EVALUATION OF THE MITIGATING EFFECTS OF ZINC OXIDE NANOPARTICLES ON *Vigna unguiculata* (L.WALP) EXPOSED TO CADMIUM STRESS

BY

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DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCE

UNIVERSITY OF BENIN.

APRIL, 2024

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B. SC. HONS) DEGREE IN PLANT BIOLOGY AND BIOTECHNOLOGY

APRIL, 2024

CERTIFICATION

This is to certify that this project was prepared and presented by Success NKUKU (Miss) of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo state, Nigeria.

.. **..**

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... **..**

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DEDICATION

This work is dedicated to God almighty and also to my beloved mother Mrs Patience Nkuku.

ACKNOWLEDGMENT

My utmost appreciation goes to God almighty for his mercies and strength.

I sincerely want to acknowledge my project supervisor, Prof. B. O. Edegbai, thank you sir for your support, guidance, encouragement and teachings all through this work. God bless you sir.

I want to also appreciate the Head of Department, Prof. D. E. Vwioko and all the lecturers of the Department for seeing me through my undergraduate programme.

To my wonderful mother, Mrs Patience Nkuku. I say a very big thank you for your love, care, support and for all the sacrifices you made all through my educational pursuit. I sincerely appreciate my siblings, Favour, Kenneth, Peace, and Good news, God bless you all.

To my amazing friend Suzan Felix, I say a very big thank you, God bless you abundantly.

To my colleagues and course mates, God bless you all for all your contributions and encouragement.

Finally, i will not fail to appreciate someone who has been a major source of encouragement and support to me, Lawani Jerry, i am grateful for your presence in my life.

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ABSTRACT

The above study explores the impact zinc oxide nanoparticles (ZnO NPs) play in cadmium (Cd) clean-up as well as their ability to affect *Vigna unguiculata* growth. Cowpea, often referred to as black-eyed pea or *Vigna unguiculata*, is a significant legume crop that is extensively grown due to its high nutritional content and resistance to environmental stresses like heavy metals. The hazardous heavy metal cadmium seriously endangers both human health and agriculture. Owing to their distinctive physicochemical characteristics, such as their high reactivity and large surface area, zinc oxide nanoparticles are considered to be excellent candidates for a range of agricultural applications. The purpose of this study is to evaluate ZnO NPs' impacts on *Vigna unguiculata* growth metrics and their capacity to reduce Cd toxicity. The experimental setup consisted of control and four treatments (25mg/kg) for the heavy metal and (10, 30 and 50mg/kg) for the Nanoparticles in three replicates. Data for growth (germination, plant height, stem girth, leaf loss and number of leaves) were collected weekly during the course of the experiment. Preliminary results show that the application of zinc oxide nanoparticles (ZnO NPs)

positively impacts the developmental processes of *Vigna unguiculata*. The plants exposed to a lower dosage of ZnO NP and Cd exhibit improved growth characteristics compared to the plants exposed to higher concentration of Zinc Oxide, suggesting a stimulatory effect on the plant's overall development. This study adds to the knowledge of the potential advantages and applications of zinc oxide nanoparticles in agriculture, specifically their ability to mitigate Cadmium toxicity and their positive influence on the growth of *Vigna unguiculata*. These results have implications for sustainable farming practices and the development of eco-friendly strategies to combat heavy metal pollution in Agricultural system.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The group of elements known as heavy metals is heterogeneous, with differences in their chemical characteristics and functions. Mostly found in the transition elements of the Periodic Table. High density (usually defined as metals denser than 5 g/cm³), low levels of toxicity, Higher concentrations of certain heavy metals can be hazardous, even though they are necessary for life in tiny amounts. Natural phenomenon (found in the crust of the Earth, frequently encased in rocks and mineral substances), Hazardous vs. Essential: Some, like copper (Cu) and iron (Fe), are essential for certain life processes in minute quantities. Some, like mercury and lead, are understood to have no biological purpose. Typical examples are lead (Pb), mercury (Hg), nickel (Ni), arsenic (As), cadmium (Cd), chromium (Cr), and zinc (Pb). Heavy metals are also described as those described that have a density that is at least five times that of water (Arif *et al*., 2016; Vaghele 2020)

Essential metals like copper, which is necessary for the synthesis of haemoglobin and the digestion of carbohydrates, support the body's metabolism; nevertheless, a large amount of these heavy metals can lead to cell destruction. Similar to the extent to which heavy metals are necessary for plants, these metals likewise function as cofactors, initiate enzyme reactions, exhibit ductility and conductivity, and maintain cation stability. But when these metals are present in higher concentrations than necessary, they become poisonous. Essential heavy metal deficiencies have an impact on both agricultural productivity and the well-being of people. Non-essential metals can be hazardous even in small amounts. They don't decompose in the surroundings as well as metabolise into different precursor molecules. Heavy metals leak into the environment as a result of industrial, household, agricultural, medical, and technological use, as well as natural occurrences such as weathering of rocks and volcanic eruptions. Heavy metals are dangerous, persistent in the environment, and non-biodegradable. They are classified as pollutants and have a detrimental effect on humans, animals, and plants. This is the main problem. An ecosystem's heavy metals create a chain of pollution.

1.2 Taxonomy Of Cowpea

The cowpea [*Vigna unguiculata* (L.) Walp] is an annual herbaceous legume from the genus *Vigna*, Family Fabaceae. Due to its ability to withstand sandy soil and little rainfall, it is a significant crop in semiarid areas of Asia and Africa. Due to the fact that the plant's root nodules can fix atmospheric nitrogen, it requires relatively little in the way of inputs, which makes it an excellent crop for resource-constrained farmers and a good fit for intercropping with other crops. Cowpeas are an important economic crop in many developing regions because of their high protein content, adaptability to various soil types and intercropping systems, tolerance to drought, and capacity to improve soil fertility and prevent erosion.

It originated and was domesticated in Southern Africa and was later moved to East and West Africa and Asia. It spread to East and West Africa, Asia, and Southern Africa after being domesticated there. Along with various vitamins and minerals, the grains provide 25% protein. For farmers, the sale of the stems and leaves for animal feed during the dry season is another important source of revenue. The entire plant is fed to animals as fodder; in fact, its name probably originated from the fact that only take between 65 to 70 days to fully mature depending on if you want to harvest them green or dried. They are not a frost-hardy plant and do better in warmer climates, making them a popular Southern crop.

The cowpea was one of the first crops to be domesticated, having originated in Africa (Gomez 2004).

1.2. 1. Scientific Classification of Cowpea

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass Rosidae

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Genus: *Vigna*

Species: *Vigna unguiculata*

1.2.2 Effects of Heavy Metal on Cowpea Production

Essential and non-essential heavy metals generally produce common toxic effects on plants, such as low biomass accumulation, chlorosis, inhibition of growth and photosynthesis, altered water balance and nutrient assimilation, and senescence, which ultimately cause plant death.

One crop that may draw toxic substances from the soil, including, lead, cadmium, and chromium, is called *Vigna unguiculata*. If certain metals are taken in significant amounts, they may be dangerous to human health. The kind and concentrations of metals in the soil, the length of exposure to it, and the plant's stage of growth are some of the variables that affect how much heavy metal build up occurs in these crops.

Certain cowpea cultivars have been proven to resist heavy metal contamination and even help clean up contaminated soil, despite the potential hazards of heavy metal contamination. This shows that these crops might be useful as phytoremediation agents, clearing contaminated soils and giving farmers a way to make money and provide food.

1.3 Nanotechnology

The science of processing and modifying materials at the nanoscale level is known as nanotechnology. It is a natural substance with a variety of scientific uses that affects materials through physiochemical processes.

From the moment of emergence, plants are constantly subjected to difficult environmental conditions. Abiotic (hot temperature, drought, salt, low temperature, heavy metals, etc.) and biotic (diseases, pests, and weeds) stresses all but prevent plants from growing, developing, and reproducing normally. Crop output and quality might suffer significant losses as a result of these unfavourable factors. Plants can now effectively combat the negative impacts of both stressors that are abiotic and biotic in nature thanks to the development of nanotechnology.

Nanoparticle technology is rapidly advancing, providing novel and effective treatments for various diseases (Emerich and Thanos, 2003).

Nanotechnology is a novel way to improve the agricultural industry by providing fresh methods for increasing productivity and promoting tolerance to various pressures. Abiotic stressors, particularly drought along with salinity, are the main factors that can negatively impact crop productivity and plant growth, directly endangering the food supply needed to feed the world's expanding population. By increasing production of hormones and photosynthesis pigments as well as minimising damage caused by oxidative stress by triggering antioxidant enzymes, salinity, and drought, nanoparticles (NPs) have shown a promising role in improving plant growth and the development for various abiotic stress tolerances. The application of the field of nanotechnology is an advance towards a modernised agricultural system. (Usman *et al*., 2020).

Like traditional approaches, Nano remediation can be used both in in situ as well as ex situ. Contaminants are remedied in the original site when using the in-situ strategy; while using the ex-situ approach, contaminants are moved to an entirely distinct region.

Specifically, ZnO nanoparticles show a lot of potential because they are inexpensive, easy to prepare, and safe for the environment. They have a great deal of potential to lessen the effects of abiotic as well as biotic providing an extensive array of potential applications and the chance to solve important problems in plant cultivation.

1.3.1 Nanoparticles

Nanoparticles are spherical, polymeric particles composed of natural or artificial polymers. They range in size between 10 and 500 nm. As a consequence of their spherical shape and high surface area to volume ratio, these particles have a wide range of potential applications (Berry & Curtis, 2003).

1.3.2 Why Are Nanoparticles Important

Nanoparticles exhibit different characteristics compared to their bulk counterparts. Their small size and high surface area-to-volume ratio give them enhanced physical, chemical, and optical properties. For example, nanoparticles may display increased reactivity, improved catalytic activity, and unique optical properties such as enhanced colour or fluorescence. (Ali *et al.* ,2021).

1.4 Interaction with Heavy Metals

As a result of their small size, large surface area, higher affinity, and elevated permeability via the smaller pores, nanoparticles have a significant deal of potential for eliminating heavy metals from the air, water, and soil.

Plant species interact with NPs through uptake, translation, and accumulation (Rico et al., 2011). Various nanomaterial kinds, including Au, Ag, Al2O3, CeO2, Cu, Fe 2 O3, Fe, SiO2, TiO2, Zn, ZnO, have been reported to have an influence on the physiological functioning of plants and plant development. Singla *et al.,* 2021).

The biological substances that readily stabilise NPs include proteins, enzymes, carbohydrates, and even entire cells. These interactions with various other biomolecules enhance the antibacterial efficacy of NPs by enhancing their contacts with microorganisms (Botes and Cloete 2010).

ZnO-based nanoparticles in particular have a wide range of unique properties, such as surface area, shape, and particle size, which make them up especially significant for plant tissues. Plant cell walls, which act as natural barriers with pores ranging in size from 3 to 8 nm and thickness from 5 to 20 nm, have an impact on these interactions. Because smaller nanoparticles can more effectively permeate plant tissues than larger ones, size is a critical factor. There are two potential pathways through which bigger nanoparticles could infiltrate plants. First, new pores that are marginally larger than the typical ones may be created by the nanoparticles in order to pass through. Second, bigger nanoparticles may be able to get through due to the weakening of cell walls brought on by reactive oxygen species (ROS). (Wang et al., 2023).

1.5 Roles Nanoparticles Play In Plant Physiology And Biochemistry

Nanoparticles plays diverse roles in plant physiology and biochemistry as a result of their diversity which enables them play varied functions.

1.5.1 Zinc Oxide Nanoparticles (ZnONPs)

By strengthening the structural integrity of membranes, scavenging reactive oxygen species produced by stress, controlling differentiation of cells, nutrient and water transport, and adjusting the production of sugars, amino acids, enzymes involved in protein synthesis, photosynthetic pigments (Wang *et al* 2018) and osmoregulators, zinc oxide nanoparticles can improve a plant's ability to withstand salt.

Proper examination has also been done on the impact of ZnO-NPs on the physiological, biochemical, and antioxidative activities in different plants. (Faizan *et* al., 2021).

According to (Javed *et al*., 2017) and (Deka *et al*., 2019), Zn-NPs have demonstrated a notable potential to enhance crop productivity under abiotic challenges and are both stable and reasonably priced to synthesise (Sharifan *et al.,*2019).

1.5.2 Silver Nanoparticles (AgNps)

They are tiny particles of silver, measuring 1-100 nanometres in size, and their interactions with plants are very unique and interesting.

Silica increases plant tolerance to drought, frost and lodging. Silica strengthens plant cells which means reduced water loss, less frost damage, more root growth (Jasim *et al.,* 2017) and a decrease in lodging. Silica increases plant resistance to fungal disease and pest attack because of harder epidermal cells. Antimicrobial activity, Growth promotion, and effective in facilitating the penetration of water and nutrients through seed coat (Savithramma *et al.,* 2012).

Wound healing: Applications of AgNps may accelerate wound healing in injured plant tissues. The effects of AgNps on plants depend heavily on factors like concentration, size, shape, and surface coating of the nanoparticles. Green synthesis methods using plant extracts offer a more Eco-friendly approach to producing AgNps compared to traditional chemical methods.

1.5.3 Silica Nanoparticles (SiO_2Nps)

Stimulate seed germination and plant growth. Increase chlorophyll content and photosynthesis efficiency. Improve nutrient uptake and utilization.

Stress tolerance (Zahedi *et al.,*2020) by Strengthening cell walls, making plants more resistant to drought, salinity, heat and heavy metal stress (Gao *et al*., 2018). Reduce damage from heavy metals and other environmental stressors. Disease resistance by Triggering plant defence mechanisms against fungal and bacterial diseases. Enhance physical barriers against pathogen penetrations.

1.5.4 Titanium Dioxide Nanoparticles (TiO_2Nps)

One positive effect of titanium oxide nanoparticles is enhanced crop growth. Titanium is the ninth most abundant element in the earth's crust, and it can increase plant productivity by about 10–20%. Studies have shown that the application of these nanoparticles can promote photosynthetic activity and nutrient uptake in tomato plants. Additionally, titanium oxide nanoparticles can improve nutrient availability in soil (Ogunkunle et *al*., 2020). These nanoparticles increase the release of phosphorus from soil particles, leading to improved nutrient availability for plants promoting growth (Zhang *et* al., 2020). Moreover, the high surface area of titanium nanoparticles allows them to absorb pollutants and filter wastewater, making them beneficial for environmental remediation purposes.

Nano TiO2 can improve light absorption, increase Rubisco and photosynthesis enzyme activity. Increase nitrate absorption, accelerate the conversion of inorganic to organic material and enhance the wet and dry weight of plants. Foliar application of TiO2 on Zea mays during the reproductive stage increased pigmentation and the yield (Morteza *et al*., 2013). A study on the influence of foliar use of titanium nanoparticles on tomato (Solanum lycopersicum L.) showed that the nanoparticles increased the antioxidant enzymes properties. (Song *et* al., 2013)

1.6 Significance of Addressing Heavy Metal Pollution In The Environment

The importance of addressing heavy metal pollution in the environment is significant for several reasons. Firstly, heavy metals pose a serious threat to human health. Exposure to high levels of heavy metals such as lead, mercury, cadmium, and arsenic can lead to various health issues, including organ damage, developmental disorders, neurological problems, and even cancer.

Secondly, heavy metals can accumulate in the food chain, affecting the safety and quality of the food we consume. Plants can absorb heavy metals from contaminated soil or water, and these metals can then be transferred to animals or humans through the consumption of contaminated food. This can result in the ingestion of toxic levels of heavy metals, compromising food safety and posing a risk to public health.

Furthermore, heavy metal pollution can have detrimental effects on the environment itself. These metals can persist in the soil and water for long periods, negatively impacting biodiversity and ecosystem health. They can disrupt natural processes, harm aquatic life, and contaminate water sources, thus impairing the overall ecological balance.

Addressing heavy metal pollution is crucial to protect human health, ensure food safety, and preserve the integrity of our ecosystems. This requires implementing strict regulations and practices to reduce the release of heavy metals into the environment, developing effective monitoring systems, and implementing remediation strategies to clean up contaminated areas. By taking these actions, we can mitigate the risks associated with heavy metal pollution and safeguard both our health and the environment.

1.7 Aim and Objective of The Research

Aim

To mitigate the effects of heavy metals

Objectives

1. To access the effect of Zinc Oxide Nanoparticles on the growth of cowpea plant.
2. Investigate the effect of Zinc Oxide Nanoparticles on stress tolerance and heavy metal uptake of *Vigna unguiculata*.

1.8 Literature Review

When cowpea (*Vigna unguiculata* L. Walp) was treated with varying amounts of cadmium, the percentage of germination, growth metrics, and biochemical contents were all reduced. From January to March 2011, the experiment was carried out at the Botanical Garden, Department of Botany, Government Arts College, Thiruvannamalai, Tamil Nadu. Cowpea plants in the pot culture experiment were examined on three separate sampling days (15, 30, and 45), respectively, in soil supplemented with varying concentrations of cadmium (10, 30, and 50 mg Kg-1). A polythene sheet was used to line the interior surfaces of the pots. Each container holds 3 kg of soil that has been air dried. In every pot, six seeds were sowed. Every day, the entire field was irrigated in pots. After a week of growth, a maximum of three plants per pot were trimmed. In comparison to untreated plants, cadmium at all tested levels (10, 30, and 50 mg kg-1) reduced the growth parameters of cowpea plants, including root and shoot length, fresh and dry weight of root and shoot, total leaf area, and biochemical constituents like chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid contents. (Vijayaragavan *et al.*, 2011).

A 2 by 2 mm sieve was used to filter the top soil that was taken from the University of Ibadan's agricultural research site. To create a homogeneous sample, SEO (spent engine oil) from the University of Ibadan's maintenance department was combined and well mixed in a container. Baseline heavy metal levels were evaluated in the soil and SEO. In seedling pots, 350g of intentionally polluted soil were subjected to seven treatments and a control. There were five replications for each group (treatment and control), totalling forty pots. After adding different amounts of SEO to each treatment group in the following orders: 0, 5, 10, 15, 20 in, 25, 50, and 75 ml, respectively, the pH of the soil was determined using a pH metre. (Bello *et al.,* 2020).

To assess the early growth performance of cowpea and groundnut seeds acquired from Uyo, Nigeria, a pot experiment was carried out. The seeds were planted in soil that had been exposed to varying levels of Zn, Fe, Cu, Cr, and Pb. In order to determine the solution of their salts, heavy metals were applied to the soil: Zn (NO_3) $CuSO_4$.5H2O, $FeSO_4.7H_2O$, K_2Cr_2O7, $2.6H_2O$, and $Pb(NO_3)_2$. There were five (5) experiments carried out, one for each metal. There were three concentration treatments (10, 100, and 1000 ppm) in each experiment, meaning that there were 15 experimental units total for the two plants. Two more trials, one for each plant in the same soil but without the addition of heavy metals, functioned as the control. The germination rates of seeds were affected differently by the metal. Using the metre rule to measure the shoot heights in centimetres, Growth Performance was tracked for a period of three weeks. Growth Performance was superior than the Control in soil that had been enhanced with Zn, Fe, and Cu. With the exception of cowpea in 10 ppm and 100 ppm Cr-enriched soil, growth performances in Cr and Pb-enriched soil were lower than those of the Control. Pb was more harmful, and at concentrations of 100 ppm, it killed groundnuts in the second week. Groundnut mortality was triggered concurrently by Fe, but only at 1000 ppm enrichment. In general, growth performance declined as soil concentrations of each heavy metal increased. In terms of growth performance and mortality, cowpeas demonstrated greater heavy metal tolerance than groundnut. (Okon *et al*., 2023).

The purpose of the study was to find out how foliar application of nano-TiO2 affected the toxicity of Cd in cowpea plants. Following a 21-day exposure to Cd toxicity at a rate of 10 mg/kg soil, cowpea plants had six foliar applications of nano-TiO2 intervention. As when compared with Zn-stressed plants without the intervention, the results demonstrated that foliar application of nano-TiO2 considerably increased the amount of chlorophyll b and total chlorophyll concentrations following Cd stress. It's interesting to note that, in comparison to Cd-stressed plants, the Cd levels of roots, shoots, and grains were dramatically reduced ($p<0.05$) after nano-TiO2 sprays. Even after treatments, the levels of Cd in fleshy tissue (leaves and seeds) were still higher than the advised level. In addition, compared to Cd-stressed plants without intervention, nano-TiO_2 interventions increased Zn, Mn, and CO levels in seeds and promoted stress enzyme activity in both roots and leaves. The estimated daily intake of Cd in leaves and seeds for the adult subpopulation exceeded the WHO recommended daily intake by a few fold in both Cd-stressed and nano-TiO_2-treated plants. The health risk quotient (HQ) for the adult subpopulation exceeded unitary in seeds from nano-TiO2 treatments (HQ = 1.75 and 1.96, respectively), but no potential risk was found for leaves. In summary, foliar application of nano-TiO_2 portends significant ameliorative potential for Cd toxicity in cowpea plants (Ogunkunle *et al.*, 2020).

Heavy metal accumulation in plants can be impacted by the repeated addition of industrial waste to the soil. In cowpea (*Vigna unguiculata* [L.] Walp.), the accumulation of Cr, Cd, Ni, and Pb was assessed in the shoots and grains in soil treated with tannery sludge compost (TSC) for three years in a row. Beginning in 2009, cowpea was sown together with yearly applications of compost made from tannery sludge at (0, 2.5, 5, 10, and 20) correspondingly. The build-up of Cr, Cd, Pb, and Ni in the grains and shoots of cowpeas was assessed 40 and 60 days after sowing, respectively. Four replicates were employed in the completely randomised design experiment, and the results were analysed using to an ANOVA and a 5% F-test. After three years of TSC amendment, only Cr accumulation in the cowpea shoots was noticeable ($P < 0.05$); accumulation increased with TSC rates. But there was no appreciable build-up of Cr, Cd, Ni, or Pb in the grains. Cr increased in the shoots during three years of continuous TSC soil amendments, but it did not go to the grains. (Marcal *et al.,* 2013).

The contamination of agricultural soil with heavy metals has grown to be a severe concern. It is now essential to establish appropriate control and remediation solutions for soil contaminated with heavy metals. The purpose of the outside pot experiment was to examine the impact of mycorrhiza, zeolite, and biochar on a decrease of heavy metal bioavailability and its consequent impacts on soil characteristics, plant bioaccumulation, and cowpea growth in extremely polluted soil. The six treatments that were employed were zeolite, biochar, mycorrhiza, zeolite with mycorrhiza, biochar with mycorrhiza, and soil that had not been altered in any way. Four replications and a fully randomised design were used in the experiment. The findings showed that both shoot and root dry weight values were highest when biochar and mycorrhiza were combined, and the root and shoot concentrations of heavy metals were lowest, along with the bio concentrations and translocation determinants for each of the heavy metals. With biochar and mycorrhiza, the availability of heavy metals was significantly reduced compared to the control; the reductions were (59.1, 44.3, 38.0, 69.7, 77.8, 77.2 and 73.6, %) for Cd, Co, Cr, Cu, Ni, Pb, and Zn, respectively. In summary, the integration of biochar with mycorrhiza inoculation is a promising and economical approach to improve heavy metal immobilisation, reduce heavy metal accessibility and plant absorption, and boost cowpea plant development. Additionally, this strategy is considered environmentally friendly. (Ibrahim *et al*., 2023).

CHAPTER TWO

2. 0 MATERIALS AND METHODS

2.1 Study Area

The experimental study was done at the undergraduate project plot of the department of plant Biology and Biotechnology, Faculty of Life Sciences complex, university of Benin, Benin City, Edo State Nigeria.

2.2 Collection and Identification of Samples and Specimen

2.2.1 Source of Soil

The soil was collected from a fallow ground in Faculty of life science complex, university of Benin.

2.2.2 Source of Seed

The seeds of *Vigna unguiculata* used in the experiment were brought from IAR&T Ibadan. The seed specie is Ife brown.

2.2.3 Source of Heavy Metals

The heavy metal used in the experiment was Cadmium which was obtained and purchased from Pyrex shop, opposite University of Benin teaching hospital Ugbowo Benin city.

2.2.4 Source of Nanoparticles

The nanoparticle used in the research was Zinc Oxide Nanoparticles which were obtained from the Melorium technologies U.S.A.

2.3 Apparatus and Equipment

2.3.1 Planting Bags

Planting bags were purchased from the Ring road market Benin City, Edo State.

2.3.2 Measuring Tape/Ruler

A tape was used to measure the plant height in centimetres (cm) at various intervals of plant growth.

2.3.3 Weighing Balance

Camry emperor (44ibs x 20oz-20kg x 50kg), made in China scale was used in the measurement of soil samples.

Electronic/ digital weighing balance was used in the measurement of the ZnO NPs and heavy metals.

2.3.4 Digital Camera

OPPO A15 Was used to take photos of the plant at various stages of the growth of cowpea plant.

2.4 Experimental Setup

This study was carried out using five treatments identified below;

A Control (plants raised in normal soil).

B Control (plants raised in normal soil treated with nanoparticles).

C Plants raised in soil treated with 25mg/kg of Cd and 10mg/kg of zinc oxide nanoparticles.

D Plants raised in soil treated with 25mg/kg of Cd and30mg/kg of zinc oxide nanoparticles.

E Plants raised in soil treated with 25mg/kg of Cd and 50mg/kg of zinc oxide nanoparticles.

Five seeds of *Vigna unguiculata* were planted in each of the 7.0kg of soil-filled bags. To prevent overpopulation, seedlings were thinned, leaving one plant that was utilised to measure growth and changes in the plant. When it was needed, watering was done. Two days following planting, germination studies were done for seven days. The stem height, girth, number of leaves, number of nodes, leaf area and loss of leaves were measured every seven days. (WAP= Week after planting; DAP = day after planting).

2.5 PARAMETERS

2.5.1 Germination Studies

The germination percentage shows the estimate of the viability of the seeds planted.

Represented mathematically as;

$$G\,P = \frac{\text{number of germinated}}{\text{Total number of seeds planted}} \times 100$$

2.5.2 Thinning

The reduction of plants planted by uprooting the plants with the exception of one plant in a bag. This allows one plant to stand as a replicate and also to prevent competition among plants in a bag.

2.5.3 Measurement of Plant Height

The height of the stem was measured from the point of emergence with a measuring tape which was done on a seven (7) day interval.

2.5.4 Measurement of Stem Girth

Leaf area was determined by measuring the length and breadth of the third leaf.

2.5.5 Measurement of leaf loss

The leaf loss was taken and recorded at seven days interval.

2.5.6 Measurement 0f Leaf Area

The stem girth was measured 3cm from the point of emergence with a measuring tape and thread which was done weekly.

2.5.7 Measurement of internode

The distance between the third and fourth leaf was measured and recorded at 7 days interval

2.5.8 Determination of Leaf Number

The leaf number was counted and recorded virtually in each of the plants on a seven (7) day interval.

2.5.9 Determination of the number of nodes

The number of nodes was counted and recorded at 7 days interval.

2.6 Statistical Analysis

The results were subjected to a mean, standard deviation and standard error analysis, to determine if there was any statistical significance between the physiological characters observed in *Vigna unguiculata*

CHAPTER THREE

RESULTS

Table 3.1 shows the effect of Cadmium on germination of *a Vigna unguiculata.* From the result above, it shows Cadmium inhibitory effects on the 75mg/kg as there was low germination rate compared to control and other treatments. From day 3- 7, the highest germination was observed in B at 86.00±0.33 compared with other treatments with 80.00±0.33, 80.00±0.33 and 46.67±0.33 respectively. The values did not follow a particular concentration gradient and there was no significant difference at 5% level of probability between control and other treatments.

From Table 3.2, it was observed that the height of *Vigna unguiculata* increased gradually. Before the application of ZnO NPS as it was seen in Week 1 and 3 that there was a progressive increase in all the various treatments, this implies that the treatments followed a particular pattern consistent with the concentration gradient, but after the application of ZnO nanoparticles in Week 4 to Week 6 the plant increased with A having the highest (58.00±16.64cm) compared to B, C and D (23.33±1.80cm, 22.67±5.23cm and 25.00±12.52cm) respectively with C having the least height, with the results shown, it implies that after the application of nanoparticles the plants did not follow a particular gradient as treatment 50mg/kg was seen with the least height. The application of Nanoparticle did not enhance the height of the plant.

Table 3.1: Germination studies of *V. unguiculata* planted in different concentration of Cadmium polluted soil.

TREATMENT/TIME	**2DAP**	**3DAP**	**5DAP**	**6DAP**	**7DAP**
A	80.00±0.33	80.00±0.33	80.00±0.33	80.00±0.33	80.00±0.33
B	86.67±0.33	86.67±0.33	86.67±0.33	86.67±0.33	86.67±0.33
C	80.00±0.57	80.00±0.57	80.00±0.57	86.67±0.67	86.67±0.67
D	40.67±0.57	46.67±0.33	46.67±0.33	46.67±0.33	46.67±0.33

The values were expressed in Mean ± SEM.

Table 3.2: The effects of zinc oxide nanoparticles on the height of *Vigna unguiculata* planted in Cadmium polluted soil.

TREATMENT/TIME	1WAP	2WAP	3WAP	4WAP	5WAP	6WAP
A	12.47±0.77	14.67±0.33	18.00±0.57	23.00±1.73	32.00±6.81	58.00±16.64
B	11.50±0.85	12.17±1.17	15.50±1.04	18.33±1.45	23.17±2.58	23.33±1.80
C	10.67±1.20	12.33±1.30	14.23±0.67	16.33±0.88	19.17±2.42	22.67±5.23
D	10.33±0.67	12.33±0.92	14.00±1.52	16.33±0.88	22.67±8.17	25.00±12.52

The values were expressed in Mean ± SEM.

Table 3.3 shows a progressive increase in the stem girth across all treatments. At third week of planting, treatment A recorded the highest with 2.20±0.45 and other treatments 1.70±0.03, 1.30±0.12, 1.37±0.08 respectively. After the application of nanoparticles, it was observed that the girth increases in all treatments with C having the least value of 1.90±0.03 and other treatments 2.77±0.38, 2.17±0.07 and 2.70±0.05 respectively in week 6. The values did not follow a particular concentration gradient.

Table 3.4 shows the effects of ZnO nanoparticles on the leaf loss of *Vigna unguiculata,* from the results shown, it was observed that Cadmium had a negative effect on the Leaf Loss in week 3 with treatments C and D having values of 2.00±0.00 and 2.00±0.00 compared to other treatments with 1.00±0.67 and 1.33±0.67 respectively. This implies that the values follow a particular concentration gradient. With the application of Nanoparticles, it was observed in week 5 that control had the highest leaf loss value 2.50±1.00 compared to other treatments with (2.00±0.00, 2.00±0.00 and 2.00±0.00) respectively. This shows that ZnO Nanoparticles has a positive effect on the Leaf loss of the plant.

Table 3. 3: The effects of zinc oxide nanoparticles on the stem girth of *Vigna unguiculata* planted in Cadmium polluted soil.

TIMETREATMENT	**1WAP**	**2WAP**	**3WAP**	**4WAP**	**5WAP**	**6WAP**
A	0.97±0.03	1.47±0.08	2.20±0.45	2.30±0.25	2.33±0.27	2.77±0.38
B	0.73±0.08	1.33±0.03	1.27±0.03	1.57±0.08	1.93±0.07	2.17±0.07
C	0.77±0.03	1.40±0.12	1.30±0.12	1.50±0.06	1.77±0.08	1.97±0.03
D	0.97±0.03	1.33±0.08	1.37±0.08	1.43±0.08	1.77±0.23	2.70±0.05

The values were expressed in Mean ± SEM.

Table 3. 4: The effects of zinc oxide nanoparticles on the leaf loss of *V. unguiculata* in Cadmium polluted soil.

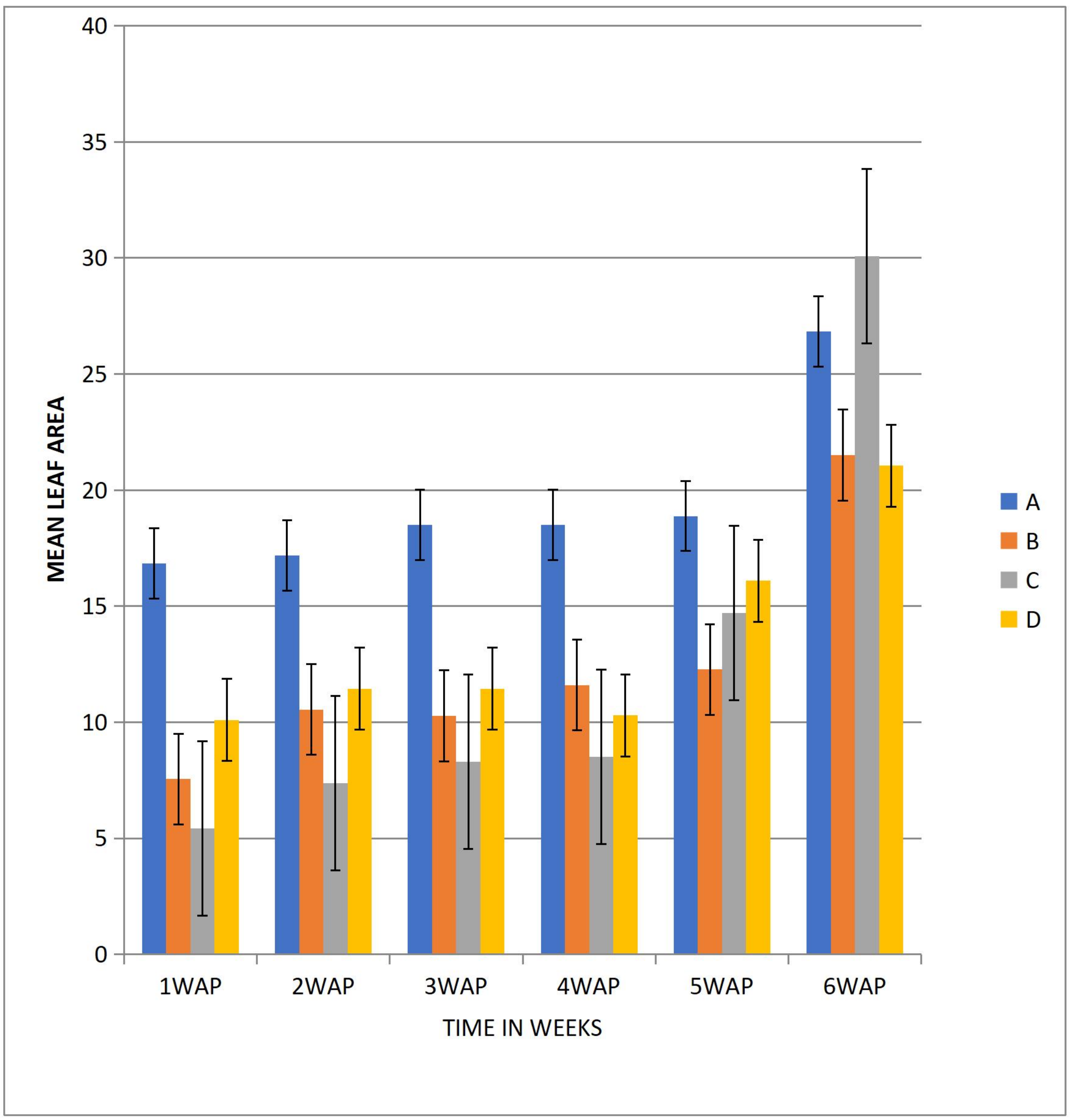
TIME/TREATMENT	1WAP	2WAP	3WAP	4WAP	5WAP	6WAP
A	0.00±0.00	1.00±0.57	1.00±0.67	1.00±0.67	2.50±0.33	1.50±1.00
B	0.33±0.33	1.33±0.67	1.33±0.67	1.67±0.33	2.00±0.00	2.00±0.00
C	0.00±00	1.33±0.67	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
D	0.00±0.00	0.33±0.33	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00

The values were expressed in Mean ± SEM.

The results for the leaf area in figure 3.1 show an increase in the leaf area across all treatments. With treatment A having the highest and treatment D, the least value in week three. (8.30 ±4.50) after the application of nanoparticle, it was observed that the various treatments grew with the application of nanoparticle in week 6,C recorded highest (30.08±10.17) with other treatments 21.00±5.34, 21.05±5.00 and 26.83±6.67 respectively. This implies that the values did not follow a concentration gradient as the treatment with 50mg/kg recorded the highest and also shows positive effect of Zinc Oxide nanoparticles on the leaf area of the plant and the negative effect of Cadmium..

Figure 3.2 shows a progressive increase in all treatments. From the results shown it was observed that treatments B, C and D had a slow internode length 1.00±0.00, 0.87±0.17, 0.17±0.17 and control 1.23±0.15 grew more than the other treatments in week 1-3 which shows the effect of Cadmium on the internode on the treated plants. With the application of the ZnO nanoparticles in week 6, A recorded 2.07±0.29 and other treatment measured 2.07±0.29, 1.37±0.32, 2.03±0.18 and 0.47±0.47 it was observed that the control grew irrespective of nanoparticles application on other treatments. This infer that zinc nanoparticles had no effect on the internode and the values obtained did not follow a particular concentration gradient.

From figure 3.3, it was observed that the number of leaves increased all through the treatments except for treatment with the highest pollutant with 2.67±0.88 in week 2 and week 3 respectively before nanoparticle application. After the nanoparticle application there was an increase in week 4 and 6 (3.67±1.3 and 6.00±1.00) respectively.



40
35
30
25
20
15
10
5
0
MEAN LEAF AREA
1WAP
2WAP
3WAP
4WAP
5WAP
6WAP
TIME IN WEEKS
A
B
C
D

Figure 3. 1 Effects of Zinc Oxide nanoparticles on the leaf area of *V. unguiculata* planted in different concentration of Cadmium polluted soil

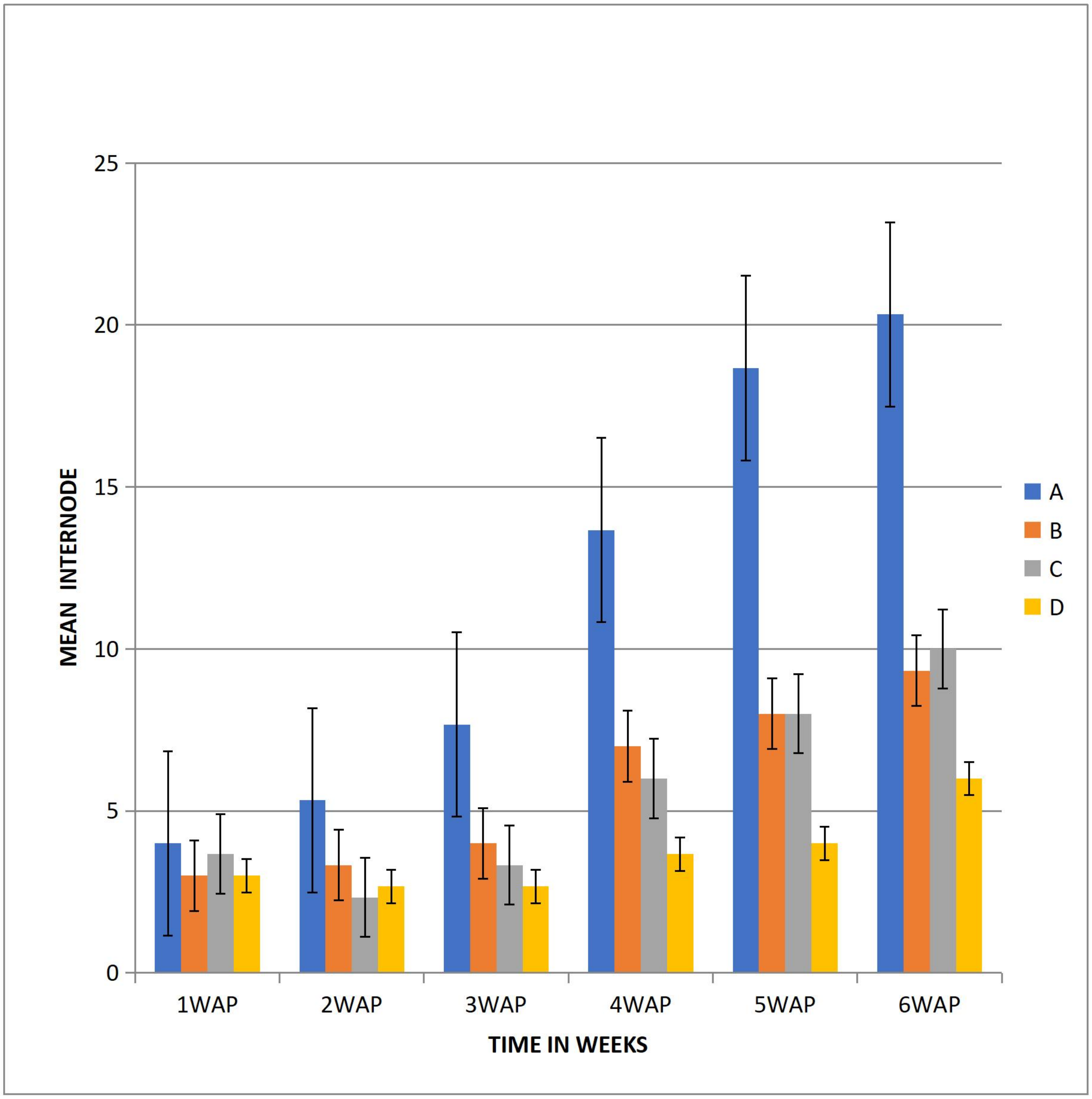


Figure 3. 2: Effects of Zinc oxide nanoparticles on the internode of *V. unguiculata p*lanted in different concentration of Cadmium treated soil.

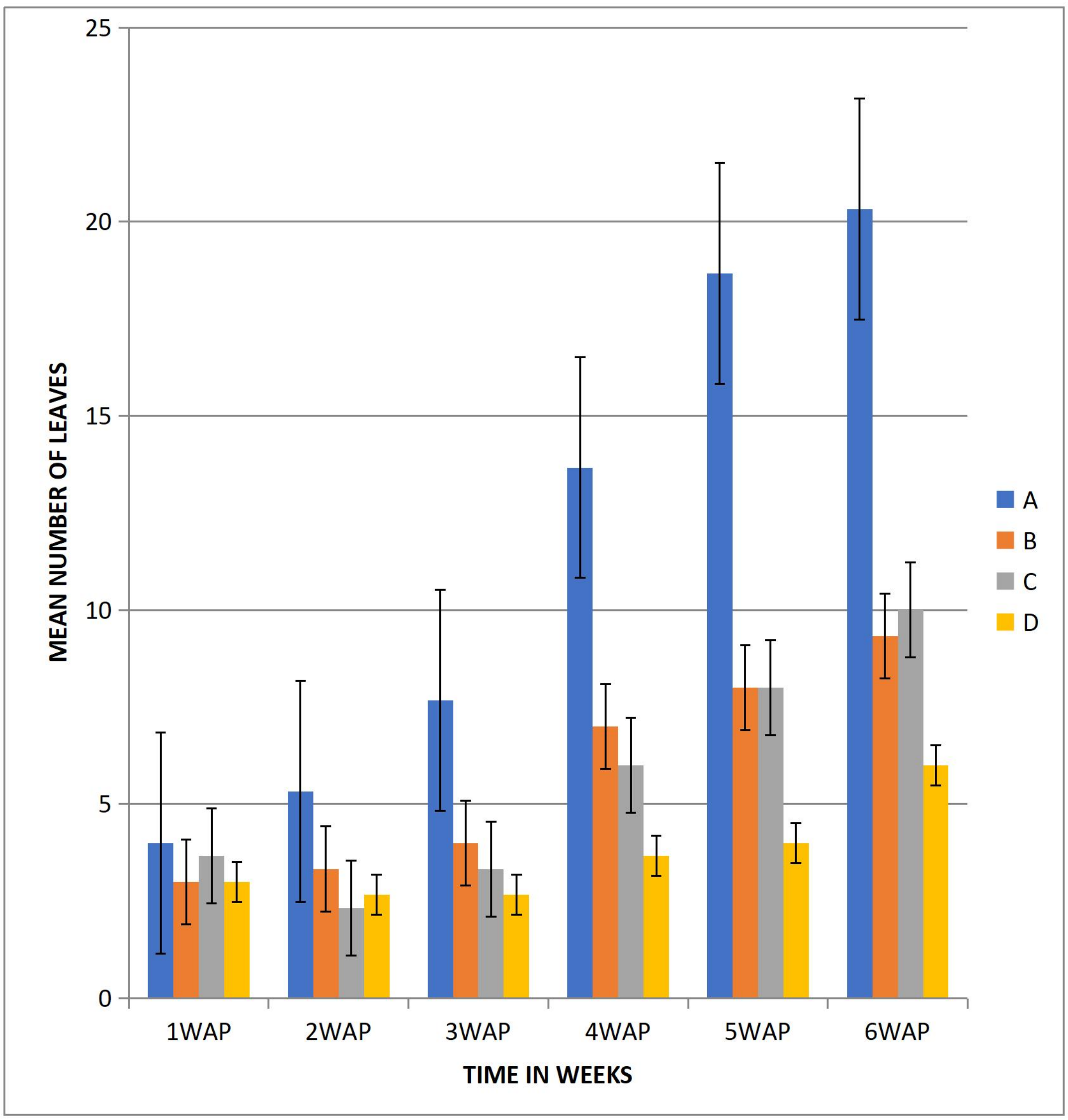


Figure 3. 3: The effects of Zinc Oxide nanoparticles on the number of leaves of *V. unguiculata* planted in Cadmium polluted soil.

Figure 3.4 show that the number of nodes increased throughout all the treatments especially in the treatment with the highest pollutant which recorded 4.33±0.88 in week 3 before nanoparticle application and other treatments and recorded 8±3.00, 6.67±0.88, 5.00±0.57, respectively. Treatment C grew exponentially in week 6 after the application of zinc oxide nanoparticles 19.67±10.17 and B recorded 19.67±2.08 compared to other treatments with 11.00±1.73 and 14.67±4.63 respectively. This shows that the values did not follow a particular gradient.

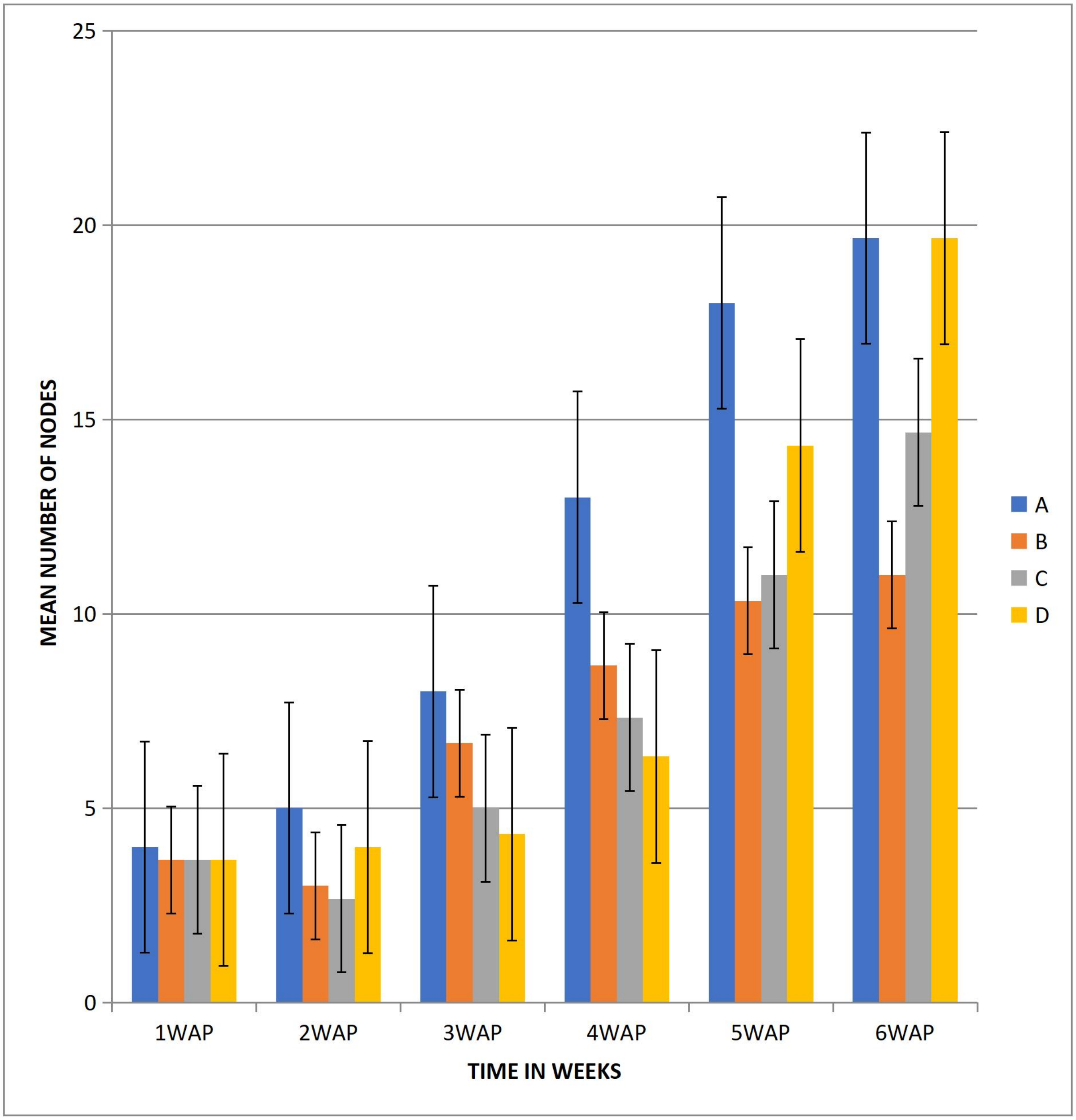


Figure 3. 4: Effects of Zinc Oxide nanoparticles on the number of nodes of *V. unguiculata* in Cadmium polluted soil.

Plate 1: *V .unguiculata* plant in bags on the field

Magnification=x5.

Plate 2: V. unguiculata at 30 DAP

Magnification=x5

Plate 3: *V. unguiculata at* 40DAP

Magnification=x5.

Plate 4: Taking Readings of *V. unguiculata*

Magnification =x5

CHAPTER FOUR

DISCUSSION

The heavy metal cadmium has a devastating effect on the growth of Vigna unguiculata (cowpea). Inhibition of Photosynthesis, Oxidative Stresses, any nutritional deficiency occurs when there is a disruption in the absorption of nutrients, such as calcium and iron, which are vital for normal growth and development. Together, these elements cause cowpea to wilt, grow more slowly, and have lower biomass (weight from shoots and roots).

The growth pattern of *Vigna unguiculata* was conducted in the field study to explore how Zinc Oxide nanoparticles impact the growth, stress tolerance and absorption mechanism of heavy metals in *Vigna unguiculata.* Before planting, the seeds were pre-treated to identify viable seeds and also to facilitate the germination procedure.

As shown in table 3.1, *unguiculata* seeds began to germinate 2 DAP, that is on the third day. Most seedlings sprouted out, except for the ones in the highest concentration of Cadmium, which did not sprout like any other treatment. This shows variations in the control and various treatments (de la Rosa *et al*., 2013). Germination studies were carried out for seven days, the result in the findings proved that Cadmium heavy metal had effect on the control and various treatments. .From control A to the different treatments, there was a steady rise in the growth performance measured in terms of height. Control A recorded the highest value whereas treatment C recorded the lowest. It was seen that the plant height grew as the experiment proceeded except for the treatment C which grew slowly in height (Prasad *et al*., 2012).

The effect of zinc oxide nanoparticles on the stem girth showed progressive increase from control A to treatments B, C and D. Treatment A recorded the highest value for girth while treatment C recorded the least value for girth. As the field experiment proceeded, it was observed that the girth increased progressively. Data pertaining to plant girth showed that the application of Zinc Oxide nanoparticles during the period of the study influenced the girth of cowpea. It was also noticed that the treatment which contains no heavy metal grew faster than the others.

The result for the leaf area showed an increase in all treatment and control. As investigated by Faizan *et al,* tomato plants facing salt stress treated with ZnO nanoparticles resulted in Significant improvements in its growth parameters including its leaf area. (Faizan *et* al.,2021). This implies that cadmium had effect on the leaf area of the plant and also the nanoparticles having significance on the leaf area of the plant by a slight increase in its area, it can be inferred that the nanoparticles slightly influences the leaf area and also the internode of the plant. The observed decrease in leaf loss following the application of ZnO nanoparticles hints at a potential positive effect of the nanoparticles on the plants. ZnO nanoparticles might have enhanced the plant's immune response, improved nutrient uptake, or provided some form of protection against pathogens or stress factors. This result aligns with existing research that suggests nanoparticles can have beneficial effects on plant growth and stress tolerance (Mustapha *et al.,* 2023), During the experiment, it was observed that the leaves of *Vigna unguiculata* began to drop due to pest attacks and environmental pressures, Senbill et al highlighted that zinc oxide nanoparticles were effective in combating pests, resulting in reduced pest infestations when applied (Senbill *et al.,*2023). The increase in internode length, number of leaves, and nodes in plants reflects their dynamic response to environmental cues and internal regulatory mechanisms. Li *et al,* also reported that the treatments of rice seeds with zinc oxide nanoparticles affects the physiological, biochemical and molecular characteristics of plants under Cd stress (Li *et al*., 2021).

Some research suggests that ZnO NPs are able to enhance the overall nutritional profile in the soil, possibly enhancing cowpea development. ZnO NPs contend with Cadmium for binding regions on the root system's surface, which could decrease cadmium absorption by the plant. ZnO NPs can additionally encourage the antioxidant defence mechanism of the cowpea, assisting defend against the radicals known as free radicals generated through Cd exposure.

Overall, the research suggests ZnO NPs might act as a potential remediation strategy to alleviate the negative effects of Cd on cowpea growth. However, more research is required to fully understand the mechanisms and optimize ZnO NP application for maximum benefit.

CONCLUSION

The findings indicated that the nanoparticles had a beneficial effect on the growth of the species of plants and gave them more resistance to environmental stressors like drought. Additionally, the nanoparticles prevented the plants from absorbing heavy metals, which protected the plants' health. These results highlight the possibilities of zinc oxide nanoparticles to transform agricultural practices by enhancing crop output and reducing the hazards related to heavy metal pollution. Promising clues about the direction of sustainable farming are provided by this research.

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