EFFECTS OF LEAF EXTRACT-BASED BIOSYNTHESIZED MANGANESE NANOPARTICLES ON GROWTH AND YIELD OF RICE (*Oryza sativa* VAR. NERICA)

BY

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SR/1861/RPR/21/112

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FACULTY OF LIFE SCIENCES
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BENIN CITY.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE (BSC), FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN. BENIN CITY, NIGERIA.

DECEMBER, 2022.

CERTIFICATION

This is to certify that this report was written by Violet Praise OKUNROBO with matriculation number LSC1708989 in accordance to the requirements of the Bachelor of Science Degree in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria for the partial fulfilment of the requirements of the award of a Bachelor of Science (BSc.) in Plant Biology and Biotechnology.

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DEDICATION

This work is dedicated to Almighty God for His grace to complete this study and to my parents Mr. and Mrs. Okunrobo Austin for their unending love.

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My sincere gratitude goes to Almighty God for His grace and providence for my academics and research work. I want to thank my supervisor, Prof. Beckley Ikhajiagbe for his contribution, supervision and advice on this research. I also want to thank the Head of the Department, Prof. E. O. Akpaja and the project coordinator, Dr. J. O. Erhabor.

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Also, to all my family members, friends, senior colleagues, PhD and MSc students; Mr. Francis Igiebor and Miss Asia Mostprecious, and my Project colleague, thank you all for your moral support and encouragement. God bless you all.

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ABSTRACT

The effects of leaf extract-based biosynthesized manganese nanoparticles on growth and yield of rice (*Oryza sativa* var. Nerica) was assessed. The study was carried out in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, utilized leaves from four plant species: Carica papaya (Pawpaw), Azadiracta indica (Neem), and the flowers of Hibiscus sabdarifa (red sorrel, commonly known as 'zobo leaves'). The rice variety employed was Nerica rice, sourced from a farm in Delta state, Nigeria. Both ferruginous and non-ferruginous soils were employed in the study. Nanoparticles were synthesized in the laboratory and applied within 3-4 hours post-synthesis to the rice plants, four weeks after planting. Application involved foliar spraying of each leaf species at room temperature, under sterile conditions. The nanoparticle treatments encompassed the control, ZM1 (5%), ZM2, and so forth (25%), ZM3 (50%), PM1 (5%), PM2 (25%), PM3 (50%), NM1 (5%), NM2 (25%), and NM3 (50%). Results from the study indicated strong correlation between the various treatments. PM2 (25%) significantly enhanced the morphological traits of the plants. There was no discernible difference between the treatments in terms of yield improvement, but the treatments had a favourable impact on the foliar chlorotic activity in the rice leaves. It is crucial to increase the plant's photosynthetic activity in order to increase productivity and yield. However, in addition to other agricultural applications, the findings from this study will help determine the potential of nanoparticles in crop growth and yield.

CHAPTER ONE

INTRODUCTION

1.1 Background

Advances in technology and increase in anthropogenic activity has brought about a paradigm shift to food availability and consumption. In recent times, bioengineered foods have found their way into the food chain supply mainly because of its availability for use in addition to its finely processed state which makes it attractive to the end user. Before now, importation of specific staple food crops like beans and rice were considered normal for a country with mass availability of land for cultivation. Limitations to the use of large farmlands in the country exists and one of such limitations is the nature of the soil in certain parts of the country which may not yield a bountiful harvest of most of our staple foods, hence, the option of importation to complement available food supply. Sequel to the ban on the importation of rice, Nigeria was recorded as the one of the leading importers of rice around the globe (Abbas *et al.,* 2018). Despite the efforts of the government to ensure steady production of staple foods in the country, crop production and yield seem to be significantly affected by environmental factors or nature (Osawaru *et al.,* 2013).

According to African Development Bank (2014), the economy of the country is primarily dependent on agriculture, despite the fact that Nigeria produces and exports oil and gas. Notwithstanding the continued vulnerability, agriculture still provides about 70% of all employment. Demand for and consumption of rice are anticipated to rise along with Nigeria's growing population. According to estimates of the Nigerian grain and field annual reports (2016), Nigerians consume 40 kg of rice per person each year. Only 1.7 million hectares of the nation's total 4.6 million hectares of rice-growing land were actually under feasible agricultural production (Nwachukwu *et al.,* 2018).

1.2 Literature Review

1.2.1 Production and Consumption of Rice in Nigeria

Rice (*Oryza sativa*) is an important food crop consumed in every household in Nigeria. It is a main source of carbohydrate providing energy for the body and it is also a highly demanded staple crop that generates income for Nigerian farmers especially small-scale producers who sell a larger percentage of their total production and consume only a few. Rice is being produced in almost all of Nigeria's agro-ecological zones. Nigeria has four different methods for growing rice: upland, lowland, irrigated, and mangrove/deep water methods (Ogumsimi *et al.,* 2016). The upland rice environment is characterized by a confined utilization technologically advanced agricultural inputs (fertilizers, pesticides, and advanced technology) during the growing season, whereas the lowland rice cultivation system can be observed in soggy lowland areas with differential water levels, and water regulation is inconsequential, but innovative farming inputs are relatively utilized (Rapu, 2016).

According to the Agricultural transformation agenda (2011), rice consumption was 5 million metric tons in 2010 and is projected to increase by 5.1% annually to 36 million metric tons by 2050. Figure 1 shows the graphical representation of these findings. The population of Nigeria is anticipated to increase annually, implying an increase in the demand for rice (Abbas *et al.,* 2018). Alfred *et al.,* (2014) reported that in Nigeria, milled rice is commonly used in industries to make other rice-based foods and medical products. Therefore, food and beverage industries (such as pasta and bread industries, beer and other liquor distilleries), as well as pharmaceutical companies, are the main industrial rice consumers in Nigeria (Rapu, 2016).

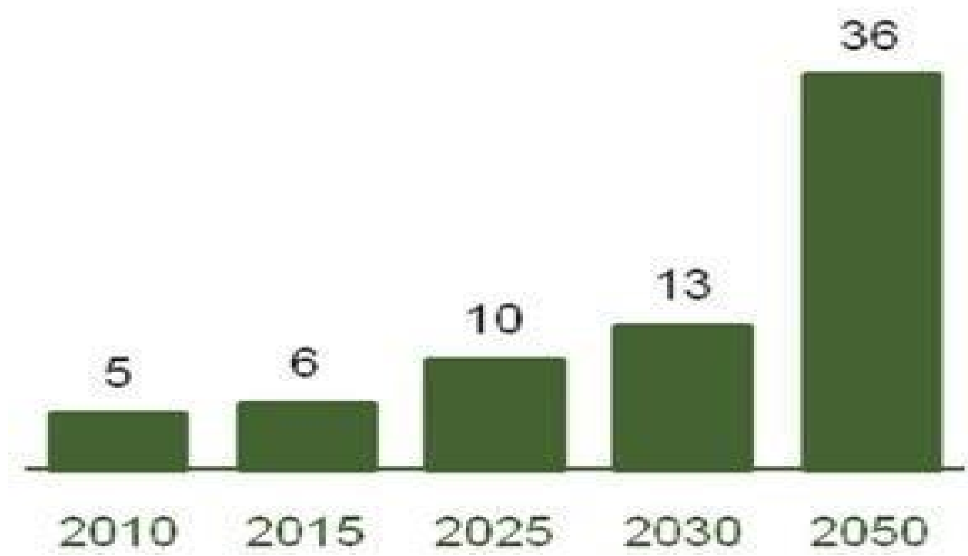


Figure 1: Projected consumption of rice (millions of metric tons)

1.2.2 Factors Affecting Rice Yield in Nigeria

The production, processing, marketing, and consumption of rice have all shifted to higher-value food items in advanced countries. It is crucial to note that, despite the emergence of technologydriven farming systems around the world, Nigeria's rice supply chain has not yet been developed to satisfy local and global market demands because there is little to no productivity improvement done to the rice, which limits its user acceptance to rural markets (Osabuohien *et al.,* 2018). Reports from a study carried out by Monturayo and Oyeleke, (2020) to evaluate factors affecting rice farming practices among farmers in Ogun and Niger States, Nigeria showed that inadequate finance, pest and diseases incidence, climate change, land tenure problem, birds disturbance, land degradation and poor soil fertility, nonavailability of quality seeds and agro-chemicals and high cost of fertilizers were significant to production output. This correlates with the report from Anthony *et al.,* (2021) that fire outbreak, thieves, cattle herdsmen attack and flood, transportation problem, poor storage facility, inadequate capital, lack of land/ tractor, means of transportation of rice seed to farm and harvested crop from farm gate to house and market; poor price, high cost of chemical, inadequate fertilizer, soil condition, lack of improved seed, inadequate extension officers, and lack of credit facilities were among the factors that influenced rice yield in Abuja.

1.2.3 Nanoparticles as a Tool for Crop Production Enhancement

Nigeria is in danger of experiencing a rice shortage, so there is a need to increase domestic rice production to help ensure food security in Nigeria (Ikhajiagbe *et al.,* 2021). In order to support Nigeria's food security, local rice production must be increased because of the possibility of supply constraints. If domestic rice production is enhanced for both national and international markets, the nation will undoubtedly generate a sufficient amount of foreign currency to advance long-term economic growth (Osawaru and Ogwu, 2020). There would be a significant increase in rice production yield if sustainable technological advancement and better management strategies utilized (Nwite *et al.,* 2010). Recent research has demonstrated that nanoparticles are created to improve crop growth and yield.

The term "nanoparticle" refers to a particle with at least one dimension smaller than 100 nm.

Different types of nanoparticles have been biosynthesized by scientists including Copper, Zinc, Silver and Manganese nanoparticles and utilized for crop production enhancement in series of research work carried out. For photosynthesis, respiration, and nitrogen (N) metabolism to occur, manganese (Mn), an essential element, must be available in trace quantities in plants (Dimkpa *et al.,* 2018). Nanoparticles of micronutrients are now used as fertilizers and agropesticides as a result of the development of nanotechnology and the increased use of nanoscale (1–100 nanometer in size) components in numerous products. The effects of nanoparticle nutrients on plant growth and productivity are discussed both negatively and favorably in recent reviews in this field (Du *et al.,* 2017; Tolaymat *et al.,* 2017; Raliya *et al.,* 2018). Notably, since nanoparticles are more reactive than their bulk-scale equivalents, these substances might be more toxic or advantageous for agricultural crops. The advantages of nano-enabled fertilizers could thus be a highly effective tool for addressing the issue of global food security with better understanding and management (Raliya *et al.,* 2018).

1.3 Taxonomy of Test Plant

Common name Rice (*Oryza sativa* L.) var Nerica

Kingdom Plantae

Superdivision Embryophyta

Division Tracheophyta

Subdivision Spermatophytina

Class Magnoliopsida

Superorder Lilianae

Order Poales

Family Poaceae

Genus *Oryza* L.

Species *Oryza sativa* L.

1.4 Justification

The country's staple food, rice, which is in high demand in every household, is significantly impacted by a number of factors, including land use restrictions and low yields per unit of land. As a result, scientists have been looking for ways to increase rice production and yield in order to balance the demand for the grain. Through carefully chosen research, scientists have found that applying nanoparticles to crop plants can increase their growth and yield while also taking into account any potential drawbacks or negative effects. Therefore, the purpose of this study was to demonstrate the importance of using biosynthesized nanoparticles to enhance rice yield and growth.

1.5 Aim of the Research

The aim of the experiment is to demonstrate the effects of leaf extract-based biosynthesized Manganese nanoparticles on growth and yield of rice (*Oryza sativa* var. Nerica).

1.6 Objectives of the Research

The objectives of this research were to:

1. Demonstrate the effects of Manganese nanoparticles on the morphological parameters of rice.

2. Investigate the impact of Manganese nanoparticles on foliar chlorosis.

3. Demonstrate the effects of Manganese nanoparticles on the overall yield of rice

CHAPTER TWO

MATERIALS AND METHODS

2.1 Description of study area

The study was carried out in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin in two phases. The first phase which was the synthesis of nanoparticles was carried out in the Environmental Biotechnology and Sustainability Research Group (EBSR) Laboratory. The second phase which was the phytoassessment of *Oryza sativa* plant exposed to biosynthesized nanoparticles was carried out at the Botanic garden of the Department of Plant Biology and Biotechnology.

2.2 Collection of Plant Samples

Leaves of four plant species including *Carica papaya* (Pawpaw), *Azadiracta indica* (Neem) and the flowers of *Hibiscus sabdarifa* (red sorrel commonly called 'zobo leaves') were used in the study. These were collected from healthy matured plant stands within the study area (Ikhajiagbe *et al.,* 2021).

2.3 Preparation of Plant Leaf/Flower Extracts

Fresh leaves of samples were collected, then washed thoroughly with distilled water several times to remove the dust particles and air dried. The dried leaves were cut into small pieces and ground to powder. About 5g of leaf powder was boiled in 100ml of distilled water at 80°C for 10 mins and filtered with Whatmann filter paper (No 1). The prepared extract solution was allowed to cool at 4°C and thereafter stored for further synthesis of nanoparticles according to the methods of

(Anandalakshmi and Venugobal, 2017).

2.4 Soil Collection and Sowing

The rice variety used was Nerica rice, which was procured from a farm in Delta state, Nigeria. Ferruginous soil, which contained high levels of iron, was obtained from the Botanic Garden for the study, and non-ferruginous soil was obtained from a composted garden at the Faculty of Agriculture, University of Benin, Benin City. The soil samples were measured into 30 plastic bowls weighing 20kg each. Seeds of *Oryza sativa* were sown into the bowls at the rate of 20 seeds per bowl. The experimental plot was a complete randomized block design with each treatment having three (3) replicates each.

2.5 Synthesis of Manganese nanoparticles (MnNP)

The method of Paul *et al.,* (2017) was adopted with slight alteration in the synthesis of Manganese oxide nanoparticles. Five (5ml) of aqueous leaf extracts were added to 50ml of aqueous solution of 0.2 M Potassium Permanganate ($KMnO_4$) while heating and stirring at 70°C and pH of 7 for 30 minutes. The solution changed from colourless to brown with the formation of a precipitate. The precipitate was thereafter centrifuged at 3000 rpm for 15 minutes and washed with distilled water for about 3 times.

2.5.1 Characterization of Manganese Nanoparticles (MnNP)

Manganese nanoparticles were characterized according to the method described by Chatterjee *et al.,* (2017) using the UV-Visible spectrophotometer at 250 – 800nm.

2.5.2 Application of Biosynthesized Nanoparticles

Nanoparticles were synthesized in the laboratory at four (4) weeks after planting of the rice. The biosynthesized nanoparticles were applied within 3-4 hours after synthesis to the rice strand plant and a booster dose was applied two weeks after initial application (Ikhajiagbe *et* *al.,* 2021). This application was done for each leaf species at room temperature using a foliar spray and under sterile conditions. The nanoparticle treatments included the control, ZM1 (5%), ZM2 (25%), ZM3 (50%), PM1 (5%), PM2 (25%), PM3 (50%), NM1 (5%), NM2 (25%), and NM3 (50%) (Nwite *et al*., 2010).

2.6 Growth and Yield Parameters Recorded

2.6.1 Plant Height

This was taken using a meter rule to measure the plant height from the base of the plant to the apex or tip of the plant.

2.6.2 Leaf Length

This was also measured using a meter rule from the stem where the leaf originated from to the tip of the leaf

2.6.3 Number of Leaves

This was recorded by physically counting the number of leaves in a particular strand of rice plant.

2.6.4 Stem Girth

This was measured using the Vernier caliper.

2.6.5 Length of Sheath

This was measured with a meter rule from the basal part of the leaf sheath to the beginning of the

first leaf.

2.6.6 Occurrence of Chlorosis

This was done by observing changes in colour of leaves using colour codes.

2.6.7 Changes in Foliar Colour

This was also done using colour codes.

2.7 Statistical Analysis

Principally, descriptive statistics was used to analyze the data obtained from the analysis using the Statistical Package for Social Sciences (SPSS) version 20 and Graph pad Prism 5. Since the soil used in the experiment was homogenized and homogeneity of the entire plot was also assumed to evaluate the data obtained.

Plate 1: Experimental plot at four weeks after planting

Plate 2: Researcher trimming and recording parameter values at eight weeks after planting

Plate 3: Experimental Plot ready for harvest

CHAPTER THREE

RESULTS

The tables and figures below show the results of the current study, which depict the effects of leaf extract-based biosynthesized manganese nanoparticles on the growth and yield of rice (Oryza sativa var. Nerica). Manganese nanoparticles biosynthesized with pawpaw, sorrel (zobo), and neem leaf extracts are shown in Figure 2 to absorb light at wavelengths between 300 and 600 nm. According to the figure below, the maximum absorption time of manganese nanoparticles produced by pawpaw was 4 hours at wavelengths of 350 nm, 400 nm, 450 nm, 500 nm, and 550 nm. Similarly, the absorption time of manganese nanoparticles biosynthesized with sorrel and neem leaf extracts was 4 hours, with wavelengths not significantly different from one another.

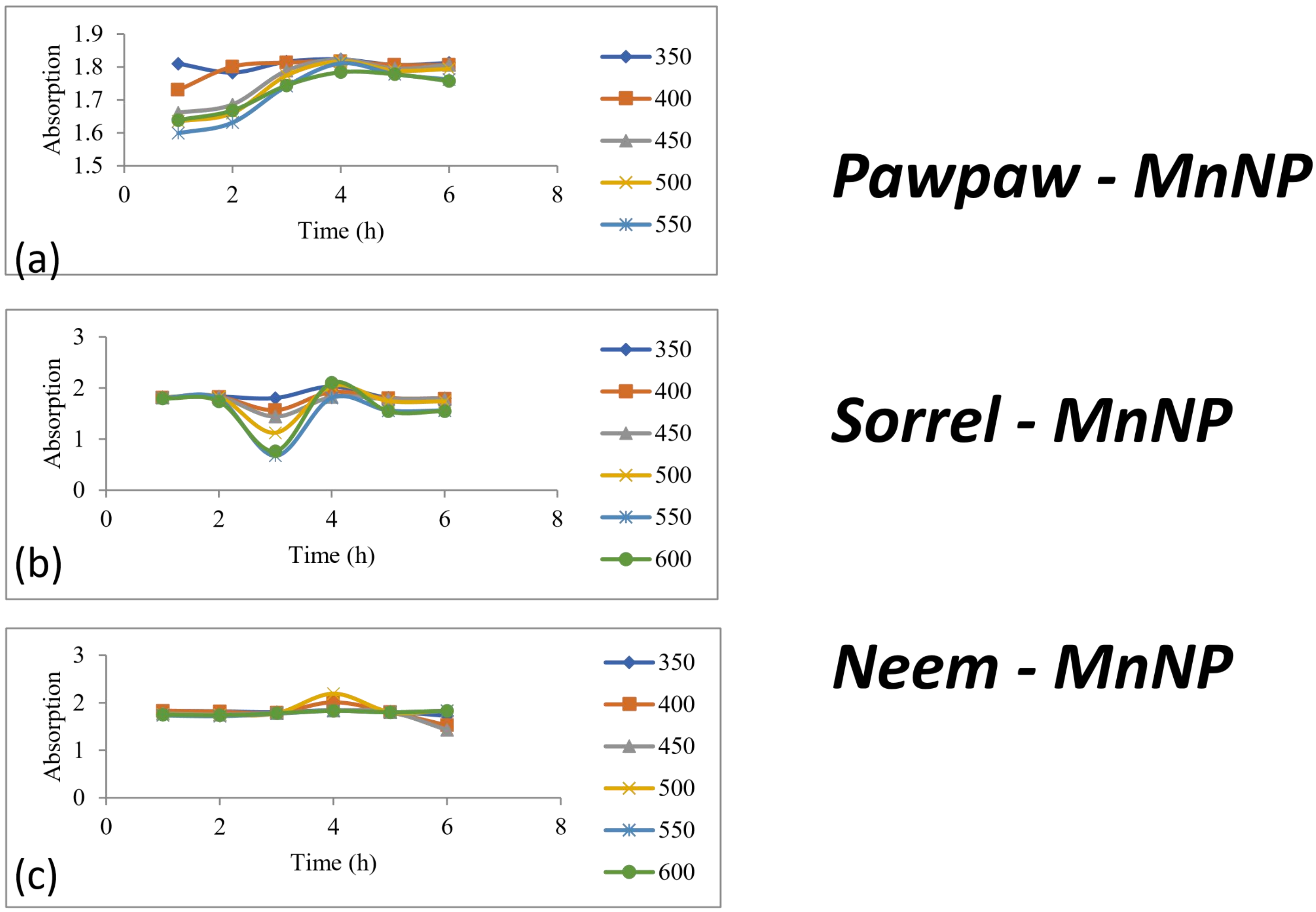


Figure 2: Absorption of manganese nanoparticles biosynthesized with (a) pawpaw (b) sorrel and (c) neem leaf extracts and measured at wavelengths of 300 - 600 nm

Over the course of eleven (11) weeks, the effects of manganese nanoparticles on plant height are depicted in Figure 3. According to the graph below, week 11 was the time when plant height was at its highest, ranging from about 17 cm in ZM3 (50%) to about 23.5 cm in PM1 (5%). The results showed that plant height increased gradually across all treatments from weeks 2 to 11, with treatment ZM3 (50%) having the smallest plant height of 17.2 cm and treatment PM1 (5%) having the highest plant height of 23.5 cm. At week 11, ZM1 (5%) had plant height of 22.3 cm, NM1 (5%) plant height of 18.5 cm, and NM3 (50%) plant height of 21 cm.

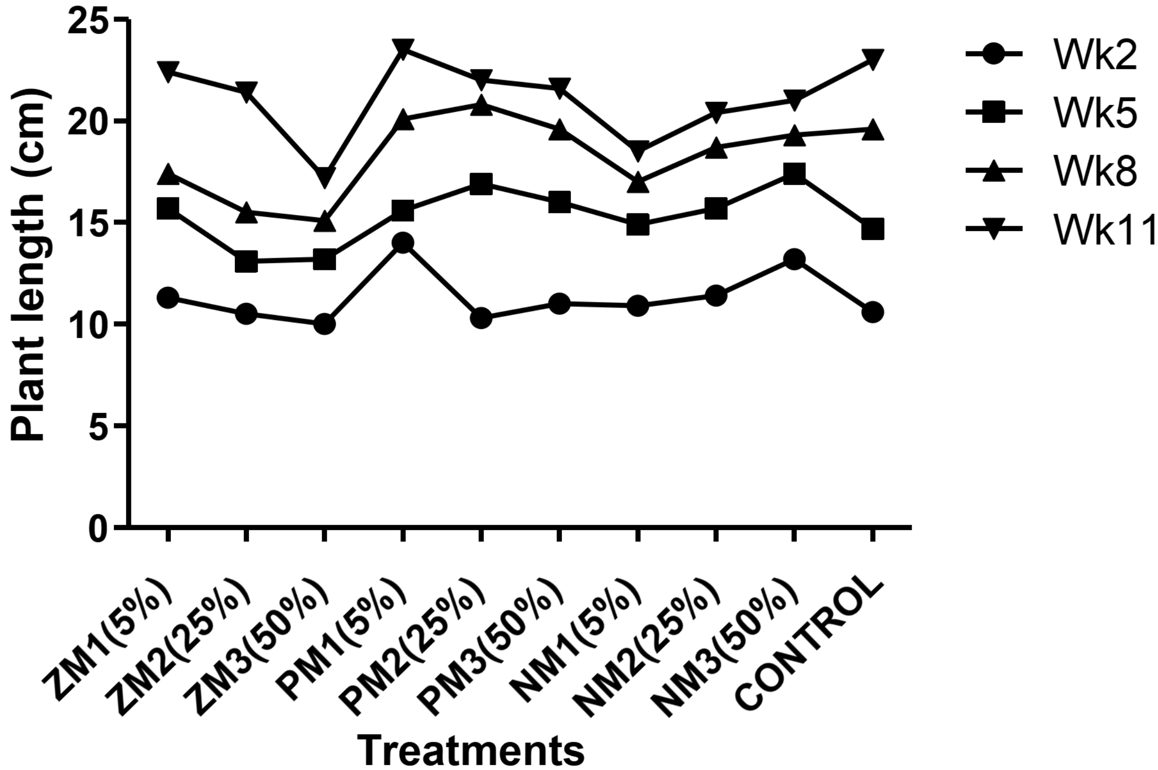


Figure 3: Impact of NP treatments on plant height over assessed period of growth

<u>KEYS</u>

ZM = Sorrel leaf extracts biosynthesized MnNP PM = Pawpaw leaf extracts biosynthesized MnNP NM = Neem leaf extracts biosynthesized MnNP

Figures 4, 5, 6, and 7 below show the findings regarding the effects of NP on leaf length, number of leaves, stem girth, and sheath length. According to the findings in figure 4, there was a significant difference between the treatments and the control in terms of leaf length. From week 2 to week 11, leaf length significantly increased across treatments, with the control having the lowest value (20.2 cm) and ZM3 (50%) having the highest value (41.0 cm). Figure 5's impact of NP on leaf count shows a gradual increase in the number of leaves, with the highest number at week 11 being 34 leaves for PM2 (25%) and the lowest at 23 for the control. Number of leaves significantly increased upon application of nanoparticles. From week 2 with a value of 9 leaves to week 8 with a value of 20 leaves and then a sudden increase at week 11 with a value of 32 leaves, ZM2 (25%) observed a progressive increase in leaf number. In the control, there were 23 leaves during harvest, however these number of leaves had increased to as much as 28 in NM3 (50%), 30 in PM1 (5%) and NM1 (5%), 31 in ZM3 (50%) and NM1 (5%), 32 in ZM1 (5%), 33 in NM2 (25%) and 34 in PM2 (25%).

The effect of NP treatments on stem girth over an 11-week period is depicted in Figure 6 All treatments showed a discernible improvement in stem girth, with the control having the highest value at week 11 (4.7 cm). With the exception of treatment NM1 (5%) and control, which had higher stem girth values at harvest of 4.1 cm and 4.7 cm, respectively, there was no discernible difference in stem girth values across treatments. The lowest value for stem girth was recorded in ZM1 (5%) with a value of 2.3 cm. Figure 7's impact of NP on sheath length showed an upward trend from weeks 2 to 11, with PM2 (25%) having the highest value of 21.3 cm and ZM3 (50%) having the lowest value of 15.1 cm at week 11.

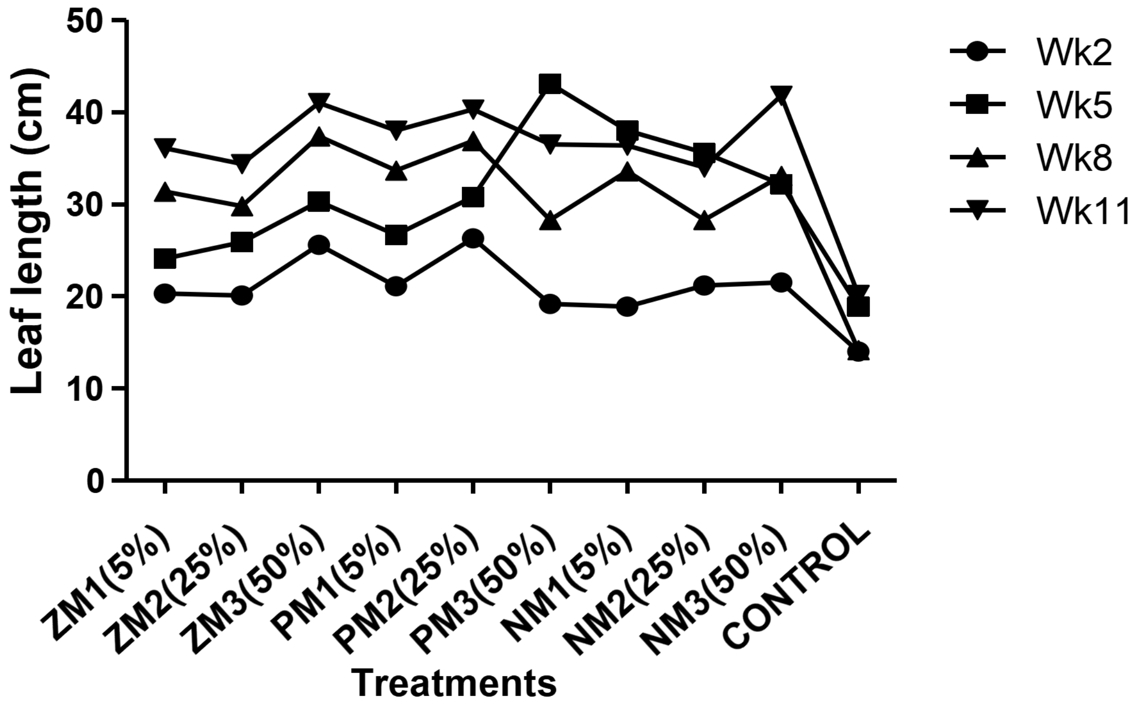


Figure 4: Impact of NP treatments on leaf length over assessed period of growth

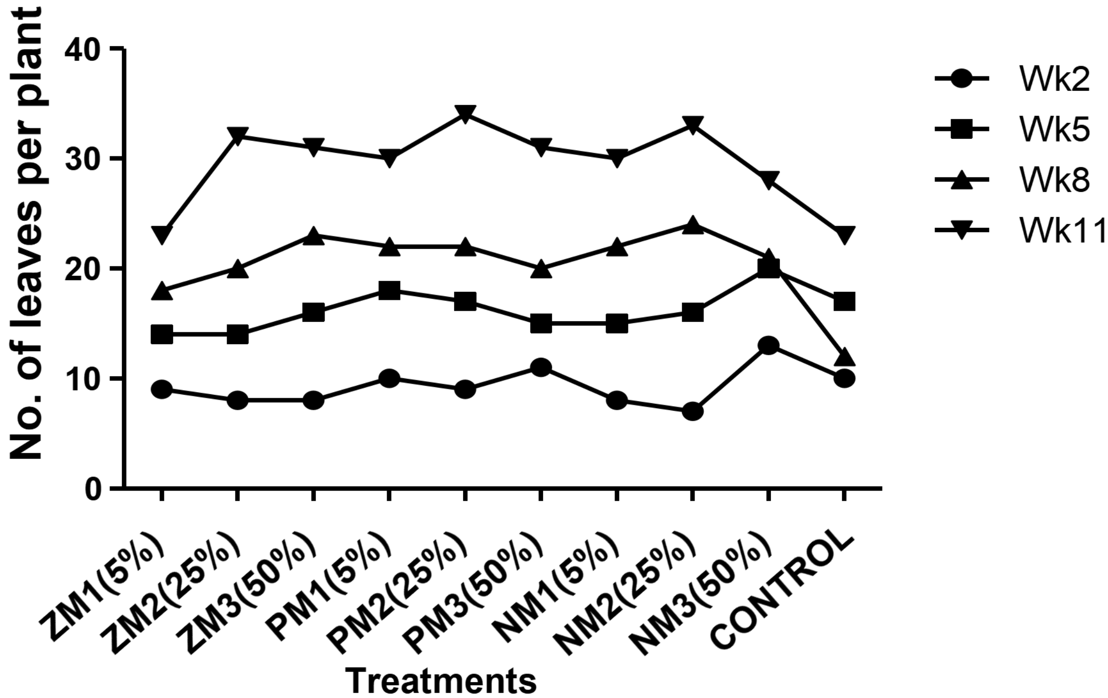


Figure 5: Impact of NP treatments on number of leaves over assessed period of growth

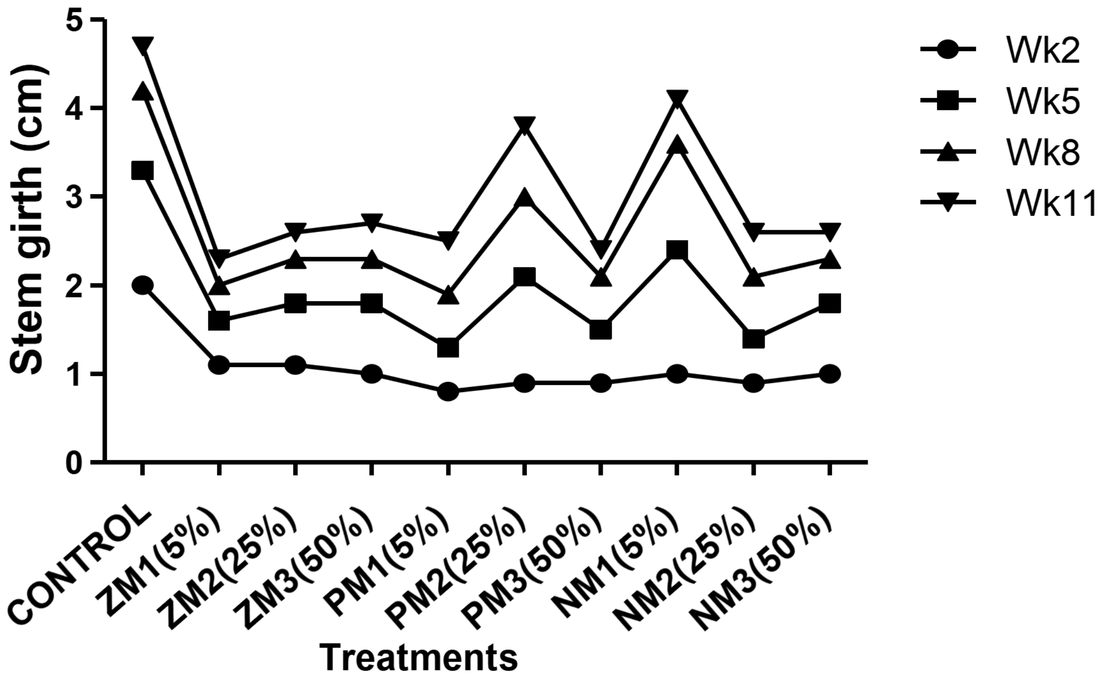


Figure 6: Impact of NP treatments on stem girth over assessed period of growth

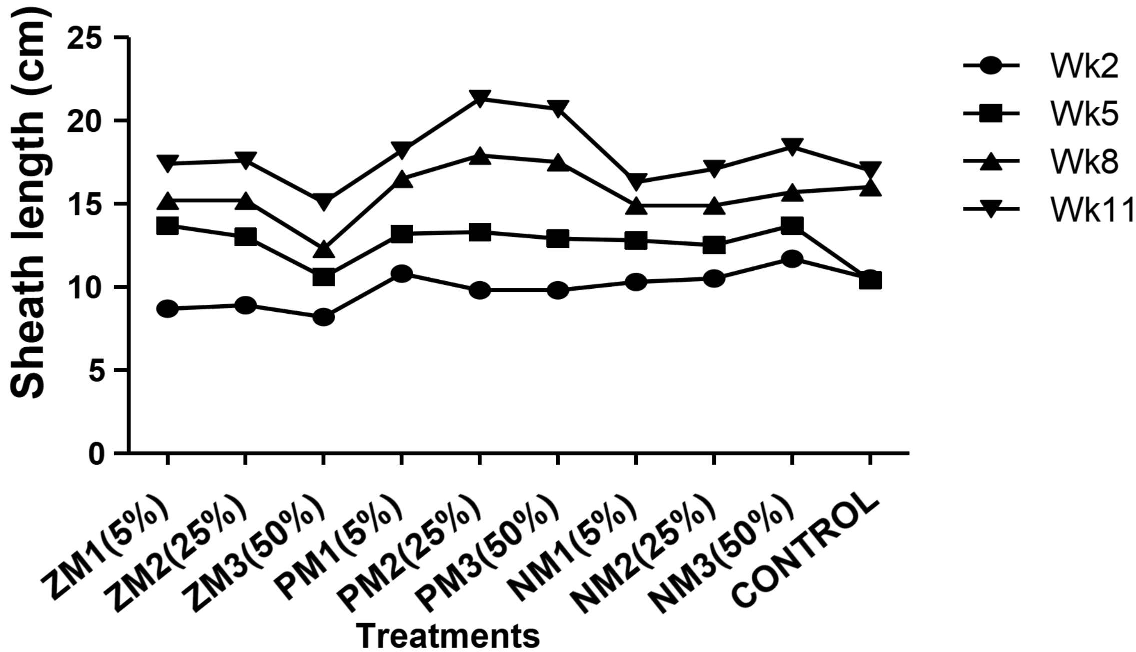
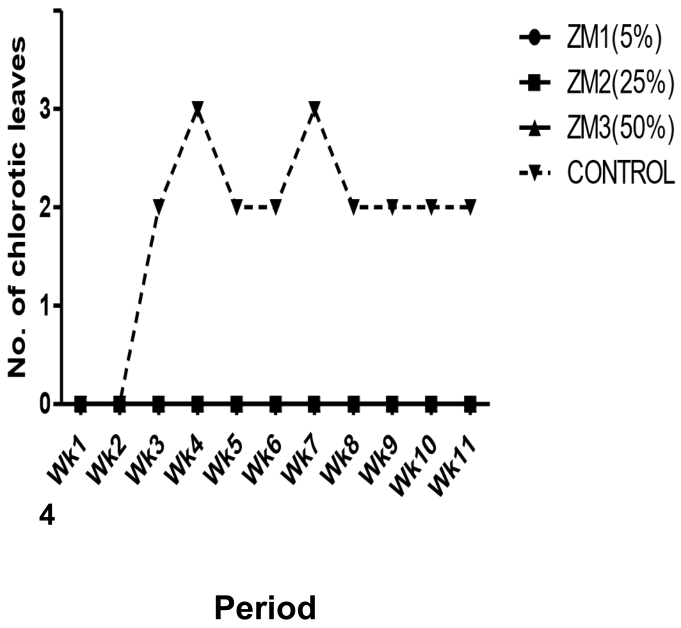


Figure 7: Impact of NP treatments on sheath length over assessed period of growth

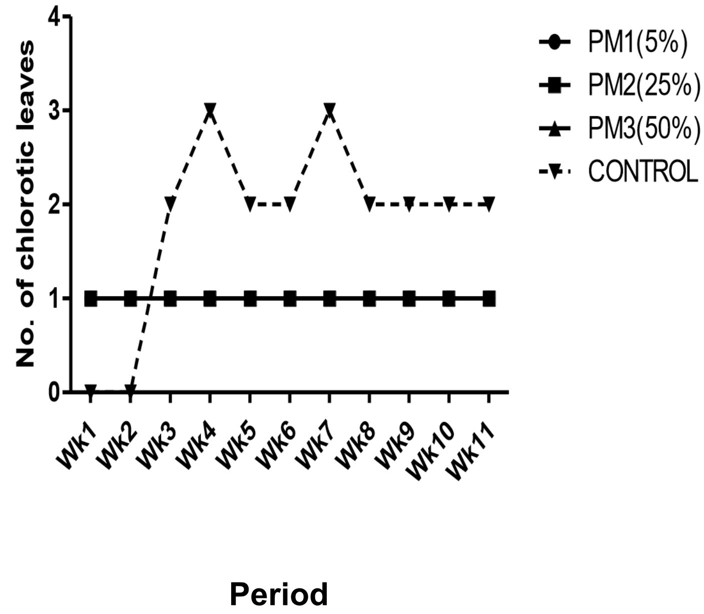
Figure 7 depicts the foliar chlorosis that resulted from the application of nanoparticles during the evaluated growth period. Results showed that the application of nanoparticles significantly changed the frequency of chlorosis. For the entire 11-week period, leaves of sorrel treated MnNP showed no signs of chlorosis, in contrast to the control, which had two chlorotic leaves at weeks 2, 5, 6, 8–11, and three chlorotic leaves at weeks 4 and 7. The subsequent decline in chlorotic leaves in the control can be attributed to leaf shedding and falling off. Only one chlorotic leaf was observed in leaves treated with pawpaw MnNP and one with neem MnNP at PM2 (25%) and NM2 (25%) respectively.

(A)



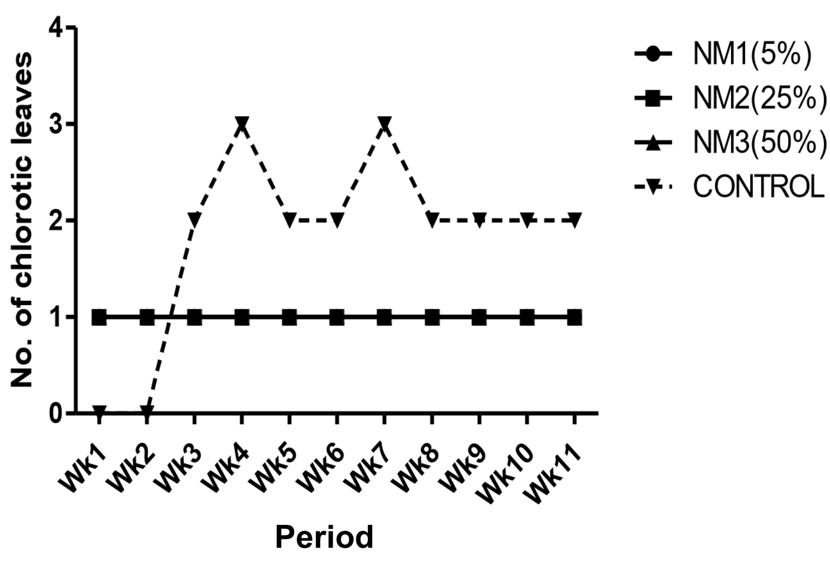
No. of chlorotic leaves
0
1
2
3
4
Wk1
Wk2
Wk3
Wk4
Wk5
Wk6
Wk7
Wk8
Wk9
Wk10
Wk11
Period
ZM1(5%)
ZM2(25%)
ZM3(50%)
CONTROL

(B)



No. of chlorotic leaves
0
1
2
3
4
Wk1
Wk2
Wk3
Wk4
Wk5
Wk6
Wk7
Wk8
Wk9
Wk10
Wk11
Period
PM1(5%)
PM2(25%)
PM3(50%)
CONTROL

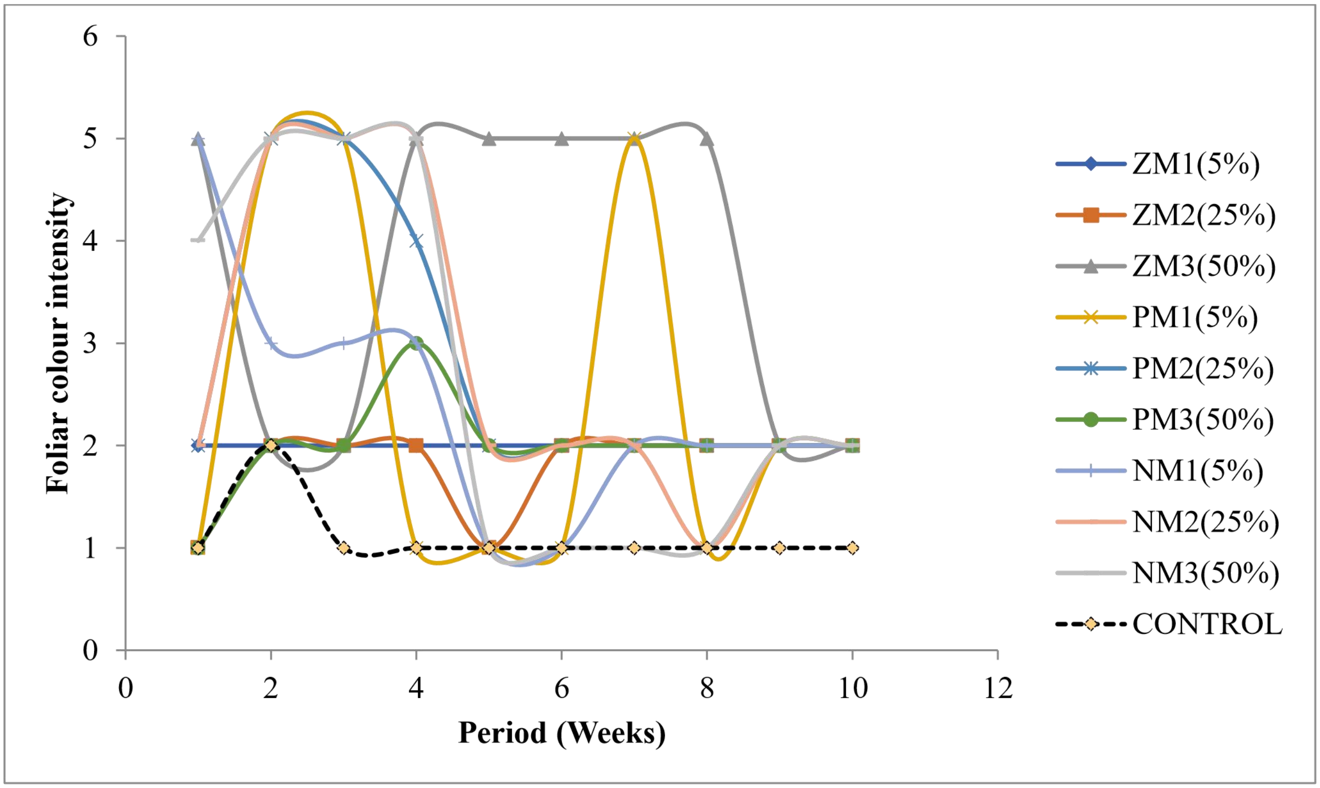
(C)



No. of chlorotic leaves
0
1
2
3
4
Wk1
Wk2
Wk3
Wk4
Wk5
Wk6
Wk7
Wk8
Wk9
Wk10
Wk11
Period
NM1(5%)
NM2(25%)
NM3(50%)
CONTROL

Figure 8: Occurrence of foliar chlorosis occasioned by application of nanoparticle biosynthesized with (A) Sorrel (B) pawpaw and (C) neem leaf extracts over assessed period of growth

Figure 9 shows the foliar color changes over an 11-week period after nanoparticle application. For the duration of the assessment period, ZM1 (5%) was lawn green. ZM3 (50%) was lawn green as of week 2, but from weeks 4 to 8, there was an increase in the shade, turning the plant drab green. At harvest, it turned back to lawn green, indicating a potential case of chlorosis. PM2 (25%) started out being a dull green color, but by week 5, it had lightened to a lawn-like shade, and it stayed that way until the experiment's end. Throughout the experiment, NM2 (25%) had a dull lawn-green hue. However, the control initially appeared lawn green as of week 2, then it gradually faded to a green-yellow color from week 3 until the end of the experiment, which could be explained by the development of chlorosis in the control. No leaf displayed the dark green color disposition during the experiment.



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

(Colour code 1 is green-yellow, 2 lawn green, 3 dark sea green, 4 lime green, 5 olive drab,6 dark olive green, 7 green, 8 dark green) Ikhajiagbe *et al.,* (2021).

Figure 9: Foliar Colour changes over the 11-week period following exposure to NP

The morphological parameters at the 11th week after sowing following exposure to MnNP are shown in Table 1. Plant height, leaf length, number of leaves, internode, stem girth, and leaf sheath are among the outcomes. There was a significant increase in leaf length in ZM3 (50%) (40.1 cm) compared to the control (20.2 cm). Similarly, number of leaves ranged from 23 in ZM1 (5%) to 34 in PM2 (25%) compared to 23 in control. After applying nanoparticles, leaf length and number significantly increased. However, there was no significant difference in plant height (p = 0.983), internode (p = 0.102), and length of sheath (p = 0.045).

Table 1: Morphological parameters at the 11th week after sowing after exposure to MnNPs

Treatment	Plant height (cm)	Leaf length (cm)	No. of leaf	Internode (cm)	Stem girth (cm)	Length of sheath (cm)
ZM1(5%)	22.4	36.1	23.0	4.0	2.3	17.4
ZM2(25%)	21.4	34.4	32.0	4.0	2.6	17.6
ZM3(50%)	17.2	41.0	31.0	4.0	2.7	15.1
PM1(5%)	23.5	38.0	30.0	3.0	2.5	18.2
PM2(25%)	22.0	40.3	34.0	3.0	3.8	21.3
PM3(50%)	21.6	31.2	31.0	3.0	2.4	20.7
NM1(5%)	18.5	29.6	30.0	3.0	4.1	16.3
NM2(25%)	20.4	31.1	33.0	3.0	2.6	17.1
NM3(50%)	21.0	24.2	28.0	5.0	2.6	18.4
ZEXTR	22.0	18.0	18.0	4.0	4.4	16.0
PEXTR	21.5	15.5	18.0	4.5	4.6	14.5
NEXTR	24.3	17.3	19.0	4.0	4.4	16.8
CONTROL	23.0	20.2	23.0	4.0	4.7	17.0
F-statistic	0.352	6.608	1.138	4.457	12.605	6.781
p-value	0.983	0.016	0.295	0.102	0.003	0.045
LSD (0.05)	6.7	7.7	9.5	1.4	0.9	5.2

Table 2 shows the yield parameters of rice occasioned by the application of MnNP over the assessed period of growth. Results for panicle branch, length of grain, diameter of grain, weight of grain, number of grains, weight of panicle length of panicleResults showed that ZM3 (50%) , PM2 (25%) and PM3 (50%) did not affect yield parameter at all, implying that the concentration may be too toxic for the plant, however all other treatments were no significantly different from the control (p = 4.7) in terms of panicle branch. All other yield parameters showed no significant difference from the control, some treatments had values lower than the control, for example, PM3

(50%) had 0.5 cm length of grain compared o the control (0.8 cm). Similarly, number of grains in ZM3 (50%) was 25 compared to the control (80).

Table 2: Yield parameters of rice occasioned by application of MnNP over assessed period of growth

Treatment	Panicle branch	Length of grain (cm)	Diameter of grain (cm)	Weight of grain (g/DW)	No of grains	Weight of panicle (g/DW)	Length of panicle (cm)
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
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
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

CHAPTER FOUR

DISCUSSION

The effects of leaf extract-based biosynthesized manganese nanoparticles on growth and yield of rice (*Oryza sativa* var. Nerica) have been assessed. To ensure food security for the anticipated 9 billion population by 2050, the world's agricultural output will need to rise by an estimated 60% (Tscharntke *et al.,* 2012; Lipper *et al.,* 2014). Utilizing cutting-edge technologies in agriculture, such as nanotechnology, may be essential for increasing productivity and sustainability (Bommarco *et al.,* 2012). Crop losses from biotic and abiotic stresses are significant and unforeseen (Jisha *et al.,* 2013). Many techniques, including conventional plant breeding, genetic recombination, polyploidy breeding, bioengineering, and seed priming, have been modified to hasten seedling emergence in the field and to give plants tolerance to unfavourable conditions (de Oliveira and Gomes-Filho, 2016). The use of nanomaterials has been reported to be safe compared to their counterparts in the improvement of crop yield and productivity (Kasote *et al,* 2021).

The results of this study are consistent with the reports of Ikhajiagbe *et al*., (2021), who provided evidence that Ag-NPs can significantly increase plant yield in ferruginous soil, a condition that was previously detrimental to rice yield tendency. Latif *et al.,* (2017) established that application of AgNPs made from *Ocimum basilicum* and *Mangifera indica* at concentrations of 20, 40 ppm showed an increase in shoot length, fresh and dry weight of shoot, chlorophyll, total carbohydrate and protein content in wheat plants' shoots. However, at concentrations higher than these, an inhibitory effect was seen which was in line with our findings that further increase in concentration of MnNP biosynthesized with pawpaw, neem and sorrel leaf extracts will not favour the yield and increase in plant height, leaf length and general growth parameters of rice. Shadak, (2019) also reported that foliar application of AgNPs (20, 40, and 60 mg/l) improved the fenugreek plant's growth parameters (such as shoot length, number of leaves per plant, and shoot dry weight) and increased some biochemical components, such as photosynthetic pigments and indole acetic acid (IAA) contents, which improved the yield quantity and quality of the yielded seeds as well as increasing antioxidant activity of the yielded seeds. Ikhajiagbe and Musa (2020) investigated the 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 and observed that with the application of AgNPs, there was significant improvements in growth responses of the plants exposed to salinity; especially at low salt stress and low AgNPs concentration.

PM2 (25%) concentration of MnNP enhanced leaf length, number of leaves and length of sheath, this concentration proved to be a moderate concentration for plant growth parameters as there was significant difference in the length of leaves and number of leaves compared to the control. This is supported by Keerthana *et al.,* (2021) confirming that biogenesis of ZnO nanoparticles has been successfully accomplished and is a safe, non-toxic, environmentally compatible material after recording a significant difference in increase of the growth parameters such as seed germination

(89 %), Total plant height (34 cm), Shoot height (18 cm), Root height (7.8 cm), Total fresh weight

(22 gm), Shoot fresh weight 914 gm), Root fresh weight (1.8 gm), Total dry weight (5.24 gm), Shoot dry weight (4.76 gm), Root dry weight (0.39 gm), Number of branches (7), Leaf area (7.8 cm), Number of pods (7) and Length of pods (13 cm).

Manganese nanoparticles had no significant difference in the yield of rice as all values were below the control, however, panicle branch was highest in NM1 (5%) (20 cm), an indication that at low concentrations, MnNP tends to have an impact on the plant. Similarly, ZM1 (5%) recorded the highest number of grains (75) amongst other treatments of MnNP and the lowest number of grains was recorded in ZM3 (50%) (25). This is a confirmation that at high concentrations of MnNP, yield of rice plant is negatively affected. This was supported by Salem *et al.,* (2016) who reported from findings in their study that in determining the potential of sulphur nanoparticles for enhancing tomato's growth, increasing the concentration of sulphur nanoparticles from 100 ppm to 300 ppm cause an increase in root and shoot lengths, while higher concentration 400 ppm and 600 ppm induced an inhibitory effect. Results of this study reveal that SNPs have the potential to enhance root and shoot growth of tomato and the effect is concentration dependent. The impact of MnNP on growth and yield of rice plant depends on the concentration being applied.

The presence of foliar necrosis was significantly different between MnNP and the control. Comparing the sorrel leaf extract biosynthesized MnNP treatments to the control revealed that the treatments had no foliar necrosis while the control had it. Comparison with the control also revealed an occurrence of foliar chlorosis in the control and one occurrence in the 25% concentration of both pawpaw leaf and neem extracts biosynthesized MnNP. These findings demonstrate that manganese biosynthesised nanoparticles promote photosynthesis by increasing the levels of photosynthetic pigments chlorophyll a, b, carotenoids and other associated pigments. This is in line with the results obtained by Sadak (2019) who reported significant increases in all photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids, and total pigments) of the fenugreek plant in response to treatment with different concentrations of AgNPs. These findings are consistent with the findings of Farghaly and Nafady (2015) and Latif et al. (2017), who reported that AgNPs significantly promote photosynthesis, and this is closely related to a change in nitrogen metabolism.

4.1 Conclusion

The study has revealed the effects of leaf extract-based biosynthesized nanoparticles on growth and yield of rice (*Oryza sativa* var. Nerica). Some chosen growth and yield parameters showed a strong correlation between the various treatments. PM2 (25%) significantly enhanced the morphological traits of the plants. There was no discernible difference between the treatments in terms of yield improvement, but the treatments had a favourable impact on the foliar chlorotic activity in the rice leaves. It is crucial to increase the plant's photosynthetic activity in order to increase productivity and yield. However, in addition to other agricultural applications, the findings from this study will help determine the potential of nanoparticles in crop growth and yield.

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