rice following foliar-applied plant-mediated nanoparticles. Notably, plants grown in ferruginous soils characterized by high iron concentrations (Ikhajiagbe and Saheed, 2020) often face nutrient imbalances and physical barriers that constrain growth (Urriago-Ospina *et al*., 2020). However, this study demonstrated that nanoparticle application could partially mitigate such constraints, leading to improved early growth performance, consistent with Sadak (2019), who reported positive effects of nanoparticles on plant growth under suboptimal soil conditions.

In particular, zinc nanoparticles (ZnNPs) consistently produced the most significant improvements across various morphological parameters. This may be attributed to enhanced nitrogen assimilation and improved stress resilience, as Zn plays a pivotal role in enzymatic functions and protein synthesis (Tripathi *et al*., 2022; Benzon *et al*., 2015). The results corroborate Hao *et al*. (2016), who reported that nanoparticle exposure enhanced rice root elongation and supported shoot development. Also, the observed increase in leaf number following nanoparticle treatment further supports the idea that NPs can influence gene expression, potentially through modulation of microRNAs regulating growth and metabolic pathways (Owusu Adjei *et al*., 2021). Furthermore, the improvement in foliar coloration after nanoparticle application (transitioning from olive drab to dark green) suggests enhanced chlorophyll synthesis, thereby improving photosynthetic efficiency.

While a general trend of improvement was observed, some variations in growth responses were noted depending on the nanoparticle type, concentration, and the plant extract used for synthesis. CuNPs and MnNPs induced moderate increases in growth parameters but were less consistent compared to ZnNPs. This differential response aligns with the findings of Ducic and Polle (2007), who reported that excessive Mn could sometimes exacerbate plant stress under certain conditions. Interestingly, a slight reduction in panicle length was observed in some nanoparticle treatments compared to the control. This is in contrast to Kamari *et al*. (2014), who found that nano-ZnO treatment led to the elongation of triticale panicles, suggesting that responses may be crop-specific and influenced by the interaction between nanoparticles and the physiological needs of the species.

Generally, the results underscore the promising potential of green-synthesized nanoparticles, particularly ZnNPs, in enhancing early seedling vigor of rice grown under ferruginous soil conditions. However, the concentration-dependent effects observed emphasize the need for careful optimization of nanoparticle dosage to avoid potential phytotoxicity. This study supports the findings of Saheed and Ikhajiagbe (2020) and Ikhajiagbe*et al*. (2021) regarding the positive effects of nanoparticle application in ferruginous soils, further validating the potential of biosynthesized nanoparticles to improve plant growth and agricultural productivity.

CONCLUSION

This study successfully demonstrated the green synthesis of Zn, Cu, and Mn nanoparticles (NPs) using leaf extracts of *Carica papaya*, *Hibiscus sabdariffa*, and *Azadirachta indica*. The nanoparticles exhibited rapid formation and stability, with a significant positive impact on the germination and early growth of *Oryza sativa* var. Nerica. Key morphological parameters, including plant height, leaf length, number of leaves, stem girth, root development, and root dry weight, were notably enhanced, with ZnNPs showing the most consistent and significant improvements. These results support the potential of nanoparticles, particularly ZnNPs, in enhancing plant growth, especially in ferruginous soils with nutrient imbalances, and suggest their usefulness in improving agricultural productivity under suboptimal conditions. Future research should be conducted to assess the sustained effects of nanoparticle applications on plant growth, soil health, and any potential accumulation of nanoparticles in plant tissues.

AUTHORS CONTRIBUTIONS

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

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ajala AS, Gana A, Analysis of Challenges Facing Rice Processing in Nigeria. Journal of Food Processing. 10, 1 – 6, 2015.

Akande T, The Rice Sector in Nigeria, United Nation Crop Project (UNCP). Country Agricultural Project on Trade Liberalization in Agricultural Sector and the Environment, Geneva, Switzerland. African Crop Science Journal, 15 (4), 223 – 235, 2003.

Anandalakshmi K, Venugobal J, Green Synthesis and Characterization of Silver Nanoparticles Using *Vitex negundo* (Karu Nochchi) Leaf Extract and its Antibacterial Activity. Journal of Medicinal Chemistry, 7, 218-225, 2017.

APHA Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C. USA. 874p, 2008.

Arnon DI, Copper enzymes in isolated chloroplast polyphenol oxidase in beta vulgaris. Plant Physiology, 24, 1-15, 1949.

Ayodele OK, Folasade MO, JoshuaOA, Chris OA, Activity of the antioxidant defense system in a typical bioinsecticide-and synthetic insecticide-treated cowpea storage beetle *Callosobrochus maculatus* F. (Coleoptera: Chrysomelidae). International Journal of Insect Science, 6, 99 – 108, 2014.

Benzon HRL, Rubenecia MRU, Ultra Jr, VU, Lee SC, Nano-fertilizer affects the growth, development, and chemical properties of rice. International Journal of Agronomy and Agricultural Research, 7 (1),105-117, 2015.

Bruinsma J, World Agriculture: Towards 2015/2030. An FAO Perspective. Earthscan Publications Ltd, London, 444p, 2003.

Chatterjee S, Subramanian A and Subramanian S (2017). Synthesis and characterization of manganese dioxide using *Brassica oleracea* (cabbage). Journal of Industrial Pollution Control, 33: 1627-1632.

Devasenan S, HajaraBeevi N and Jayanthi SS, Green synthesis and Characterization of Zinc nanoparticle using *Andrographis paniculata* leaf extract. International Journal of Pharmaceutical Sciences Review and Research, 39 (1): 243-247, 2016.

Ducic T and Polle A, Manganese toxicity in two varieties of Douglas fir (*Pseudotsuga menziesii* var. viridis and glauca) seedlings as affected by phosphorus supply. Functional Plant Biology, 34: 31-40, 2007.

Ekugbe G, Nigeria is Largest Producer of Rice, Sorghum in Africa. Available [online] at:

https://www.thisdaylive.com/index.php/2021/11/30/nigeria-is-largest-producer-of-rice-sorghum-in-africa/ Accessed January 14, 2023.

FAO, A regional rice strategy for sustainable food security in Asia and the pacific. FAO Regional Office for Asia and the Pacific, Bangkok, 2003.

Hao Y, Zhang, Z, Rui, Y, Ren, J, Hou, T, Wu, S, Rui M, Jiang F and Liu L, Effects of different nanoparticles on seed germination and seedling growth in rice. In: Proceedings of the 2nd annual international conference on advance material engineering. Atlantis Press, Netherland, 2016.

Igiebor FA, Ikhajiagbe B and Anoliefo GO, Growth and Development of Salinity-Exposed Rice (*Oryza sativa*) Rhizo-inoculated with *Bacillus subtilis* under Differing pH Levels. Studia Universitatis Babeş – Bolyai, Biologia 64 (2): 41-53, 2019.

Igiebor FA, Asia M and Ikhajiagbe B, Green Nanotechnology: A Modern Tool for Sustainable Agriculture – a Review. International Journal of Horticultural Science and Technology, 10 (4): 269-286, 2023.

Ikhajiagbe, B, Possible adaptive growth responses of *Chromolaena odorata* during heavy metal remediation. Ife Journal of Science, 18(2): 403 – 411, 2016.

Ikhajiagbe B and Ogwu MC, Hazard quotient, microbial diversity, and plant composition of spent crude oil-polluted soil. Beni-Suef University Journal of Basic and Applied Sciences, 9 (26): 1-9, 2020.

Ikhajiagbe B and Saheed M, Application of biosynthesized nanoparticles in the enhancement of growth and yield performances of rice (*Oryza sativa* var. nerica) under salinity conditions in a ferruginous ultisol. FUDMA Journal of Sciences, 4 (1): 120 – 132, 2020.

Ikhajiagbe B, Igiebor FA and Ogwu C, Growth and yield performances of rice (*Oryza sativa* var. nerica) after exposure to biosynthesized nanoparticles. Bulletin of the National Research Centre, 45 (62):1-13, 2021.

Kamari H, Seyed Sharifi R and Sedghi M, Effect of Nano Zinc oxide foliar application and application of free living nitrogen fixing bacteria on yield and morphophysiological characteristics of Triticale. Crop Physiology Journal, 6 (22): 37-52, 2014.

Maxwell K and Johnson GN, Chlorophyll fluorescence - A practical guide. Journal of Experimental Botany, 51: 659-668, 2000.

Ngo QB, Dao TH, Nguyen HC., Tran XT, Nguyen TV, Khuu TD and Huynh TH, Effects of nanocrystalline powders (Fe, Co and Cu) on the germination, growth, crop yield and product quality of soybean (Vietnamese species DT-51). Advances in Natural Sciences: Nanoscience and Nanotechnology, 44 (19): 7315–7320, 2014.

Nwite JC, Essien BA, Essien JB, Eke BA, Keke CI, Igwe CA and Wakatsuki T, The contribution of different sources of ash on the soil improvement and rice yield in a sawah rice farming in southeastern Nigeria. In: Proceedings of the 44th annual conference of the Agricultural Society of Nigeria held at LAUTECH, Ogbomosho, Oyo State, pp 988–992, 2010.

Orjiude A, Rice production hits nine million MT in 2021 – Emefiele. Available [online]https://punchng.com/rice-production-hits-nine-million-mt-in-2021-emefiele/. Accessed 17th November, 2022.

Osawaru ME and Ogwu MC, Survey of plant and plant products in local markets within Benin City and environs. In: Filho LW, Ogugu N, Ayal D, Adelake L, da Silva I (eds) African handbook of climate change adaptation. Springer, Berlin. pp. 1-23, 2020.

Osawaru ME, Ogwu MC and Braimah L, Growth responses of two cultivated Okra species (*Abelmoschus caillei* (A. Chev.) Stevels and *Abelmoschus esculentus* (Linn.) Moench) in crude oil contaminated soil. Nigerian Journal of Basic and Applied Sciences, 21 (3): 215–226, 2013a.

Osawaru ME, Ogwu MC and Chime AO, Assessment of growth performance of two Okra species (*Abelmoschus esculentus* [L.] Moench and *Abelmoschus caillei* [A. Chev.] Stevels) exposed to crude oil contaminated soil. Nigerian Journal of Biotechnology, 26:11–20, 2013b.

Owusu Adjei M, Zhou X, Mao M, Rafique F and Ma J, MicroRNAs Roles in Plants Secondary Metabolism. Plant signaling and behavior, 16(7): 191 – 205, 2021.

Ozalkan CJ, Sepetoglu HT, Daur I and Sen OF, Relationship between some plants growth parameters and grain yield of chickpea (*Cicer arietinum* L.) during diferent growth stages. Turkish Journal of Field Crops, 15(1): 79-83, 2010.

Paul JJ, Sakunthala M and IniyaUdhaya C, Green synthesis of Manganese nanoparticles using the aqueous extract of *Ctenolepis garcini* (Burm. f.) C.B Clarke. International Journal of Botany Studies, 2 (5): 71-75, 2017.

Rosenberg HR, Chemistry and physiology of the vitamins. Interscience Publisher, New York. pp 452-453, 1992.

Sadak MS, Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenumgraecum*). Sadak Bulletin of the National Research Centre, 43 (38): 1-6, 2019.

Saheed M and Ikhajiagbe B, Assessment of physico-chemical properties of ferruginous utisol in Benin City, Edo State: possible impact on plant distribution. Studia Universitatis Vasile Goldis Arad Seria StiinteleVietii, 30(2):88–95, 2020.

Sims JR and Haby VA, Simplified colorimetric determination of soil organic matter. Soil Science, 112: 137–141, 1971.

Tripathi R, Tewari R, Singh KP, Keswani C, Minkina T, Srivastava AK, De Corato U and Sansinenea E, Plant mineral nutrition and disease resistance: A significant linkage for sustainable crop protection. Frontiers in plant science, 13, 883970. https://doi.org/10.3389/fpls.2022.883970, 2022.

Urriago-Ospina LM, Jardim CM, Rivera-Fernández G, Kozovits AR, Leite MGP and Messias MCTB, Traditional ecological knowledge in a ferruginous ecosystem management: lessons for diversifying land use. Environ Dev Sustain, 2020.

USD, Soil Survey Field and Laboratory Methods Manual. Soil Survey Investigations Report No. 51, Version 1.0. R. Burt (ed.). U.S. Department of Agriculture, Natural Resources Conservation Service, Lincoln, Nebraska, 435p, 2009.

Wu S, Rajeshkumar S, Madasamy M and Mahendran V, Green synthesis of copper nanoparticles using Cissusvitiginea and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artificial Cells, Nanomedicine, and Biotechnology, 48 (1): 1153-1158, 2020.

Zakaria H,Simpson K, Use of reversed phase HPLC analysis for the determination of provitamin A, carotene in tomatoes. Journal of Chromatography, 176, 109-117, 1979.